

Interactive comment on “The trophic biology of the holothurian *Molpadia musculus* at 3500 m in the Nazaré Canyon (NE Atlantic)” by T. Amaro et al.

Anonymous Referee #2

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Review of Amaro et al. The trophic biology of the holothurian *Molpadia musculus* at 3500m in the Nazare Canyon (NE Atlantic)

The manuscript by Amaro et al. addresses feeding ecology, and biochemical transformation of sediments in the gut of a major ecosystem engineer, *Molpadia musculus* in a deep-sea submarine canyon system. I believe that this is a very useful contribution and should eventually be accepted in Biogeosciences as it provides unique data about a topic which is very poorly understood, and any information extracted from this type of study will be a valuable addition to our understanding of deep-sea megafaunal food-webs and trophic ecology. It is generally well structured, but I do have some minor comments that I would like to see addressed prior to its acceptance in BG.

Specific comments: Abstract Line 1: Change to ‘Megafaunal organisms play a key
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role in ecosystem functioning in the deep-sea through bioturbation and organic matter cycling’ Line 3: No ‘lead-in’ is given with regards to why sediments and holothurians were collected. Just an example, To quantify the role of *M. musculus* in sediment cycling, sediment samples...’ Line 11: Insert ‘by the time sediment reached’ between ‘were digested’ and ‘the mid-gut’. Line 14: C-digestion rate(s) (plural). Line 16: Change to ‘the entire holothurian’s population could remove about XX- biopolymeric C and XX g N m⁻² d⁻¹ from the sediments. Line 17: ‘plays a key role in benthic tropho-dynamics’ Line 18: Change ‘of the’ to ‘in the’.

Introduction Page 3063, Line 1: I think that some v. important papers are missing from this reference list. Fabio De leo, Craig Smith, Ashley Rowden, David Bowden, and Malcolm Clark have a very nice paper in Proceedings of the Royal Society B ‘Submarine-canyons: hotspots of benthic biomass and productivity in the deep-sea, doi: 10.1098/ rspb.2010.0462. Also, the authors should include Eric Vetter, Craig Smith, Fabio De Leo’s paper ‘Hawaiian hotspots: enhanced megafaunal abundance and diversity in submarine canyons on the oceanic islands of Hawaii in Marine Ecology, vol. 31, 183-199. Line 8: Change to ‘is a major conduit of organic matter on the Portuguese margin’ Line 12: Insert ‘may act as a sink for OM (reference)’ Line 16: Again, the two previously stated papers are missing and are very important to include. Line 20: Change to ‘occurs in’ Line 22: Change to ‘The reasons for such massive abundances are unknown’ Line 24: Describe how food-supply is an important factor controlling the abundance and composition of holothurian assemblages. What type of correlation??? positive??? negative??? Be a little more specific. I would also suggest that you include other papers that have found correlations between POC flux and animal biomass (e.g. Craig Smith, Fabio De Leo, Angelo Bernadino, Andrew Sweetman, Pedro Martinez 2008. Abyssal food limitation, ecosystem structure and climate change. Trends in Ecology and Evolution 23(9): 518-528. Ruhl, HA, JA Ellena, and KL Smith Jr, 2008. Connections between climate, food limitation, and carbon cycling in abyssal sediment communities: a long time-series perspective. Proceedings of the National Academy of Sciences, USA, 105, 17006–17011. Ruhl, HA, 2007. Abundance

and size distribution dynamics of abyssal epibenthic megafauna in the northeast Pacific. Ecology 88: 1250-1262.) Line 25: Change to 'in the Pacific Ocean' Line 28: What do you mean by 'decries'? Do you mean 'debris'? Page 3064, Line 21-26: The authors shift between past and present tense. Please find a tense and settle on it. Don't keep shifting between the two as can be seen where the authors write in line 22 'we investigated' (i.e. past tense) and in line 25 where they write 'we quantify' (present tense) Introduction in general: No hypotheses have been mentioned with regards to the feeding study. I think that the paper would benefit a lot from the author's stating their original null hypotheses and discussing the data in the context of these hypotheses.

Material and methods Page 3065: Line 9: change to 'to the canyon and out onto the abyssal plain' Line 18: Change to 'redistribution of particulate matter' Line 20: Define 'surface burrower'. This is a very unusual term. I would change to 'head-down deposit feeder' Line 23: 'Change to 'shallow waters' Line 24: Change to 'their tails' Page 3066: Line 2: Change to 'using data' Line 5: Change to 'Molpadiids holothurians were enumerated and their density m⁻² determined from replicate multicores' Line 9-11: Delete entire sentence 'Intact animals. ...' Line 14: Delete 'deep' Line 15: Change 'about' to 'approximately'. Please continue this throughout the manuscript. Line 17: Changed 'dugged' to 'dug'. Line 18: Delete 'and collected the specimens' Line 21: Change to 'a bio-box attached to a retractable tray' Line 22: Change to 'Once the ROV was back on-board, Molpadia ...' Line 23: Change to 'were placed at in-situ temperature' Line 24: Describe how you identified and separated the oesophagus, the mid-gut, and hind gut. Was it done using faint partitions in the gut lining as points of reference. Page 3067: Which method? Megacoring or using an ROV. Please clarify. If using an ROV, were the holothurians collected, brought to the surface and then brought down to the seafloor for the in-situ experiments, or were they collected and placed in the chambers during the same dive? Page 3068: Lines 20-26: It appears as though a One-Way ANOVA has been used to test for differences in extracellular enzymatic activities between different gut sections. I don't think ANOVA can be used here, as one of the basic assumptions of ANOVA is interdependence between and within samples. This is clearly not the

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case here, where extracellular enzymatic activities in the oesophagus, mid gut and hind gut will almost certainly be dependent on one another. I would suggest that the authors re-analyse the data using a One-Way ANOVA on ranked data (i.e. a Kruskal Wallis test). Significant results can then be tested separately using Mann Whitney U tests and P-values corrected for multiple tests using a Bonferroni or sequential Bonferroni correction. If the authors have good reason for using an ANOVA here I would be happy to hear about it. It should be given along with details of how normality and equal variances were tested. Page 3069: Line 2: What statistics program was used?

Results section Page 3069: Line 14: What is the error term here, a range, standard deviation, standard error? Please define, and include numbers of replicates. Furthermore, what was the biomass of these animals. I think it is important from an OM cycling point-of-view to provide a biomass estimate. Line 18: Remove 'patterns' and insert 'profiles'. Lines 23-Page 3070 (line 9): I don't think that the authors can use a parametric test here to test for differences between different depths as they are essentially testing for differences between dependent samples. Page 3071: Line 7: I see from the figure 6 that the carbohydrates had the 2nd highest digestion rate, followed by the lipids, but in the text it appears to be the other way around. Which is correct? The text or the figure?

Discussion Page 3071: Line 23-25. Change to significant effects were observed in the processing of OM on the seabed'. Page 3072 Line 1: Change to 'Although the reason for such a rise in holothurians. ...' Line 2: 'hypothesize' Change to 'is associated with changing quantity and quality. ...' Lines 5-8: These data-sets could be discussed in the context of the studies by McClain and Barry (2010) that found a remarkable degree of faunal turnover at scales of 100 m, and often 10 m related to geographic features of a canyon complex (Craig McClain and James Barry (2010): Habitat heterogeneity, disturbance, and productivity work in concert to regulate biodiversity in deep submarine canyons, Ecology, 91(4), pp. 964-976) as well as De Leo et al. (2010). See previous citation. I also think that it would be a good idea to give a biomass estimate and Corg

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data in the results and discuss the abundances and biomass in relation to sediment chemistry and the total C-inventory of the sediments. Line 26: Change 'availability' to 'inventory'. Page 3073 Line 19: I would suggest the authors read and cite Smith et al. (2008) TREE (see previous citation here). Line 20: Change to 'can be considered as a bioreactor'. I would also suggest that the authors cite the 'modelling animal guts as chemical reactors' literature here (e.g. Penry and Jumars 1985, 1987). Page 3074 Line 5: Change to 'This enzyme is responsible for the hydrolysis of proteins, breaking them down into ...'. Line 10: I believe that the carbohydrate and lipid digestion rates are reversed. Please double-check. Line 17: Change to 'so it could be hypothesized that this enzyme is very important for P-cycling by holothurians in deep-sea sediments' Line 19: 'Hypothesized' not 'hypothesised'. Change throughout manuscript. Line 22: Sorry, but I could not find the data concerning the prokaryotic biomass contribution to total protein. Where is it? Line 28: Change to 'in which the organism preferentially selects food-rich particles'. Page 3075 Line 1: Insert 'selectively' between 'organism' and 'digests'. Line 10: Change to 'using the geochemical tracer ^{234}Th as a proxy for recently deposited OM...'. Line 14: Delete 'the' between 'on' and 'rich' Line 16: Define what you mean by 'horizontal food-rich particles' Line 21: Again, here I think you should include some estimates of megafaunal biomass, so C-turnover rates can be compared to biomass (in units of C), and the abundance/ biomass data should be discussed in the context of McClain and Barry (2010). Page 3076: Line 1: Change to 'influencing the entire benthic community and each other by feeding. Line 3: Change to 'primary consumers of fresh OM and they may possess advantages over other taxa when foraging for it'. Line 4: From what I can remember, Witte et al. (2003) studied macrofauna, so this should be stated instead of writing 'large sedimentary fauna'. Line 6: Which do you mean as being very active? Macrofauna or megafauna? Line 7: Delete 'the' between 'influencing' and 'other' Line 13: Change to 'Therefore, we hypothesize that the impact of *M. musculus* affects not only the degradation of OM, but its redistribution and availability for other fauna at specific locations in the largest submarine canyon of the European margins'. Line 17: Change to 'may be overestimated' Line 19: Change

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to 'important to note that faeces production can redistribute OM' Line 21: Change to 'In summary we suggest that whilst feeding, these deep-sea holothurians play a potentially important role in sediment mixing, and modify the structure and geochemistry of sediments. Thus, they appear to be key ecosystem engineers within the Nazare canyon ecosystem.'

Table 1 legend: List of stations sampled during cruises XXX and XXX. Table 2 legend: Change to '...PERMANOVA tests for changes in the quantity and biochemical composition of sediment OM with sediment depth in the Nazare Canyon. Table 3 and 4 legends: Change to '...PERMANOVA tests' Table 4 legend: Change to 'DF = degrees of freedom' Figure 1 legend: Change to '...burrowing animals as seen by the burrows and feeding pits in the photograph'

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