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

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### 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<sup>-2</sup> d<sup>-1</sup>. 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).

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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 detritus and dead or decaying animals), microorganisms (prokaryotes, diatoms, protozoans) and

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faecal pellets of other animals (Massin, 1982; Moriarty, 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.

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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  $-80^{\circ}\text{C}$  (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 ( $450^{\circ}\text{C}$  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 per-

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centage 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,  $\beta$ -glucosidase and alkaline phosphatase) were determined by cleavage of analogue fluorogenic substrates (L-Leucine-4-methylcoumarinyl-7-amide, Leu-MCA; 4-MUF- $\beta$ -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  $\mu\text{l}$  of MUF-Glu and Leu-MCA (final concentration 200  $\mu\text{M}$ ) and 50  $\mu\text{l}$  of MUF-P (final concentration 50  $\mu\text{M}$ ) in a final volume of 5 ml of seawater. Substrate incubations were performed in the dark at in situ temperature for 1 h. 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 ( $60^{\circ}\text{C}$ , 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

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tration 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.

5 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 ( $67\pm 1\%$ ), followed by lipids ( $50\pm 11\%$ ) and carbohydrates ( $23\pm 11\%$ ). Overall, biopolymeric C digestion rate was  $62\pm 3\%$  (Fig. 5).

### 3.4 Enzymatic activities

10 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  $\beta$ -glucosidase activity was almost  
15 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

20 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 (Laueremann et al., 1996; Ruhl and Smith, 2004). Significant effects were observed on the process of the OM on the seabed when species changed  
25 in density by over 3 orders of magnitude in a period of two and a half years (Bett et

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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  $220\pm 75.4$  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*  
5 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.  $1.4 \text{ mg g}^{-1}$  (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  
10 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  
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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<sup>-1</sup> 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<sup>-1</sup>) 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

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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

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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,  $^{234}\text{Th}$ , 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  $220 \pm 75.4$  holothurians  $\text{m}^{-2}$ , the population of *M. musculus* at 3500 m depth in the Nazaré Canyon could be able to remove from the sediment ca.  $0.49 \pm 0.13$  g C and  $0.13 \pm 0.03$  g N  $\text{m}^{-2} \text{d}^{-1}$ . 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.  $\text{m}^{-2}$  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

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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 ( $4.04 \pm 0.61$  mg  $\text{g}^{-1}$ ). 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.

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**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

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**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.

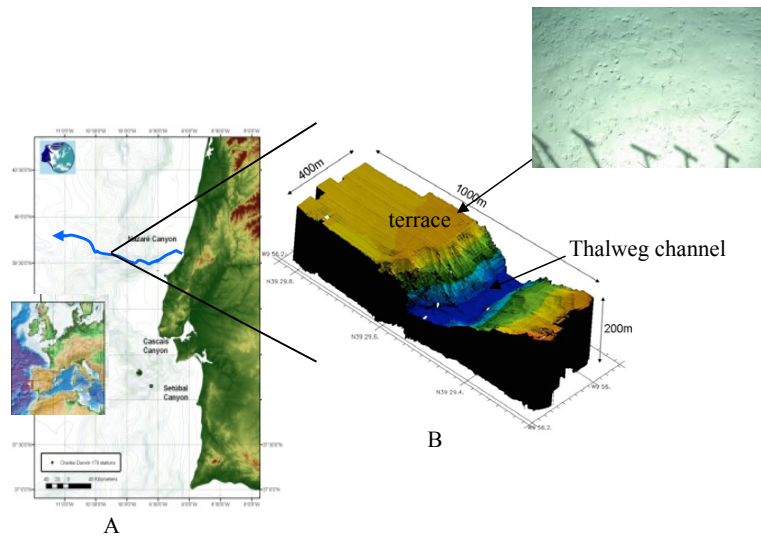
ANOVA							
	Source	SS	DF	MS	<i>F</i>	<i>P</i>	SNK
Protein	Depth	71.94	4	17.99	30.92	***	1>2–5
	Residual	5.82	10	0.58			
	Total	77.76	14				
Carbohydrate	Depth	0.25	4	0.06	0.21	ns	na
	Residual	2.96	10	0.30			
	Total	3.21	14				
Lipid	Depth	0.20	4	0.05	1.23	ns	na
	Residual	0.41	10	0.04			
	Total	0.61	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	<i>P</i>
Depth	4	1025.10	256.27	16.61	**
Residual	10	154.28	15.428		
Total	14	1179.30			

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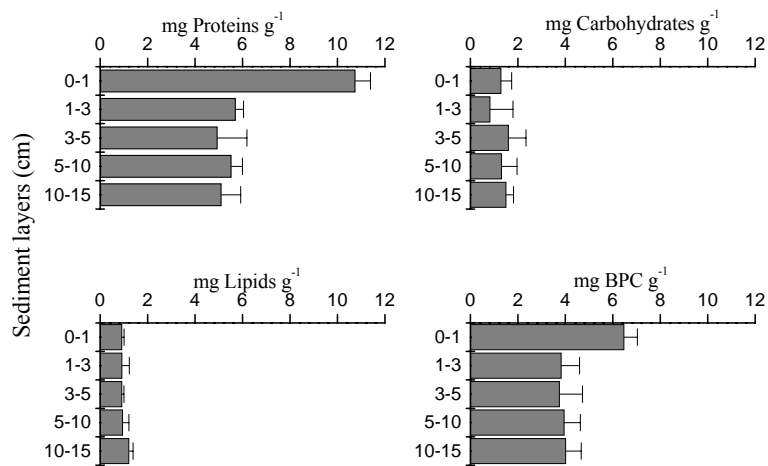
**Fig. 1.** Nazaré Canyon, 3500 m site. **(A)** Chart of the bathymetry of the Portuguese margin (GEBCO, 2003), showing the location the Nazaré Canyon; **(B)** ROV high resolution swath bathymetry of the central thalweg channel of the Nazaré canyon at 3500 m. The steep channel wall is composed of rock 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).

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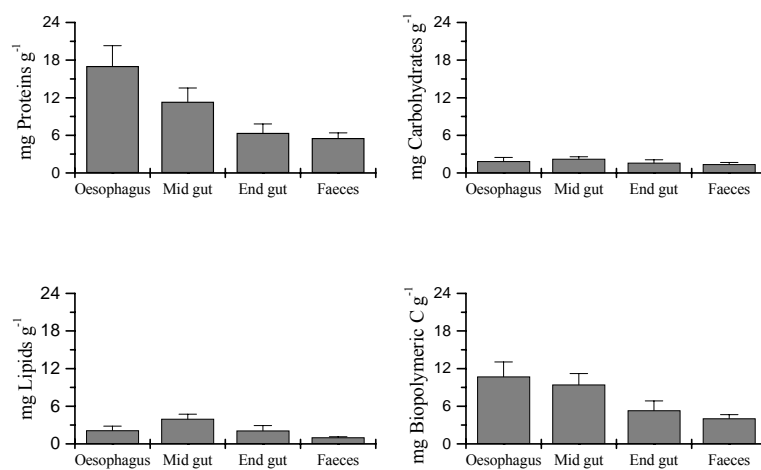
**Fig. 2.** A picture of a specimen of *Molpadia musculus* immediately after collection, when analysed for biometric variables.

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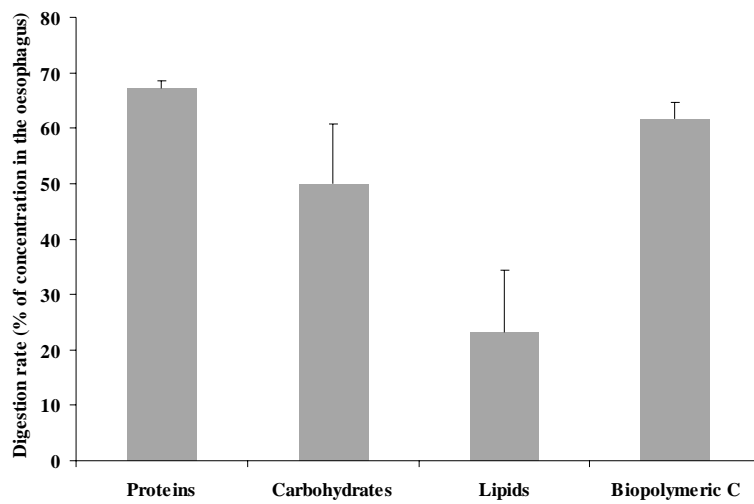
**Fig. 3.** Vertical distributions of the main biochemical classes of organic compounds (proteins, carbohydrates, lipids) and biopolymeric C (the sum of carbon within these compounds) in the sediment. Standard deviations are indicated. Data are expressed in milligrams per g of sediment dry weight.

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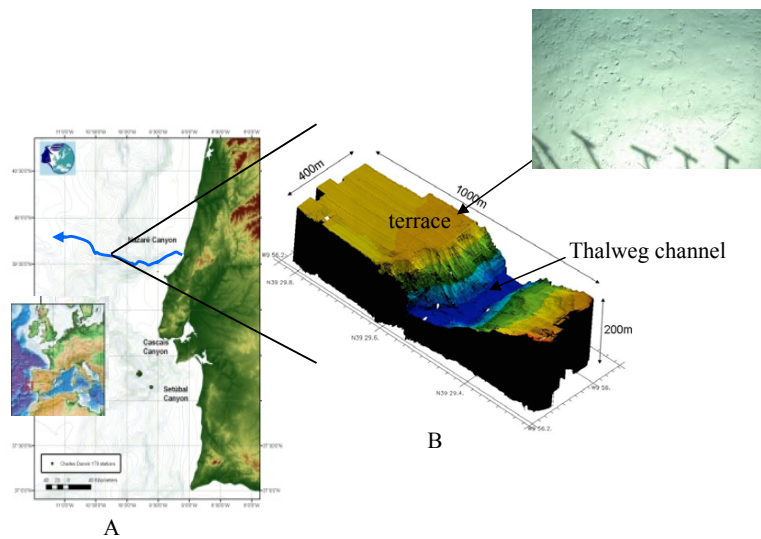
**Fig. 4.** Distributions of the main biochemical classes of organic compounds (proteins, carbohydrates, lipids) and biopolymeric C through the gut of *M. musculus* (oesophagus, mid gut, hind gut and faeces). Standard deviations are indicated. Data are expressed in milligrams per g of sediment dry weight.

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**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.

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**Fig. 6.** Distributions of aminopeptidase,  $\beta$ -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.

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