Biogeosciences Discuss., 7, 3061–3094, 2010
www.biogeosciences-discuss.net/7/3061/2010/
doi:10.5194/bgd-7-3061-2010

Biogeosciences
Discussions

The trophic biology of the holothurian *Molpadia musculus* at 3500 m in the Nazaré Canyon (NE Atlantic)

T. Amaro[1,3], S. Bianchelli[2], D. S. M. Billett[3], M. R. Cunha[1], A. Pusceddu[2], and R. Danovaro[2]

[1]CESAM e Departamento de Biologia, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal
[2]Dipartimento di Scienze del Mare,Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italy
[3]National Oceanography Centre, Southampton, University of Southampton, Waterfront Campus, European Way, Southampton SO14 3ZH, UK

Received: 9 April 2010 – Accepted: 12 April 2010 – Published: 28 April 2010

Correspondence to: T. Amaro (amaro@ua.pt)

Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

Megafaunal organisms play a key role in the deep-sea ecosystem functioning. At 3500 m depth in the Nazaré Canyon, NE Atlantic, very high abundances of the infaunal holothurian *Molpadia musculus* were found. Sediment samples and holothurians were collected by ROV and experiments were conducted in situ in incubation chambers. The biochemical composition of the sediment (in terms of proteins, carbohydrates and lipids), the holothurians' gut contents and holothurians' faecal material were analysed. In the sediments, proteins were the dominant organic compound, followed by carbohydrates and lipids. In the holothurian gut contents, conversely, protein concentrations were higher than the other compounds and decreased significantly as the material passed through the digestive tract. About 33±1% of the proteins were digested already in the mid gut, with a final digestion rate equal to 67±1%. Carbohydrates and lipids were ingested in smaller amounts and digested with lower efficiencies (23±11% and 50±11%, respectively). As a result, biopolymeric C digestion rate was on average 62±3%. We also calculated that the entire holothurians' population could remove from the sediment about 0.49±0.13 g biopolymeric C and 0.13±0.03 g N m^{-2} d^{-1}. These results suggest that the *M. musculus* plays a key role in the benthic tropho-dynamics and biogeochemical processes of the Nazaré Canyon.

1 Introduction

The deep sea is the largest ecosystem on Earth, covering about 60% of the globe's surface. Deep-sea ecosystems provide the largest reservoirs of biomass and non-renewable resources (Gage and Tyler, 1991), which are linked to their biodiversity (Danovaro et al., 2008b). They act as the ultimate sink for organic material derived from the upper ocean's primary production and are essential in the biogeochemical cycling of organic matter (OM) on a global scale (Dell'Anno and Danovaro, 2005; Danovaro et al., 2008a).

Deep-sea submarine canyons host hotspots of OM and biomass (Gage et al., 1995; Vetter and Dayton, 1998; Duineveld et al., 2001; Bianchelli et al., 2008; Ingels et al., 2009; Tyler et al., 2009; Pusceddu et al., 2010). Within canyon, biodiversity can be high, because of their topographic complexity and spatio-temporal variability (Bianchelli et al., 2008; Ingels et al., 2009; Tyler et al., 2009) . These features also make canyons difficult environments to investigate. As a result, knowledge of their ecological functioning, including OM supply and remineralisation are almost entirely unknown.

The Nazaré Canyon is a major sediment pathway on the Portuguese margin, and one of the most spectacular topographic features of the NE Atlantic margin (de Stigter et al., 2007; Arzola et al., 2008, Masson et al., 2010). Large concentrations of organic C and biopolymeric C (BPC) were found at the middle section of the Nazaré Canyon, suggesting this canyon to act as a sink for OM burial (de stigter et al., 2007; Pusceddu et al., 2010; Masson et al., 2010). Sediments and OM are transported from the continental shelf down the canyon and into the deep sea (de Stigter et al., 2007; Oliveira et al., 2007). Enrichment of OM in canyon sediments leads to enhanced benthic abundance and biomass (Rowe et al., 1982; Soetaert et al., 1991; Vetter and Dayton, 1998, 1999; Palanques et al., 2005; Bianchelli et al., 2008), and increase metabolic activities (Duineveld et al., 2001; Accornero et al., 2003).

In the central part of the Nazaré Canyon (3500 m depth) the deep-sea burrowing sea cucumber, *Molpadia musculus* (Riso, 1826), occurs on consistently high abundances, which is unusual for a deep-sea burrowing species at this depth (Amaro et al., 2009). The reasons of such a massive occurrence are still unknown. Food supply can be an important factor controlling the abundance and composition of holothurian assemblages (Sibuet, 1985). For example, Ruhl and Smith (2004) found correlations between food supply and megafaunal abundance over a 14-year time series at a deep-sea site of the Pacific Ocean. Similar correlations have been reported also in the Porcupine Abyssal Plain (PAP) in the northeast Atlantic (Billett et al., 2001). The food sources for benthic organisms may include organic detritus (including seagrass/algae decries and dead or decaying animals), microorganisms (prokaryotes, diatoms, protozoans) and faecal pellets of other animals (Massin, 1982; Moriarity, 1982; Jumars, 1993; Roberts et al., 2001). The relative proportions of these potential food items determine the nutritional value of the ingested material, which, in turn, regulates the potential utilization rates by deposit-feeding holothurians (Purinton et al., 2008). A proportion of the material ingested is fresh (i.e., recently deposited detritus from the surface ocean). Some of the OM is degraded partially by benthic metazoans and prokaryotes (Smith et al., 1993; Levin et al., 1997; Miller et al., 2000). The largest fraction of OM in deep-sea sediments is composed of refractory compounds and becomes available to higher benthic organisms only after ageing and microbial breakdown (Danovaro et al., 1993; Pusceddu et al., 2009). Although there have been several investigations of the nutrition of deposit-feeding holothurians (Deming and Colwell, 1982; Sibuet et al., 1982; Billett et al., 1988; Roberts et al., 1991, 2000; Ginger et al., 2001; Witbaard et al., 2001; Hudson et al., 2005), the debate concerning the biological and chemical composition of the material ingested and assimilated by these organisms is still open. Khripounoff and Sibuet et al. (1980) suggested assimilation efficiencies of 15% for total organic carbon (TOC) and 22% for total nitrogen (TN) in some abyssal species. Likewise, Ginger et al. (2001) estimated assimilation efficiencies from 15 to 46% for TOC and from 11 to 53% for TON in four abyssal holothurians. Moreover, it is still largely unknown the contribution these large organisms make to the biogeochemical processes and organic matter cycling in deep-sea ecosystems.

The aim of our study is to understand the role of *M. musculus* in the ecosystem functioning of the Nazaré Canyon at 3500 m. To do so, we investigated the biochemical composition of potential food sources (in terms of proteins, carbohydrates and lipids) and estimated enzymatic digestion and potential utilization by *M. musculus*. In addition, owing to the enormous abundance of this deep-sea holothurian, we quantify the impact of holothurians on C and N cycling at mid canyon depths.

2 Material and methods

2.1 Study area

The Nazaré Canyon is a very narrow and elongated system. It extends for more than 210 km, from the canyon head less than 1 km from the Portuguese coastline to the abyssal plain at 5000 m depth (Fig. 1). Unlike many submarine canyons, the Nazaré Canyon is not connected to a major river system. However, it is a major sediment pathway on the Portuguese margin (de Stigter et al., 2007; Masson et al., 2010). The sediment is predominantly of terrigenous silt and clay origin (Alt-Epping et al., 2007). It is transported actively from the upper shelf to the canyon to the abyssal plain by sediment gravity flows that occur on annual or longer timescales and are particularly vigorous during periods of winter storms (de Stigter et al., 2007). The down canyon currents lead to cycles of resuspension and deposition, alternating with intervals during which the sediment is deposited on the sea bed (de Stigter et al., 2007). The finer sediment resuspended by bottom currents, settles on the terraces adjacent to the thalweg, whereas some sand or even coarser material generally travels through the canyon. In the upper and middle canyon there are moderately strong tidal currents (max. currents up to 35 cm s^{-1}) which can cause sediment resuspension and, consequently, transport and redistribution particulate matter (Quaresma et al., 2007).

2.2 Species studied

Molpadia musculus (Phylum Echinodermata) is a surface burrowing deposit-feeder holothurian. It has a stout body and a posterior region which narrows to form a “tail”. There are no tube feet (Fig. 2). Species of the genus *Molpadia* inhabits muddy environments from shallow water to abyssal depths (Rhoads and Young, 1971). These organisms feed head down in the sediment and use the tail to keep contact with the sediment surface (Pawson et al., 2001).

2.3 Sampling

Densities of *M. musculus* were determined using the data collected during RRS *Charles Darwin* cruise 179 during 12 April and 17 May 2006. Eight megacores, fitted with 100-mm diameter core tubes, were deployed at ca. 3500 m (Table 1) in the Nazaré Canyon. Molpadiids holothurians were counted and their density determined from the cores.

Sediment samples, holothurians and their faecal material were collected using the *Isis* Remotely Operated Vehicle (ROV) at 3500 m in the Nazaré Canyon during the RRS *James Cook* cruise 10, from the 3 June to the 7 July 2007 (Table 1). Intact animals were collected using scoops and undisturbed sediment samples were taken with the cores.

Sediment samples were collected using 3 push cores fitted with 57 mm inner diameter core tubes. Upon recovery, each of three cores was divided in 5 layers: 0–1, 1–3, 3–5, 5–10, 10–15 cm and deep frozen at −80 °C until analysis. The water temperature at the sampling depth was about 3 °C.

As *M. musculus* individuals were not visible in ROV video footage of the seafloor, these specimens were collected with a ROV scoop that digged into the sediment and collected the specimens. The mud collected at the same time as the holothurians was removed by shaking the scoop until the sediment fell away. A total of 10 holothurians were collected at the same location as the sediment samples (Table 1) and returned to the ship inside the bio-box held on the retractable tool tray (Hughes et al., 2010). Only intact animals were selected for the study. *Molpadia musculus* specimens were put immediately at in situ temperature (3 °C), in a temperature-controlled laboratory. Each specimen was dissected in a sterilized Petri dish. The gut was opened and sediment samples were taken from three different parts of the gut (the oesophagus, the mid gut and the hind gut) making certain to exclude all gut tissues (Amaro et al., 2009). Each sediment sample from the gut was transferred to a vial and stored at −80 °C until analysis.

Faecal material was collected during an in situ experiment placed on the seabed. The in situ experiment consisted of a circular chamber with 6 individual funnels that allowed each specimen to defecate to an individual sterilized vial. Five specimens were collected with the same method described above. Each *M. musculus* was placed into an individual funnel. A sixth funnel was used as a control. Once each specimen was on the funnel, no evisceration or defecation was evident during the time of the set up of the experiment. The experiment lasted three days, the time necessary for these organisms to defecate (Amaro, personal observation). Once on board, the faecal material was collected in a sterilized vial attached to the end of each funnel and stored at −80 °C (Hughes et al., 2010).

2.4 Analysis of potential food sources

The protein, carbohydrate and lipid contents of 1) the sediments, 2) the material extracted from the gut and 3) the faecal material, were determined spectrophotometrically, and their concentrations were calculated from calibration curves of serum albumin, D-glucose and tripalmitine equivalents, respectively, and normalised to sediment dry weight (Pusceddu et al., 2009). For each biochemical assay, blanks were obtained using pre-combusted sediments (450 °C for 4 h). The detailed protocols are described in Danovaro (2010). All of the analyses were performed on three replicates, with about 0.2–1 g of wet sediment per sample.

Biopolymeric carbon was defined as the sum of the carbon equivalents of total proteins, carbohydrates and lipids (using conversion factors of 0.49, 0.40 and 0.75, respectively) and has been often reported as the fraction of total organic C potentially available to benthic consumers (Fabiano et al., 1995; Danovaro et al., 2001; Dell'Anno et al., 2002, 2003).

As *M. musculus* feeds head down in the sediment, it was assumed that all of the biochemical compounds ingested by these holothurians occurred in the top 15 cm depth of the sediment.

For all of the biochemical compounds, the digestion rate was estimated as the percentage of the difference in concentration between the oesophagus and hind gut. Based on the concentrations of the different biochemical compounds in the gut, faeces and sediment layers deeper than 1 cm, we determined the concentration factor as the ratio between concentrations in the oesophagus and in the sediment.

2.5 Analyses of exo-enzymatic activities

Extracellular enzyme activities (aminopeptidase, β-glucosidase and alkaline phosphatase) were determined by cleavage of analogue fluorogenic substrates (L-Leucine-4-methylcoumarinyl-7-amide, Leu-MCA; 4-MUF-β-D-glucopyranoside, MUF-Glu and 4-MUF-P-phosphate, MUF-P respectively; all substrates from SIGMA) as described in Danovaro et al. (1998). After evaluation of saturation concentrations, measurements were carried out in triplicate by adding 100 µl of MUF-Glu and Leu-MCA (final concentration 200 µM) and 50 µl of MUF-P (final concentration 50 µM) in a final volume of 5 ml of seawater. Substrate incubations were performed in the dark at in situ temperature for 1h. Samples were analyzed fluorometrically (at 380 nm excitation, 440 nm emission for Leu-MCA and 365 nm excitation, 455 nm emission for MUF-Glu and MUF-P) as reported in Danovaro et al. (2005). The detailed protocols of all enzymatic analyses are described in Danovaro (2010). Data were normalized to dry weight (60 °C, 24 h) and reported as nanomoles of fluorescein released per gram of sediment per hour.

2.6 Data analysis

Differences in the quantity of the different potential food sources between sediments and the holothurian gut material and faeces, and between the different layers in the sediment, were investigated separately by means of one-way analyses of variance (ANOVA). One-way ANOVA was also applied to test for differences in extracellular enzymatic activities between the different gut sections (oesophagus, mid gut and end gut) and the faeces. When significant differences were observed, then post-hoc Tukey's tests were performed. For all ANOVA and Tukey's tests, an alpha level of 0.05 was used as the threshold for statistical significance.

The differences in 1) the biochemical composition (protein, carbohydrate and lipid) of the different sediment layers, 2) the biochemical composition and enzymatic activities within the holothurian gut material and 3) the top cm of the sediment, were investigated by a distance-based permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001; McArdle and Anderson, 2001). PERMANOVA was carried out using the PERMANOVA package included in the Primer 6+ software. The analysis was based on Euclidean distances of normalized data using 4999 random permutations of the appropriate units (Anderson and Ter Braak, 2003). The pseudo-multivariate variance components for each term in the model were calculated using direct multivariate analogues to the univariate ANOVA estimators (e.g., Searle et al., 1992).

3 Results

3.1 Abundance of molpadiids holothurians

We estimated a total abundance of 220±75.4 *M. musculus* per m^2 based on the number of organisms present in the total area sampled by the cores.

3.2 Analyses of potential food sources in sediments and in holothurian gut contents

Vertical patterns of protein, carbohydrate, lipid and biopolymeric C concentrations in the top 15 cm of sediment are illustrated in Fig. 3. Proteins were the dominant biochemical compound (70%, on average of all sediment layers), followed by lipids (18%) and carbohydrates (12%). Total protein concentrations ranged from 10.76 ±0.63 $\mathrm{mg\,g^{-1}}$ in the top cm of the sediment to 5.11±0.80 $\mathrm{mg\,g^{-1}}$ in the 10–15 cm layer.

Sedimentary protein content decreased significantly from the 0–1 cm layer to the deeper sediment layers (Table 2). No significant differences were observed between all other layers. Carbohydrate concentrations in the sediment ranged from

$1.29 \pm 0.44\,\mathrm{mg\,g^{-1}}$ in the top 0–1 cm layer to $1.51 \pm 0.31\,\mathrm{mg\,g^{-1}}$ in the 10–15 cm layer. There were no significant changes with increasing depth in the sediment (Table 2). Lipid concentrations ranged from $0.92 \pm 0.09\,\mathrm{mg\,g^{-1}}$ in the top cm of the sediment to $1.21 \pm 0.16\,\mathrm{mg\,g^{-1}}$ in the deepest sediment layer. No significant changes with increasing depth in the sediment were detected (Table 2). Biopolymeric C sediment content displayed the same trend as proteins with a significant difference between the top 1 cm and the rest of the sediment core (Table 2). As for the different classes of organic compounds, no significant differences were observed between the layers from 1 to 15 cm depth in the sediment core.

3.3 Organic matter in holothurians guts and faeces

Changes in protein, carbohydrate and lipid contents through the gut and in the faeces of *M. musculus* are illustrated in Fig. 4. Protein concentrations ranged from $17.05 \pm 3.26\,\mathrm{mg\,g^{-1}}$ in the oesophagus to $5.57 \pm 0.83\,\mathrm{mg\,g^{-1}}$ in the faeces. In all holothurians gut tracts and in the faeces, protein content was 5 to 17 times greater than the other biochemical compounds. Protein concentrations were significantly different between the oesophagus to the other holothurian gut sections (Table 3). Carbohydrate content ranged from $1.85 \pm 0.64\,\mathrm{mg\,g^{-1}}$ in the oesophagus to $2.24 \pm 0.34\,\mathrm{mg\,g^{-1}}$ in the mid gut, and $1.38 \pm 0.29\,\mathrm{mg\,g^{-1}}$ in the faeces, but there were no significant differences between the different gut sections and the faeces (Table 3). Lipid concentrations varied from $2.14 \pm 0.68\,\mathrm{mg\,g^{-1}}$ in the oesophagus to $3.97 \pm 0.75\,\mathrm{mg\,g^{-1}}$ in the mid gut and to $1.02 \pm 0.11\,\mathrm{mg\,g^{-1}}$ in the faeces. There was no specific trend from the oesophagus, the mid gut and the terminal part of the holothurians' guts (Table 3). Biopolymeric C concentrations ranged from $10.70 \pm 2.24\,\mathrm{mg\,g^{-1}}$ in the oesophagus to $4.04 \pm 0.61\,\mathrm{mg\,g^{-1}}$ in the faeces.

Based on the concentrations of the different biochemical compounds in the gut, faeces and sediment layers deeper than 1 cm, we determined the concentration factor as the ratio between concentrations in the oesophagus and in the sediment. The concentration factor of proteins in the oesophagus was about 3.1. For carbohydrate and lipid the concentration factors were about 1.3 and 2.0 respectively. The mean concentration of biopolymeric C in the oesophagus was about 2.7 greater than in the sediment.

Digestion rates of the different biochemical compounds were then calculated as the difference in concentration between the material in the oesophagus and that in the faeces. These calculations revealed that proteins had the highest digestion rate (67±1%), followed by lipids (50±11%) and carbohydrates (23±11%). Overall, biopolymeric C digestion rate was 62±3% (Fig. 5).

3.4 Enzymatic activities

Changes in extracellular enzymatic activities within the holothurians gut are presented in Fig. 6. The PERMANOVA revealed that, overall, enzymatic activity changed considerably between the different gut sections and surface sediments (Table 4). Aminopeptidase and alkaline phosphatase activities displayed significant differences between the different gut sections and the surface sediment, but β-glucosidase activity was almost invariant. In particular, values of aminopeptidase and alkaline phosphatase activities were significantly higher in the mid gut than in the other gut sections and in the top 1 cm of the sediment (Table 4).

4 Discussion

Deep-sea holothurians are common in most deep-sea soft bottoms and dominate the megafaunal abundance and biomass over large parts of the deep-sea floor (Sibuet et al., 1982; Billett 1991; Roberts et al., 2000). In recent years, large scale changes have been noticed in the density of abyssal megafauna species both in the NE Atlantic (Billett et al., 2001) and NE Pacific (Lauermann et al., 1996; Ruhl and Smith, 2004). Significant effects were observed on the process of the OM on the seabed when species changed in density by over 3 orders of magnitude in a period of two and a half years (Bett et al., 2001). Although the reason of such a rise of holothurians abundance is still uncertain, it is possible to hypothesise that this phenomenon is associated to favourable quantity and availability of trophic resources (Billett et al., 2010). In the present study, very high abundances of the infaunal holothurian *M. musculus* were found at 3500 m in the Nazaré Canyon. For example, *Molpadia blakei* was found with abundances of ca. 0.0044 ind. per m^2 at depths of ca. 3900 m at the PAP site (Billett, 1991). In contrast the abundances here were 220±75.4 ind. per m^2, which is unprecedented in deep-sea infaunal holothurians. To investigate the potential food sources that might sustain such a large population, the biochemical composition of the sediment, the holothurians' gut contents and holothurians' faecal material were analysed. We used the labile portion of OM in the sediment as the possible food source for these organisms. This labile portion of OM mainly consists of simple and/or combined compounds (i.e. biopolymers) like proteins, carbohydrates and lipids, which are rapidly mineralised (Danovaro et al., 2001; Pusceddu et al., 2009). Organic matter in the sediments where *M. musculus* exists in high densities is composed mainly by proteins, followed by carbohydrates and lipids (Fig. 3). High concentrations of proteins (and biopolymeric C) were found in the top 0–1 cm layer and despite a significant decrease to 1–3 cm layer, large quantities were observed also down to 15-cm depth in the sediment core. For example, at the PAP, sedimentary proteins were lower when compared to our results. The highest estimated value in the top 0.5 cm was ca. 1.4 mg g^{-1} (Danovaro et al., 2001). In other studies, the availability of proteins in deep-sea sediments was even lower than the one mentioned above (Sibuet, 1984; Pfannkuche and Thiel, 1987; Danovaro et al., 1993; Boetius et al., 1996, Tselepides et al., 2000). Carbohydrates and lipids were in the same range as for the PAP deep-sea sediments (Danovaro et al., 2001). This indicates that the subsurface sediment layers of the Nazaré Canyon are characterised by a relatively large availability of compounds with high nutritional quality (i.e. N rich). These results were consistent with previous studies that reported that sediments here are enriched in OM and chlorophyll-*a* and present a significant accumulation of labile and high quality OM, compared with other sites along the Portuguese margin and canyons in the Mediterranean regions (Danovaro et al., 1999; Tselepides et al., 2000; García and Thomsen, 2008; García et al., 2008; Amaro et al., 2009; Pusceddu et al., 2010; Masson et al., 2010). The Nazaré Canyon displayed sediment accumulation rates higher than those found in other regions located along the west Iberian continental slope (between 5 and 17 mm y^{-1} on various different terraces) (de Stigter et al., 2007; Arzola et al., 2008; Pusceddu et al., 2010). The rates found at ca. 3500 m are higher than expected for these depths (14 mm y^{-1}) and are closer to those found in the canyon head (de Stigter et al., 2007). This suggests this part of the canyon to be a mid-canyon depocenter of sediment and OM (Arzola et al., 2008; Masson et al., 2010). The cause for this is still unknown, however it is thought to be related to a number of regional oceanographic and local climatic processes like wind, internal waves, tidal currents (Schmidt et al., 2001, Vitorino et al., 2002a, b; Canals et al., 2006; Masson et al., 2010), floods and storms (de Stigter et al., 2007), which have been observed in other deep-sea canyons along the European continental margins (Pusceddu et al., 2010). The export of organic C from the upper to the mid part of the canyon is a recurrent mechanism fuelling the benthos of this area with a significant amount of OM of high nutritional quality. For example, an accumulation of labile organic compounds on the seabed is often associated with unprecedented abundance, biomass and biodiversity of metazoan meiofauna (Danovaro et al., 2002, 2003; Gambi et al., 2003).

The gut of a holothurian is a sort of bioreactor (Jumars, 2000), in which the animals quickly extract assimilable food from the ingested sediments. A wide spectrum of hydrolytic gut enzymes has evolved to fit the diet of each species, reflecting the types of food items that are available in the sediment (Féral, 1989).

Sibuet et al. (1982), investigating the trophodynamics of the holothurians *Deima validum validum* and *Pseudostichopus villosus* from the Demera Abyssal Plain, estimated assimilation efficiencies of ca. 18% for proteins, ca. 2.7% for carbohydrates and ca. 1.1 % for lipids. A total of ca. 22.3% and 20.5% of the ingested material was assimilated by *D. validum validum* and *P. villosus*, respectively. In our study we found high concentrations of proteins in the oesophagus of *M. musculus* (5–17 times higher than the other biochemical compounds) while lipids and carbohydrates were present in low concentrations (Fig. 4). Protein concentrations decreased significantly between the oesophagus and the rest of the holothurian gut, concomitantly with the highest aminopeptidase activities found in the mid gut (Fig. 6). Since this enzyme is responsible of the hydrolysis of proteins and to break them down into oligomers which are more easily assimilated by the animal (Roberts et al., 2001), we conclude that the mid gut is the portion of the holothurians where the highest fraction (about 33±1%) of the proteins is digested, with a final digestion rate equal to 67±1%. Carbohydrates and lipids were not removed in the mid gut, but were preferentially digested in the end gut, with final digestion rates equal to 23±11% and 50±11%, respectively (Figs. 4, 5, 6). This result reflects the preferential utilization of organic N and lipids in the dietary requirement of these holothurians. Moreover, the significantly higher alkaline phosphatase activity in the oesophagus and mid gut when compared to the hind gut (Figs. 4, 6) supports the hypothesis of a potential and quick exploitation of organic P in the mid gut. Phosphatase activity contributes to degradation and cycling of organic phosphorus. The activity of this enzyme has been found to be consistently high in abyssal holothurians (Roberts et al., 2001), so that it could be hypothesised a potentially important contribution of holothurians in the P cycling in deep-sea sediments.

Previous studies have hypothesised a key role of the prokaryotic biomass in the diet of deep-sea holothurians (Deming and Colwell, 1982; Sibuet et al., 1982; Roberts et al., 2001; Amaro et al., 2009). However, overall prokaryotic biomass contributed for less than 0.1% of the total protein absorbed. Holothurians would appear not to rely on microbes for their direct nutrition. However, prokaryote activity on organic substrates might indirectly provide essential nutrients (Deming and Colwell, 1982; Eardly et al., 2001).

Deposit-feeders may have two ways to ingest and assimilate food using foraging and digestive strategies, which involve: 1) particle selection, in which the organism preferentially selects food-rich material during the pickup and ingestion (Billett et al., 1988; Levin et al., 1997; Ferner and Jumars, 1999), and 2) assimilatory selection in which the organism digests and/or assimilates food in its guts (Penry and Jumars, 1990; Mayer et al., 1997; Jumars, 2000). Based on the composition and concentration of biochemical compounds in the sediment and in the oesophagus, we determined the average food selection. In our study, we estimated the average concentration of biopolymeric C in the oesophagus of *M. musculus* as being approximately 3-times greater than in the sediment. However, according to Billett et al. (1988), this concentration factor might depend on season, so it may vary. McClintic et al. (2008) and Puriton et al. (2008) using radiocarbon assimilations as tracers of labile organic matter, hypothesised that *M. musculus* have the highest digestive selective index and the least index for particle selection. Also, Miller et al. (2000) explored the selectivity and rates of some megafaunal deposit feeding (including molpadiids) by comparing a geochemical tracer, ^{234}Th, for recently settled material in the guts of these animals. These authors suggested that subsurface deposit feeders like molpadiids, either bring the surface sediment to their feeding depth (ca. 7 cm), or they feed selectively on the rich subsurface sediments. On the contrary, surface deposit-feeding holothurians, like elasipodids, selectively feed from the surface, which resulted from the rapid exploitation of horizontal food-rich particles. Assuming that *M. musculus* is a head-down feeder like *Molpadia oolitica* (Rhoads and Young, 1971) we hypothesize that in the Nazaré Canyon, *M. musculus* has a high efficiency in exploiting sedimentary proteins and complements its feeding requirements by an optimal foraging strategy.

At the time of sampling, given the average abundance to be 220±75.4 holothurians m^{-2}, the population of *M. musculus* at 3500 m depth in the Nazaré Canyon could be able to remove from the sediment ca. 0.49±0.13 g C and 0.13±0.03 g N m^{-2} d^{-1}. However, we cannot disregard the importance of other deep-sea benthic species that have been found in large numbers in this system. For instance, the holothurian *Ypsilothuria bitentaculata* (Ludwig, 1893) has abundances of 136 ind. m^{-2} in the same location as *M. musculus* (Cunha et al., 2010). *Ypsilothuria bitentaculata* is a surface feeder that might be responsible in exploiting horizontally food-rich particles and might have a higher selectivity in exploiting food from the sediment. Therefore, both these deep-sea deposit feeders might be influencing the entire benthic community and influence each other on their own feeding strategy. According to Billett et al. (2010) deep-sea megafauna are primary consumers for fresh food and they may have advantage when foraging for it. Larger sedimentary fauna preferentially rework the food that arrives on the seabed, ahead of the meiofauna and bacteria (Witte et al., 2003). These animals can be very active contributing to sediment reworking and potentially influencing the other infaunal assemblages through bioturbation and remineralization (Ruhl and Smith, 2004). Cunha et al. (2010), showed that macrofauna biodiversity values are lower in the sediments characterised by the presence of *M. musculus* and *Y. bitentaculata* than in other Nazaré Canyon areas. Smallwood et al. (1999), Ginger et al. (2001) and Witbaard et al. (2001), suggested that megafauna can significantly affect the food resources available to the rest of the benthos. Therefore, we can conclude that the impact of *M. musculus* affects not only the degradation of OM, but also its redistribution in the deep-sea floor of the largest submarine canyon of the European margins. Our data also shows that the sediment from the faeces still contain a considerable amount of biopolymeric C ($4.04 \pm 0.61\,\mathrm{mg\,g^{-1}}$). Although this value can be overestimated, as the faecal material may contain artifacts due to disturbance or handling of the experiment, it is still important to notice that faeces can redistribute the OM. This consequently, will affect the infaunal community. We can therefore suggest that these deep-sea holothurians, while feeding, provide a potentially important contribution to sediment mixing. They modify substantially the structure and geochemistry of sediments and might be, therefore, key components that influence the ecosystem functioning of this peculiar deep-sea region.

Acknowledgements. This research was partially supported by the HERMES project, EC contract no GOCE-CT-2005-511234, funded by the European Commission's Sixth Framework Programme under the priority "Sustainable Development, Global Change and Ecosystems". This study was supported by a grant (SFRH/BPD/21459/2005) awarded by Fundação Para a Ciência e Tecnologia (FCT) to Teresa Amaro. The officers and crew of RRS *James Cook* and Ben Boorman are acknowledged for their help in collecting the samples.

References

Accornero, A., Picon, P., de Bovee, F., Charriere, B., and Buscail, R.: Organic carbon budget at the sediment–water interface on the Gulf of Lions continental margin, Cont. Shelf Res., 23, 79–92, 2003.

Alt-Epping, U., Mil-Homens, M., Hebbeln, D., Abrantes, F. and Schneider, R. R.: Provenance of organic matter and nutrient conditions on a river- and upwelling influenced shelf: A case study from the Portuguese Margin, Mar. Geol., 243, 169–179, 2007.

Amaro, T., Witte, H., Herndl, G. J., Cunha, M. C., and Billett, D. S. M.: Deep-sea bacterial communities in sediments and guts of deposit-feeding holothurians in Portuguese canyons (NE Atlantic), Deep-Sea Res. I, 56, 1834–1843, 2009.

Anderson, M. J.: A new method for non-parametric multivariate analysis of variance, Aust. J. Ecol., 26, 32–46, 2001.

Anderson, M. J. and ter Braak, C. J. F.: Permutation tests for multi-factorial analysis of variance, J. Stat. Comp. Simul., 73, 85–113, 2003.

Arzola, R. G., Wynn, R. B., Lastras, G., Masson, D. G., and Weaver, P. P. E.: Sedimentary features and processes in the Nazaré and Setúbal submarine canyons, west Iberian margin, Mar. Geol., 250(1–2), 64–88, 2008.

Bett, B. J., Malzone, M. G., Narayanaswamy, B. E., and Wigham, B. D.: Temporal variability in phytodetritus and megabenthic activity at the seabed in the deep Northeast Atlantic, Prog. Oceanogr., 50, 349–368, 2001.

Bianchelli, S., Gambi, C., Pusceddu, A., and Danovaro, R.: Trophic conditions and meiofaunal assemblages in the Bari Canyon and the adjacent open slope (Adriatic Sea), Chem. Ecol., 24, 101–109, 2008.

Billett, D. S. M., Llewellyn, C., and Watson, J.: Are deep-sea holothurians selective feeders?, in: Echinoderm Biology, edited by: Burke, R., Maldenov, P., Lambert, P., and Parsley, R., Balkema, Rotterdam, 421–429, 1988.

Billett, D. S. M.: Deep-sea holothurians, Oceanogr. Mar. Biol. Annu. Rev., 29, 259–317, 1991.

Billett, D. S. M., Bett, B. J., Rice, A. L., Thurston, M. H., Galéron, J., Sibuet, M., and Wolff, G. A.: Long-term change in the megabenthos of the Porcupine Abyssal Plain (NE Atlantic), Prog. Oceanogr., 50, 325–348, 2001.

Billett, D. S. M., Bett, B. J., Reid, W. D. K., Boorman, B., and Priede, M.: Long-term change in the abyssal NE Atlantic: The "*Amperima* Event" revisited, Deep-Sea Res. II, doi:10.1016/j.dsr2.2009.02.001, in press, 2010.

Boetius, A., Scheibe, S., Tselepides, A., and Thiel, H.: Microbial biomass and activities in deep-sea sediments of the Eastern Mediterranean: trenches are benthic hotspots, Deep-Sea Res, 43, 1439–1460, 1996.

Cunha, M. R., Paterson, G. F., Alves, D., Amaro, T., Blackbird, S., De Stitger, H., Ferreira, C., Glover, A., Hilário, A., Kiriakoulakis, K., Nealova, L., Ravara, A., Rodrigues, C. F., Tiago, A., and Billett, D. S. M.: Macrofauna assemblages from three Portuguese submarine canyons (NE Atlantic), Deep-Sea Res., Special Issue, submitted, 2010.

Canals, M., Puig,, P., Durrieu de Madron, X., ,Heussner, S., Palanques, A., and Fabres, J.: Flushing submarine canyons, Nature, 444, 354–357, 2006.

Danovaro, R.: Methods for the Study of Deep-Sea Sediments, Their Functioning and Biodiversity, CRC Press, Boca Raton, 428 pp., 2010.

Danovaro, R., Fabiano, M., and Della Croce, N.: Labile organic matter and microbial biomasses in deep-sea sediments (Eastern Mediterranean Sea), Deep-Sea Res. I, 40, 953–965, 1993.

Danovaro, R., Marrale, D., Della Croce, N., Dell'Anno, A., and Fabiano, M.: Heterotrophic nanoflagellates, bacteria, and labile organic compounds in continental shelf and deep-sea sediments of the eastern Mediterranean, Microb. Ecol., 35, 244–255, 1998.

Danovaro, R., Dinet, A., Duineveld, G., and Tselepides, A.: Benthic response to particulate fluxes in different trophic environments: a comparison between the Gulf of Lions–Catalan Sea (W Mediterranean) and the Cretan Sea (E Mediterranean), Prog. Oceanogr., 44, 287–312, 1999.

Danovaro, R., Dell'Anno, A., and Fabiano, M.: Bioavailability of organic matter in the sediments of the Porcupine Abyssal Plain, northeastern Atlantic, Mar. Ecol. Prog. Ser., 220, 25–32, 2001.

Danovaro, R., Gambi, C., and Della Croce, N.: Meiofauna hotspot in the Atacama Trench, eastern South Pacific Ocean, Deep-Sea Res. I, 49, 843–857, 2002.

Danovaro, R., Della Croce, N., Dell'Anno, A., and Pusceddu, A.: A depocenter of organic matter at 7800-m depth in the Atacama trench sediments (SE Pacific Ocean), Deep-Sea Res. I, 50, 1411–1420, 2003.

Danovaro, R., Armeni M., Luna, G. M., Corinaldesi, C., and Dell'Anno, A.: Exo-enzymatic activities and dissolved organic pools in relation with mucilage development in the Northern Adriatic Sea, Sci. Total Environ., 353, 189–203, 2005.

Danovaro, R., Dell'Anno, A., Corinaldesi, C., Magagnini, M., Noble, R., Tamburini, C., and

Weinbauer, M.: Major viral impact on the functioning of benthic deep-sea ecosystems, Nature, 454, 1084–1087, 2008a.

Danovaro, R., Gambi, C., Dell'Anno, A., Corinaldesi, C., Fraschetti, S., Vanreusel, A., Vincx, M., and Gooday, A. J.: Exponential decline of deep-sea ecosystem functioning linked to benthic biodiversity loss, Curr. Biol., 18, 1–8, 2008b.

Dell'Anno, A., Mei, M. L., Pusceddu, A., and Danovaro, R.: Assessing the trophic state and eutrophication of coastal marine systems: a new approach based on the biochemical composition of sediment organic matter, Mar. Pollut. Bull., 44, 611–622, 2002.

Dell'Anno, A., Incera, M., Mei, M. L., and Pusceddu, A.: Mesoscale variability of organic matter 617 composition in NW Adriatic sediments, Chem. Ecol., 19, 33–45, 2003.

Dell'Anno, A. and Danovaro, R.: Extra-cellular DNA plays a key role in deep-sea ecosystem functioning, Science, 309(5744), 2179, 2005.

Deming, J. W. and Colwell, R. R.: Barophilic bacteria associated with digestive tracts of abyssal holothurians, Appl. Environ. Microbiol., 44, 1222–1230, 1982.

de Stigter, H. C., Boer, W., de Jesus Mendes, P. A, Jesus, C. C., Thomsen, L., van den Bergh, G. D., and van Weering, T. C. E.: Recent sediment transport and deposition in the Nazaré Canyon, Portuguese continental margin, Mar. Geol., 246, 144–164, 2007.

Duineveld, G., Lavaleye, M., Berghuis, E., and De Wilde, P.: Activity and composition of the benthic fauna in the Whittard Canyon and the adjacent continental slope (NE Atlantic), Oceanol. Acta, 24, 69–83, 2001.

Eardly, D. C., Gallagher, J. M., and Patching, J.:Bacterial abundance and activity in deep-sea sediments from the eastern North Atlantic, Prog. Oceanogr., 50, 245–259, 2001.

Fabiano, M., Danovaro, R., and Fraschetti, S.: A three-year time series of elemental and biochemical composition of organic matter in subtidal sandy sediments of the Ligurian Sea (northwestern Mediterranean), Cont. Shelf Res., 15, 1453–1469, 1995.

Féral, J.-P.: Activity of the principal digestive enzymes in the detritivorous apodous holothuroid *Leptosynapta galliennei* and two other shallow-water holothuroids, Mar. Biol., 101, 367–379, 1989.

Ferner, M. C. and Jumars, P. A.: Responses of deposit-feeding spionid polychaetes to dissolved chemical cues, J. Exp. Mar. Biol. Ecol., 236, 89–106, 1999.

Gage, J. D. and Tyler, P. A.: Deep-Sea Biology: a Natural History of Organisms at the Deep-Sea Floor, Cambridge University Press, Cambridge, UK, 504 pp., 1991.

Gage, J. D., Lamont, P. A., and Tyler, P.A.: Deep-sea macrobenthic communities at contrasting sites off Portugal, preliminary results: 1 Introduction and diversity comparison, Int. Rev. Ges. Hydrobiol., 80, 235–250, 1995.

Gambi, C., Vanreusel, A., and Danovaro, R.: Biodiversity of nematode assemblages from deep-sea sediments of the Atacama Slope and Trench (South Pacific Ocean), Deep-Sea Res. I, 50, 103–117, 2003.

García, R. and Thomsen, L.: Bioavailable organic matter in surface sediments of the Nazaré 650 canyon and adjacent slope (Western Iberian Margin), J. Mar. Syst., 74, 44–59, 2008.

García, R., van Oevelen, D., Soetaert, K., Thomsen, L., de Stigter, H. C., and Epping, E.: Deposition rates, mixing intensity and organic content in two contrasting submarine canyons, Prog. Oceanogr., 76, 192–215, 2008.

Ginger, M. L., Billett, D. S. M., Mackenzie, K. L., Kirakoulakis, K., Neto, R. R., Boardman, D. K., Santos, V. L. C. S., Horsfall, I. M., and Wolff, G. A.: Organic matter assimilation and selective feeding by holothurians in the deep sea: some observations and comments, Prog. Oceanogr., 50, 407–421, 2001.

Hudson, I. R., Wigham, B. D., Solan, M., and Rosenberg, R.: Feeding behaviour of deep-sea dwelling holothurian: Inferences from a laboratory investigation of shallow fjordic species, J. Mar. Syst., 57, 201–218, 2005.

Hughes, S. J. M., Amaro, T., Ingels, J., Boorman, B., Hawkins, E. L., Billett, D. S. M., Vanreusel, A., and Cunha, M. R.: Benthic Abyssal Research using a Remotely Operated Vehicle (ROV): Novel Experimentation in the Nazaré Canyon, Deep-sea Res Part II, special issue, submitted, 2010.

Ingels, J., Kiriakoulakis, K., Wolff, G. A., and Vanreusel, A.: Nematode diversity and its relation to the quantity and quality of sedimentary organic matter in the deep Nazaré Canyon, Western Iberian Margin, Deep-Sea Res. I, 56, 1521–1539, 2009.

Jumars, P. A.: Gourmands of mud: Diet selection in marine deposit feeders, in: Mechanisms of Diet Choice, edited by: Hughes, R. N., Blackwell Scientific Publishers, 124–156, 1993.

Jumars, P. A.: Animal guts as non-ideal chemical reactors: partial mixing and axial variation in absorption kinetics, Am. Nat., 155, 544–555, 2000.

Khripounoff, A. and Sibuet, M.: La nutrition d'echinodermes abyssaux I. Alimentation des holothuries, Mar. Biol., 60, 17–26, 1980.

Lauermann, L. M. L., Kaufmann, R. S., and Smith, K. L.: Distribution and abundance of epibenthic megafauna at a long-term time series station in the abyssal northeast Pacific, Deep-Sea Res. I, 43, 1075–1104, 1996.

Levin, L., Blair, N., DeMaster, D., Plaia, G., Fornes, W., Martins, C., and Thomas, C.: Rapid subduction of organic matter by maldanid polychaetes on the North Carolina Slope, J. Mar. Res., 55, 595–611, 1997.

Massin C.: Effects of feeding on the environment: Holothuroidea, in: Echinoderm Nutrition, edited by: Jangoux M. and Lawrence J. M., Rotterdam: A.A., Balkema, 493–497, 1982.

Masson, D. G., Huvenne, V. A. I., de Stigter, H., Wolff, G. A., Kiriakoulakis, K., Arzola, R. G., and Blackbird, S.: Efficient burial of carbon in a submarine canyon, Geology, submitted, 2010.

Mayer, L. M., Schick, L. I., Self, R. F. L., Jumars, P. A., Findlay, R. H., Chen, Z., and Sampson, S.: Digestive environments of benthic macro-invertebrate guts: enzymes, surfactants and dissolved organic matter, J. Mar. Res., 55, 785–812, 1997.

McArdle, B. H. and Anderson, M. J.: Fitting multivariate models to community data: a comment on distance-based redundancy analysis, Ecology, 82, 290–297, 2001.

McClintic, M. A., DeMaster, D. J., Thomas, C. J., and Smith, C. R.: Testing the FOODBANCS hypothesis: Seasonal variations in near-bottom particle flux, bioturbation intensity, and deposit feeding based on ^{234}Th measurements. Deep-Sea Res. II, 55, 2425–2437, 2008.

Miller, R. J., Smith, C. R., DeMaster, D. J., and Fornes, W.: Feeding selectivity and particle processing by deep-sea megafaunal deposit feeders: a ^{234}Th approach, J. Mar. Res., 58, 653–673, 2000.

Moriarty, D. J.: Feeding of *Holothuria atra* and *Stichopus chloronotus* on bacteria, organic carbon and organic nitrogen in sediments of the Great Barrier Reef, Aust. J. Mar. and Freshw. Res., 33, 255–263, 1982.

Oliveira, A., Santos, A. I., Rodrigues, A., and Vitorino, J.: Sedimentary particle distribution and dynamics on the Nazaré Canyon system and adjacent shelf (Portugal), Mar. Geol., 246, 105–122, 2007.

Palanques, A., Garcia-Ladona, E., Gomis, D., Martin, J., Marcos, M., Pascual, A., Puig, P., Gili, J. M., Emelianov, M., Monserrat, S., Guillen, J., Tintore, J., Segura, M., Jordi, A., Ruiz, S., Basterretxea, G., Font, J., Blasco, D., and Pages, F.: General patterns of circulation, sediment fluxes and ecology of the Palamo (La Fonera) submarine canyon, northwestern Mediterranean, Prog. Oceanogr., 66, 89–119, 2005.

Pawson, D., Vance, D. J., and Ahearn, C.: Western Atlantic sea cucumbers of the Order Molpadiida (Echinodermata: Holothuroidea), Bull. Biol. Soc. Washington, 10, 311–327, 2001.

Penry, D. L. and Jumars, P. A.: Gut architecture, digestive constraints and feeding ecology of deposit-feeding and carnivorous polychaetes, Oecologia, 82, 1–11, 1990.

Pfannkuche, O. and Thiel, H.: Meiobenthic stocks and benthic activity on the northeast Svalbard Shelf and in the Nansen Basin, Polar Biol., 7, 253–266, 1987.

Purinton, B. L., DeMaster, D. J., Smith, C. R., and Thomas, C. J.: ^{14}C as a tracer of labile organic matter in Antarctic benthic foodwebs, Deep-Sea Res. II, 55, 2438–2450, 2008.

Pusceddu, A., Dell'Anno, A., Fabiano, M., and Danovaro, R.: Quantity and bioavailability of sediment organic matter as signatures of benthic trophic status, Mar. Ecol. Prog. Ser., 375, 41–52, 2009.

Pusceddu, A., Bianchelli, S., Canals, M., Sanchez-Vidal, A., Durrieu De Madron, X., Heussner, S., Lykousis, V., de Stigter, H., Trincardi, F., and Danovaro, R.: Organic matter in sediments of canyons and open slopes of the Portuguese, Catalan, Southern Adriatic and Cretan Sea margins, Deep-Sea Res. I, 57, 441–457, 2010.

Quaresma, L. S., Vitorino, J., Oliveira, A., and Silva, J.: Evidence of sediment resuspension by nonlinear internal waves on the western Portuguese mid-shelf, Mar. Geol, 246, 123–143, 2007.

Rhoads, D. C. and Young, D. K.: Animal-sediment relations in Cape Cod Bay, Massachusetts II. Reworking by *Molpadia oolitica* (Holothuroidea), Mar. Biol., 11, 255–261, 1971.

Roberts, D., Billett, D. S. M., McCartney, G., and Hayes, G. E.: Prokaryotes on the tentacles of deep-sea holothurians - a novel form of dietary supplementation, Limnol. Oceanogr., 36, 1447–1452, 1991.

Roberts, D., Gebruk, A. V., Levin, V., and Manship, B. A. D.: Feeding and digestive strategies in deposit-feeding holothurians, Oceanogr. Mar. Biol. Annu. Rev., 38, 257–310, 2000.

Roberts, D., Moore, H. M., Berges, J., Patching, J. W., Carton, M. W., and Eardly, D. F.: Sediment distribution, hydrolytic enzyme profiles and bacterial activities in the guts of *Oneirophanta mutabilis*, *Psychropotes longicauda* and *Pseudostichopus villosus*: what do they tell us about digestive strategies of abyssal holothurians?, Prog. Oceanogr., 50, 443–458, 2001.

Rowe, G. T., Polloni, P. T., and Haedrich, R. L.: The deep-sea macrobenthos on the continental-margin of the Northwest Atlantic-Ocean, Deep-Sea Res. I, 29, 257–278, 1982.

Ruhl, H. A. and Smith, K. L.: Shifts in deep-sea community structure linked to climate and food supply, Science, 305, 513–515, 2004.

Smallwood, B. J., Bett, B. J., Smith, C. R., Gage, J. D., Patience, A., Hoover, D., and Wolff, G. A.: Megafauna can control the quality of organic matter in marine sediments, Naturwissenschaften, 86(7), 320–324, 1999.

Schmidt, S., de Stigter, H. C., van Weering, T. C. E.: Enhanced short-term sediment deposition

743 within the Nazaré Canyon, North-East Atlantic, Mar. Geol., 173, 55–67, 2001.

Searle, S. R., Casella, G., McCulloch, C. E. (eds.): Variance components, John Wiley and Sons, New York, New York, USA, 1992.

Sibuet, M., Khripounoff, A., Deming, J., Colwell, R., and Dinet, A.: Modification of the gut contents in the digestive tract of abyssal holothurians, in: Proceedings of the International Echinoderm Conference, edited by: Lawrence, J. M., Balkema, Rotterdam, Tampa Bay, 421–428, 1982.

Sibuet, M.: Deposit-feeding invertebrates in the deepsea ecosystem. Selectivity in feeding and diet of holothurians, Oceanis, 10, 623–876, 1984.

Sibuet, M.: Quantitative distribution of echinoderms (Holothuroidea, Asteroidea, Ophiouroidea, Echinoidea) in relation to organic matter in the sediment, in deep sea basins of the Atlantic Ocean, in: *Echinodermata*. Proceedings of the Fifth International Echinoderm Conference, edited by: Keegan, B. F. and O'Connor, B. D. S., Balkema, Rotterdam, Galway 24–29 September 1984, Galway, Ireland, 99–108, 1985.

Smith Jr., K. L., Kaufman, R. S., and Wakefield, W. W.: Mobile megafaunal activity monitored with a time-lapse camera in the abyssal North Pacific, Deep-Sea Res. I, 40, 2307–2324, 1993.

Soetaert, K., Heip, C., and Vincx, M.: The meiobenthos along a Mediterranean deep-sea transect off Calvi (Corsica) and in an adjacent canyon, Mar. Ecol., 12, 227–242, 1991.

Tselepides, A., Polychronaki, T., Marrale, D., Akoumianaki, I., Dell'Anno, A., Pusceddu, A., and Danovaro, R.: Organic matter composition of the continental shelf and bathyal sediments of the Cretan Sea (NE Mediterranean), Prog. Oceanogr., 46, 311–344, 2000.

Tyler, P., Amaro, T., Arzola, R., Cunha, M. R., de Stigter, H., Gooday, A., Huvenne, V., Ingels, J., Kiriakoulakis, K., Lastras, G., Masson, D., Oliveira, A., Pattenden, A., Vanreusel, A., Van Weering, T., Vitorino, J., Witte, U. and Wolff, G.: Europe's Grand Canyon: Nazaré Submarine Canyon, Oceanography, 22(1), 46–57, 2009.

Vetter, E. W. and Dayton, P. K.: Macrofaunal communities within and adjacent to a detritus-rich submarine canyon system, Deep-Sea Res. II, 45, 25–54, 1998.

Vetter, E. W. and Dayton, P. K.: Organic enrichment by macrophyte detritus, and abundance patterns of megafaunal populations in submarine canyons, Mar. Ecol. Prog. Ser., 186, 137–148, 1999.

Vitorino, J., Oliveira, A., Jouanneau, J.-M., and Drago, T.: Winter dynamics on the northern Portuguese shelf. Part 2: bottom boundary layers and sediment dispersal, Prog. Oceanogr., 52, 155–170, 2002a.

Vitorino, J., Oliveira, A., Jouanneau, J. M., and Drago, T.: Winter dynamics on the northern Portuguese shelf Part 1: physical processes, Prog. Oceanogr., 52, 129–153, 2002b.

Witbaard, R., Duineveld, G. C. A., Kok, A., van der Weele, J., and Berghuis, E. M.: The response of *Oneirophanta mutabilis* (Holothuroidea) to the seasonal deposition of phytopigments at the Porcupine Abyssal Plain in the Northeast Atlantic, Prog. Oceanogr., 50, 423–441, 2001.

Witte, U., Wenzhöfer, F., Sommer, S., Boetius, A., Heinz, P., Aberle, N., Sand, M., Cremer, A., Abraham, W.-R., Jørgensen, B. B., and Pfannkuche, O.: In situ experimental evidence of the fate of a phytodetritus pulse at the abyssal sea floor, Nature, 424, 763–766, 2003.

Table 1. List of the stations data concerning the samples taken on two different cruises.

Station	Gear	Dive	Date	Latitude	Longitude	Depth (m)
CD56848#1	Megacorer	–	08-05-2006	39°30′00	09°56′01	3517
CD56848#2	Megacorer	–	08-05-2006	39°30′00	09°55′60	3523
CD56848#3	Megacorer	–	09-05-2006	39°29′24	09°56′03	3512
CD56851#1	Megacorer	–	09-05-2006	39°29′24	09°55′58	3517
CD56851#2	Megacorer	–	09-05-2006	39°29′24	09°56′01	3517
CD56851#3	Megacorer	–	10-05-2006	39°29′24	09°56′01	3522
CD56856#1	Megacorer	–	11-05-2006	39°29′24	09°55′60	3519
CD56856#2	Megacorer	–	11-05-2006	39°30′00	09°55′60	3522
JC10-090	ISIS experiment	46	09-06-2007	39°29′82	09°55′85	3534
JC10-091	ISIS experiment	48	12-06-2007	39°29′82	09°55′89	3534
JC10-099	ISIS suction sampler	50	13-06-2007	39°29.85	09°56′91	3535
JC10-099	ISIS suction sampler	50	14-06-2007	39°29.52	09°56′14	3648
JC10-099	ISIS suction sampler	50	14-06-2007	39°29.53	09°56′14	3649
JC10-099	ISIS suction sampler	50	14-06-2007	39°29.53	09°56′14	3651
JC10-106	ISIS Push core	51	15-06-2007	39°29.76	09°55′94	3529
JC10-106	ISIS Push core	51	15-06-2007	39°29.76	09°55′94	3529
JC10-106	ISIS Push core	51	15-06-2007	39°29.76	09°55′93	3529
JC10-106	ISIS Push core	51	15-06-2007	39°29.77	09°55′92	3529
JC10-106	ISIS Push core	51	15-06-2007	39°29.76	09°55′93	3529

Title Page
Abstract | Introduction
Conclusions | References
Tables | Figures
◂◂ | ▸▸
◂ | ▸
Back | Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

Table 2. Outputs of the ANOVA, SNK and PERMANOVA testing for changes with depth in the sediment of the quantity and biochemical composition of sediment organic matter in the Nazaré Canyon. DF = degree of freedom, SS = sum of squares; MS = mean square; F = F statistic; P = probability level; *** $P < 0.001$; ** = $P < 0.01$; ns = not significant; na = not applicable.

ANOVA

	Source	SS	DF	MS	*F*	*P*	SNK
Protein	Depth	71.94	4	17.99	30.92	***	1>2–5
	Residual	5.82	10	0.58			
	Total	77.76	14				
Carbohydrate	Depth	0.25	4	0.06	0.21	ns	na
	Residual	2.96	10	0.30			
	Total	3.21	14				
Lipid	Depth	0.20	4	0.05	1.23	ns	na
	Residual	0.41	10	0.04			
	Total	0.61	14				
Biopolymeric C	Depth	16.20	4	4.05	7.45	**	1>2–5
	Residual	5.44	10	0.54			
	Total	21.63	14				

PERMANOVA

Source	DF	SS	MS	Pseudo-F	*P*
Depth	4	1025.10	256.27	16.61	**
Residual	10	154.28	15.428		
Total	14	1179.30			

BGD

7, 3061–3094, 2010

The trophic biology of the holothurian *Molpadia musculus*

T. Amaro et al.

Table 3. Outputs of the ANOVA, SNK, and PERMANOVA testing for changes in the quantity and biochemical composition of organic matter in the various sections of the holothurian gut. Acronyms as in Table 1.

ANOVA						
	Source	DF	MS	*F*	*P*	SNK
Protein	Gut section	5	650.56	18.11	***	Oesophagus > Mid gut > Others
	Residuals	12	35.92			
	Total	17				
Carbohydrates	Gut section	5	0.411	2.68	ns	na
	Residuals	12	0.154			
	Total	17				
Lipid	Gut section	5	40.67	14.18	***	Mid gut > (Oesophagus = End gut) > Others
	Residuals	12	0.287			
	Total	17				
Biopolymeric C	Gut section	5	266.64	13.11	***	Oesophagus > Mid gut > Others
	Residuals	12	20.34			
	Total	17				

PERMANOVA					
Source	df	SS	MS	Pseudo-F	*P*(MC)
Gut section	3	267.15	89.052	17.161	***
Residual	8	41.513	51.891		
Total	11	308.67			

Table 4. Outputs of the ANOVA, SNK and PERMANOVA testing for changes in extracellular enzymatic activities in the various sections of the holothurians' gut (ANOVA and SNK tests) and between the gut and the sediment (PERMANOVA). *D* = degree of freedom, SS = sum of squares; MS = mean square; *F* = F statistic; *P* = probability level; ** = *P* < 0.01; ns = not significant; na = not applicable.

ANOVA

Variable	Source	DF	SS	MS	*F*	*P*	SNK (only significant tests)
Aminopeptidase	Gut/sediment	3	1.42E+10	4.72E+09	83.74	**	Mid gut > End gut and sediment
	Residual	8	4.51E+09	56 383			
	Total	11	1.87E+10				
β-glucosidase	Gut/sediment	3	12.417	0.41389	13.78	ns	na
	Residual	8	24.024	0.3003			
	Total	11	36.441				
Alkaline phosphatase	Gut/sediment	3	7.99E+09	2.66E+09	45.31	*	Mid gut > End gut
	Residual	8	4.70E+09	58 789			
	Total	11	1.27E+10				

PERMANOVA

Source	DF	SS	MS	Pseudo-F	*P*
Gut/sediment	3	2.22E+10	7.39E+09	64.123	**
Residual	8	9.21E+09	1.15E+09		
Total	11	3.14E+10			

Title Page
Abstract Introduction
Conclusions References
Tables Figures
◀◀ ▶▶
◀ ▶
Back Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

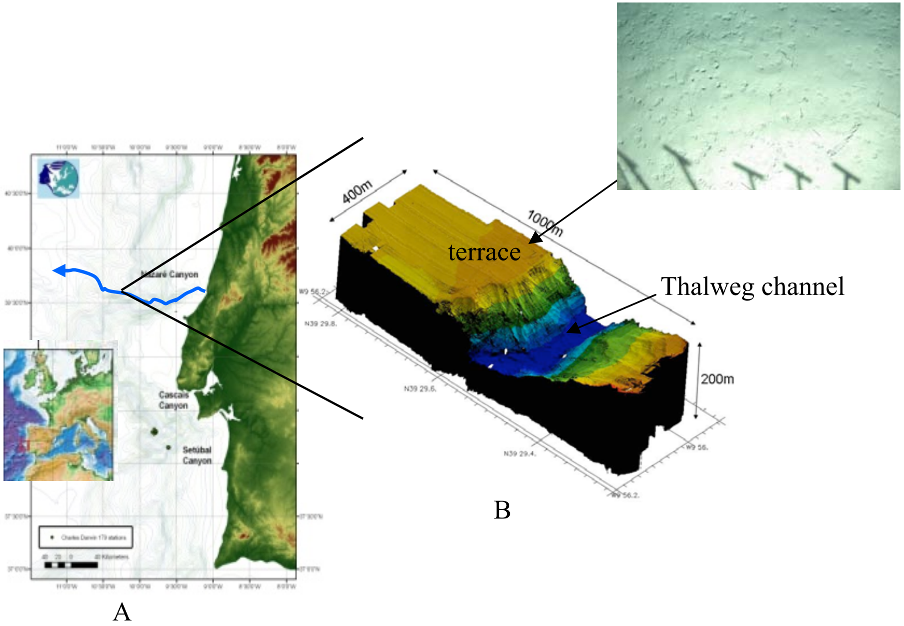

Fig. 1. Nazaré Canyon, 3500 m site. **(A)** Chart of the bathymetry of the Portuguese margin (GEBCO, 2003), showing the location the Nazaré Canyon; **(B)** ROV high resolution swath bathymetry of the central thalweg channel of the Nazaré canyon at 3500 m. The steep channel wall is composed of rock outcrop. The channel floor has boulders with a thin drape of mud. The terrace to the north of the thalweg is characterised by high rate of mud deposition with many burrowing animals (photograph top right) (Modified after, Masson et al., 2010).

Fig. 2. A picture of a specimen of Molpadia musculus immediately after collection, when analysed for biometric variables.

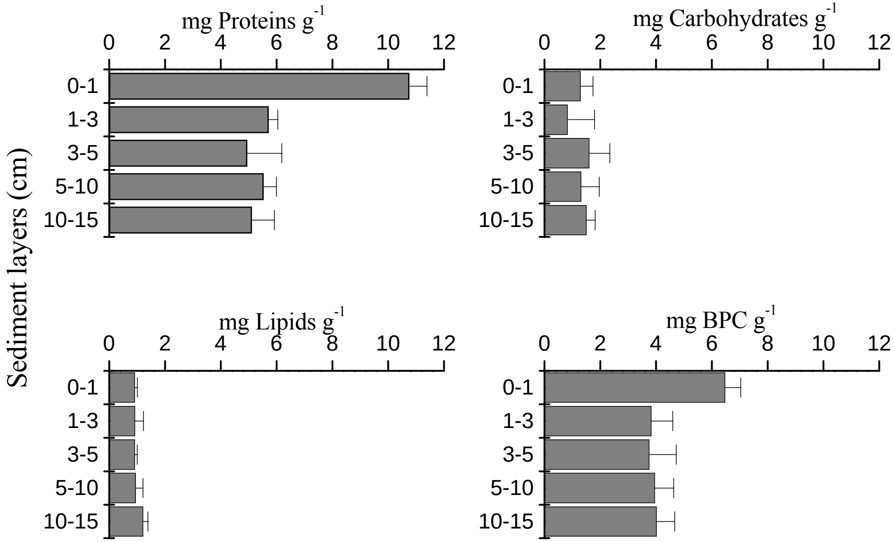

Fig. 3. Vertical distributions of the main biochemical classes of organic compounds (proteins, carbohydrates, lipids) and biopolymeric C (the sum of carbon within these compounds) in the sediment. Standard deviations are indicated. Data are expressed in milligrams per g of sediment dry weight.

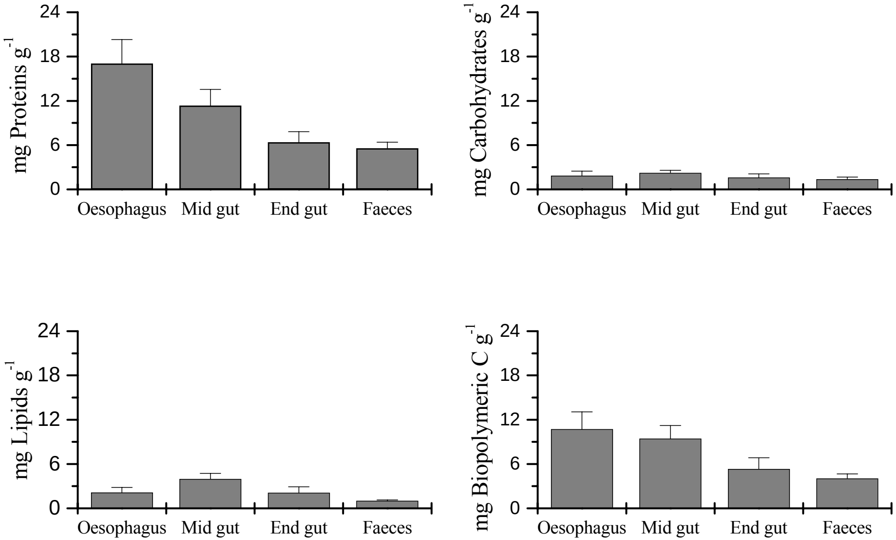

Fig. 4. Distributions of the main biochemical classes of organic compounds (proteins, carbohydrates, lipids) and biopolymeric C through the gut of *M. musculus* (oesophagus, mid gut, hind gut and faeces). Standard deviations are indicated. Data are expressed in milligrams per g of sediment dry weight.

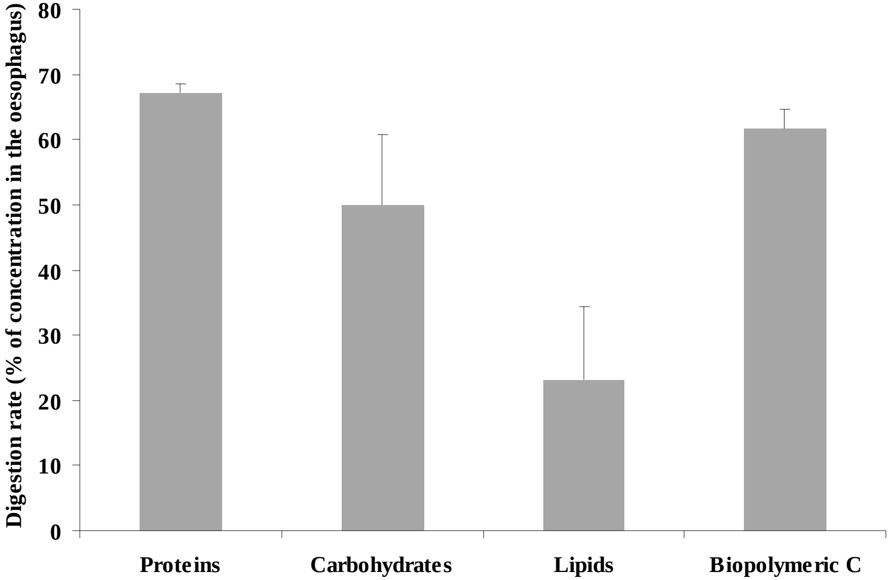

Fig. 5. Digestion rate in % of the main biochemical classes of organic compounds (proteins, carbohydrates, lipids) and biopolymeric C in *M. musculus*. Standard deviations are indicated. Data are mg per g of sediment dry weight.

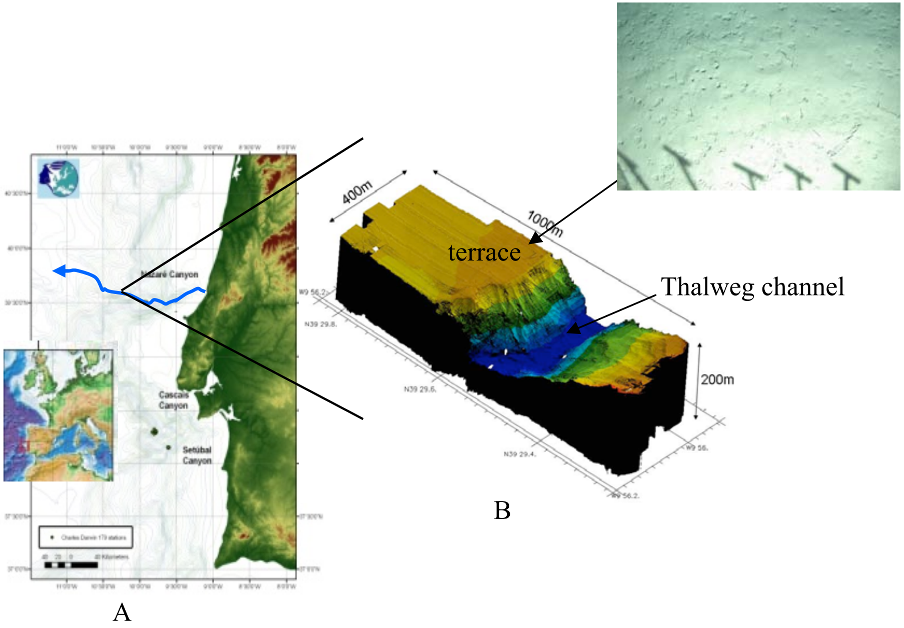

Fig. 6. Distributions of aminopeptidase, β-glucosidase and alkaline phosphatase activity through the gut of *M. musculus* and in sediment samples. Standard deviations are indicated. Data are expressed in nanomole of substrated released per g of sediment dry weight per hour.