



■ Object:
Submission of a **revised** manuscript to Biogeosciences special issue *Assessing environmental impacts of deep-sea mining – revisiting decade-old benthic disturbances in Pacific nodule areas*

■ From Paulo,
To Editor and Associate Editor of the Biogeosciences

Brest, January 16th 2020.

Dear Dr. Treude,

We are pleased to submit our **revised** manuscript entitled “*Alpha and beta diversity patterns of polychaete assemblages across the nodule province of the eastern Clarion-Clipperton Fracture Zone (Equatorial Pacific)*” by P. Bonifácio, P. Martinez-Arbizu & L. Menot for consideration to be published in Biogeosciences special issue “Assessing environmental impacts of deep-sea mining – revisiting decade-old benthic disturbances in Pacific nodule areas”.

We are glad that our corrections mostly pleased both the referees. We are thankful for the positive comments and minors’ corrections suggested which were completely accepted in this version. Following all suggested modifications, our revised manuscript has been improved mainly with: the addition of abundance and number of species per box core at table 1; and developing important thoughts in the conclusions; but also detailing and correcting few typos, excluding exceeding references and calling the new columns added in table 1.

As solicited by you, below you can find the answers to each comment for the two referees who suggested minor revisions. Ours answers are in green and make references to the pages/lines of the revised manuscript (marked-up and submitted separately).

We thank you and we are looking forward to hearing from you.

Best regards,
Paulo Bonifácio, Pedro Martinez-Arbizu & Lénaïck Menot

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Author's response to Referee's comments

Dear Authors,

I appreciate your careful consideration of all my questions and suggestions. Thanks a lot for doing a very thorough job here! I have no objections if the paper is published as is.

We're glad to read that and we thank the referee for the substantial and important suggestions given.

It seems to me, however, that some valuable thoughts are lost in the rather condensed edits you did to the manuscript. I have the impression that the paper would still benefit if some more content of your very detailed responses would also enter the text of the manuscript.

As I said - I accept if you leave as is but suggest that you to read through your answers and check whether some content could be transferred to the manuscript. Below I am quoting the answers where I had most strongly felt that important thoughts were not fully considered in your edits to the manuscript text.

"the main unknown is most likely about the biology and biotic interactions of species: how long do they live, how do they reproduce and disperse, do they interact and how are they interacting between others. These would be key questions to answer, although much more challenging than looking at correlations of abiotic factors and biological variables."

> This seems important information showing that the environmental-variable-approach underlying the regional management plan but maybe even studies on species diversity and turnover are in the end not sufficient to fully assess the risk and provide guidance if and how mining projects should be carried out.

The following sentence was added in the Conclusions (page 17 lines 1 to 2):

"Furthermore, there are vast gaps in knowledge regarding the life cycle and population dynamics that would need to be better constrained to fully assess the risks and provide guidance in mining management.

"If the aim is to monitor and preserve all levels of biological diversity, from gene, to species, to functions then polychaetes are likely not enough."

> You explained in the MS why you've chosen Polychaetes but the information that investigations (not only to preserve everything but also to fully understand the risks associated with mining) should consider all groups and size classes is not fully conveyed.

Answered with next comment

"We agree that these general recommendations would need to be more specific. There is a need to carefully think the sampling design and sampling effort together with statisticians. This would be a topic for another paper."

> To me this is an important point: recommendations on sampling design and effort for a specific region or site have to evolve from a scientific / statistical (and potentially iterative)

process and cannot be prescribed. Good to mention that future studies need to address this.

We have changed and added both suggestions in the Conclusions (page 17 lines 20 to 26):

From “In the framework of an ambitious and collective effort to inventory species richness in the CCFZ, a stratified random sampling at nested scales, from region down to seascapes, would provide the scales of species turn-over while intensive sampling of selected habitats up to the point where the number of singletons decreases with sample size would provide accurate estimates of species diversity. Both strategies are needed to assess the potential risks and scales of biodiversity loss due to nodule mining in the CCFZ.”

*To “In the framework of a similarly ambitious and collective effort to inventory species richness in the CCFZ, a stratified random sampling at nested scales, from region down to seascapes, would provide the scales of species turn-over while intensive sampling of selected habitats up to the point where the number of singletons decreases with sample size would provide accurate estimates of species diversity. Both **strategies should consider different taxonomic and functional groups of the abyssal fauna, which are likely to show different responses to nodule mining. Such an approach, based on standardized sampling methods and statistical-wise sampling strategies** is needed to assess the potential risks and scales of biodiversity loss due to nodule mining in the CCFZ.”*

"A meta-analysis is going to be conducted that should provide insight onto species richness and species ranges (<https://www.isa.org.jm/news/deep-ccz-biodiversity-synthesis-workshop>). By the end of this Deep CCZ Biodiversity Synthesis we should be able to tell where we collectively stand in terms of what we know and what we don't know. In order to provide an accurate estimate of species richness, we would look for a decreasing trend in the accumulation curve of singletons."

> the take home message to include in the MS may be that joint efforts combining data from independent science and contractors are needed to get to more accurate data on species richness and turnover - and that there are promising initiatives underway to get this started.

The following sentence was added in the conclusions (page 17 lines 16 to 20):

“Under the auspice of the ISA, the synthesis of ongoing studies from independent science and contractors in the CCFZ will certainly contribute in filling some knowledge gaps on species richness and turn over but differences in objectives, strategies and methodologies among studies are also likely to put some limits on the usefulness of the exercise. The JPI Oceans pilot action “Ecological aspects of deep-sea mining” demonstrated how powerful such a joined and coordinated initiative can be.”

Author's response to Referee's comments (Dr Dando)

Review of "Alpha and beta diversity patterns of polychaete assemblages across the nodule province of the eastern Clarion-Clipperton Fracture Zone (Equatorial Pacific)" BG bg-2019-255

The manuscript is greatly improved on revision. The main findings, that almost half the polychaete species sampled in the nodule zone, of the Clarion-Clipperton Fracture Zone, were only represented by a single individual and only a single species was common to all five areas need to be widely reported.

Thank you for the suggestions and we're glad that the improvements made are approved by you. The following sentence was included in the discussion section 4.2 Species turnover and geographic ranges (page 14 lines 2 to 4):

"Our observations about Aurospio sp. 249 which was the only species sampled in all five areas confirm the potential to disperse across large geographic distances of some spionids (Guggolz et al., in press)."

It is still not entirely clear what happened to nodule-associated polychaetes. Epifauna visible on the surface were picked off before washing and sessile polychaetes still on the nodules after washing were later removed. It appears that these these were not included in the dataset but what was their percentage contribution to the total polychaete numbers. In addition it would be useful to know if the nodules were preserved for later dissection to extract their polychaete infauna. I think that it is important to give an indication of what fraction of the polychaete fauna is included in this paper.

To our knowledge, the nodule epifauna, including polychaetes, has not been processed and the nodules were not preserved to study the nodule infauna. We thus can't provide an indication of the nodule-associated fauna. However, according to Thiel et al. (1993), the fraction of polychaetes found in nodule crevices has low significance and representativity.

We have changed (page 5 lines 9 to 13):

From "Sessile polychaetes, if present, remained attached to the nodules and were not considered in this study."

*To "Sessile **and crevice-inhabitant** polychaetes, if present, remained **with the** nodules and were not considered in this study. **According to Thiel et al. (1993) who washed and broke 26 nodules, the fraction of crevice inhabitant polychaetes has low significance and representativity (i.e. only 29 specimens belonging to six species) when compared with those living in sediments surrounding the nodules (i.e. 864 polychaetes).**"*

Infauna are important in nutrient recycling and deeper-burrowing infauna are particularly important. While appreciating the difficulties in sampling these, the rare observations on them, such as the maldanid found at 50 cm depth, should be mentioned to show how little we know about the deeper abyssal infauna. In addition any observations on deep burrows would be of interest.

The following sentence was added in results section 3.1 Abundance and alpha diversity (page 8 lines 29 to 30):

“Interestingly, only a large specimen identified as Bathyasychis sp. 150 was found deeper than 50 cm (bottom of box core) and so not included in the analyses.”

Table 1 lists the data for all the box core stations. The only actual result listed in the table is the “nodules density”, actually the nodule wet weight, extrapolated to the weight per m². Firstly this is not the density and secondly all the biological data is recorded per box core area, i.e. in 0.25 m². The table should include the basic polychaete data per box core, i.e. total numbers and number of “species” so that readers can follow the author’s analysis rather than having to extract the individual core data from the database in PANGEA.

The columns “Total abundance (ind. 0.25 m²)” and “Number of species (taxa 0.25 m²)” were added in Table 1 (below on page 28) and the caption was changed accordingly.

From “Table 1. Area, locality, station, date, depth, geographical position and nodule density of all 34 box corer deployments across the CCFZ during the SO239 cruise. “” indicates box cores considered as non-quantitative, not included in the analyses.”*

To “Table 1. Details of sampling, nodule density and descriptors of alpha diversity of all 34 box corer deployments across the CCFZ during the SO239 cruise. “” indicates box cores considered as non-quantitative, not included in the analyses.”*

Additionally, the asterisk indicating non-quantitative boxes were transferred from the column “Area” to the column “Station” where they are clearer.

Area	Locality	Station	Date	Depth (m)	Latitude	Longitude	Nodule density (kg m ²)	Total abundance (ind. 0.25 m ²)	Number of species (taxa 0.25 m ²)
BGR	BGR-PA	12	20/03/15	4118	11.8471667	-117.05933	26.40	32	24
BGR	BGR-PA	15	21/03/15	4133	11.8443333	-117.05217	26.80	67	40
BGR	BGR-PA	16	21/03/15	4122	11.8573333	-117.052	24.00	52	34
BGR	BGR-PA	21	22/03/15	4120	11.8535	-117.0595	22.80	43	28
BGR	BGR-PA	23	22/03/15	4122	11.85	-117.05267	20.80	69	47
BGR	BGR-RA	51*	27/03/15	4348	11.8236667	-117.52367	0.00	22	12
BGR	BGR-RA	57	28/03/15	4370	11.8075	-117.52433	8.00	43	24
BGR	BGR-RA	58	28/03/15	4350	11.8205	-117.54167	1.60	89	47
BGR	BGR-RA	60	29/03/15	4325	11.8076667	-117.55033	18.00	65	48
IOM	IOM-control	88	02/04/15	4433	11.079	-119.65883	0.00	53	33
IOM	IOM-control	89	02/04/15	4437	11.0758333	-119.66083	1.20	38	29
IOM	IOM-control	90	03/04/15	4434	11.074	-119.66417	0.00	42	24
IOM	IOM-disturb	94	03/04/15	4414	11.0736667	-119.6555	0.40	38	28
IOM	IOM-disturb	95	03/04/15	4418	11.0735	-119.65583	0.80	43	28
IOM	IOM-disturb	97	04/04/15	4421	11.0728333	-119.65617	0.20	22	16
IOM	IOM-resed	105*	05/04/15	4423	11.0711667	-119.65533	0.00	13	9
IOM	IOM-resed	106	05/04/15	4425	11.0716667	-119.65483	0.20	23	18

IOM	IOM-resed	107	05/04/15	4425	11.0721667	-119.6545	0.30	38	26
GSR	GSR	119	08/04/15	4516	13.8591667	-123.25267	26.47	46	29
GSR	GSR	127	09/04/15	4514	13.8443333	-123.246	27.10	59	32
GSR	GSR	128	09/04/15	4511	13.8516667	-123.252	27.10	58	32
GSR	GSR	137	11/04/15	4510	13.856	-123.238	25.20	60	34
GSR	GSR	138	11/04/15	4503	13.8481667	-123.23467	26.47	74	48
lfremer	lfremer	159	15/04/15	4921	14.049	-130.13433	19.80	30	23
lfremer	lfremer	162	16/04/15	4951	14.049	-130.126	20.20	34	21
lfremer	lfremer	169	17/04/15	4964	14.0421667	-130.12733	24.10	25	15
lfremer	lfremer	180	18/04/15	4936	14.0416667	-130.13633	16.00	19	17
lfremer	lfremer	181	18/04/15	4896	14.0465	-130.1415	16.80	38	27
lfremer	lfremer	182	18/04/15	4957	14.0423333	-130.1275	22.40	19	13
APEI#3	APEI#3	195	21/04/15	4833	18.7958333	-128.36217	6.28	4	3
APEI#3	APEI#3	196	21/04/15	4847	18.7971667	-128.34617	1.80	7	5
APEI#3	APEI#3	203*	23/04/15	4843	18.774	-128.35317	2.88	3	2
APEI#3	APEI#3	204	23/04/15	4816	18.7733333	-128.33617	3.65	3	2
APEI#3	APEI#3	209*	24/04/15	4819	18.7845	-128.3725	3.65	3	3

The authors recognise that the estimated number of polychaete species and the average species range in the nodule province of the CCFZ are not well constrained as a result of the limited sampling possible on this cruise. It would therefore be helpful if the authors could discuss what sampling effort might be required to obtain reasonable estimates of these.

At the moment, there is no example of a sampling effort allowing for rarefaction curves to level off, not even at DOMES A where 41 box-cores have been sampled. To our knowledge, there is no robust statistics allowing to extrapolate the number of samples required to obtain a reasonable estimate of the number of species at local or regional scales. We have suggested the following sentences in the Conclusions (page 17 lines 20 to 26):

“In the framework of a similarly ambitious and collective effort to inventory species richness in the CCFZ, a stratified random sampling at nested scales, from region down to seascapes, would provide the scales of species turn-over while intensive sampling of selected habitats up to the point where the number of singletons decreases with sample size would provide accurate estimates of species diversity. Both strategies should consider different taxonomic and functional groups of the abyssal fauna, which are likely to show different responses to nodule mining. Such an approach, based on standardized sampling methods and statistical-wise sampling strategies is needed to assess the potential risks and scales of biodiversity loss due to nodule mining in the CCFZ.”

References added in the answers:

Thiel, H., Schriever, G., Bussau, C. and Borowski, C.: Manganese nodule crevice fauna, Deep Sea Res. Part 1 Oceanogr. Res. Pap., 40(2), 419–423, doi:10.1016/0967-0637(93)90012-R, 1993.