Response to Reviewer 1: Dr. Cene Fiser

Manuscript Title: Biogeography and community structure of 1 abyssal scavenging Amphipoda (Crustacea) in the Pacific Ocean.

Ref: bg-2018-347

Journal: BioGeosciences

Dear Dr Fiser,

We would like to thank you for the clarification of your previous comments. The authors agree, and in accordance with your suggestion, revised the CPUE effort plot to show uncorrected time, and added further details for the rarefaction analysis.

Overleaf, you will find a point by point reply on how we addressed your comments in the revised version of our manuscript. Attached also is a copy of the manuscript with track changes highlighted in green.

With Kind Regards, also on behalf of the co-authors,

Tasnim Patel.

RC1. Catch per unit analysis: the correction for sampling effort per time you used, dividing number of individuals with time the trap was deployed, may be inappropriate. The correction would be fine, if amphipods were dripping into the trap constantly, and if the densitiy of ampihpods was even. However, time that amphipods reach the trap depends on time the odor is spread and walking speed. The first hours amphipods may rapidly reach the trap, but more distant individuals arrive with larger and larger lag. This might mean that in your correction nominator increases eg logarithmically and denominator increases linearly, and that traps deployed for longer periods will be corrected "disproportionally more" than traps deployed for shorter periods. Please consider this aspect in your discussion

Our reply: *Line 374 onward, shows an amended CPUE graph which has not been corrected for time. The paragraph describing the CPUE results was also amended (line 505 onward).*

RC2. Rarefaction curves: describe your procedure better: how many subsamples were taken, what was the sizes of random subsamples, how many times you run rarefactions and so on, hence the parameters of the procedure.

Our reply: *Line 295, paragraph describing the rarefaction was amended to state it was run on the longer than 15 mm fraction indicated in Table 2.0, and curves were generated using the default parameters of the "rarefy" function in R.*

All comments from the edits directly within the PDF were addressed.

Other comments:

Tectovallopsis -> *Tectovalopsis* changed throughout.

Valettietta tenuipes -> *Paralicella tenuipes* changed throughout

Species authorities added throughout.

Figures 5a and 5b redone.

Biogeography and community structure of abyssal scavenging Amphipoda (Crustacea) in the Pacific Ocean.

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Abstract

In 2015, we have collected more than 60,000 scavenging amphipod specimens during two expeditions to the Clarion-Clipperton fracture Zone (CCZ), in the Northeast (NE) Pacific and to the DISturbance and re-COLonisation (DisCOL) Experimental Area (DEA), a simulated mining impact disturbance proxy in the Peru basin, Southeast (SE) Pacific. Here, we compare biodiversity patterns of the larger specimens (> 15 mm) within and between these two oceanic basins. Eight scavenging amphipod species are shared between these two areas, thus indicating connectivity. We further provide evidence that disturbance proxies seem to negatively affect scavenging amphipod biodiversity, as illustrated by a reduced alpha biodiversity in the DEA (Simpson Index (D) = 0.62), when compared to the CCZ (D = 0.73) and particularly of the disturbance site in the DEA and the site geographically closest to it. Community compositions of the two basins differs, as evidenced by a Non-Metric Dimensional Scaling (NMDS) analysis of beta biodiversity. The NMDS also shows a further separation of the disturbance site (D1) from its neighbouring, undisturbed reference areas (D2, D3, D4 and D5) in the DEA. A single species, *Abyssorchomene gerulicorbis* (Shulenberger & Barnard, 1976), dominates the DEA with 60% of all individuals.

Keywords

JPIO Ecological Aspects of Deep-sea mining, Clarion Clipperton Fracture Zone, CCZ, DisCOL Experimental Area (DEA), Amphipoda.

Introduction

The abyssal deep sea (3000 - 6000 m) represents the largest ecosystem on the planet, with the abyssal seafloor covering approximately 54% of the Earth's solid surface (Rex et al. 1993; Gage & Tyler, 1991). Since it is one of the least investigated ecosystems, there are still extensive gaps in our knowledge of deep-sea fauna (German et al. 2011). Marine research has thus far focused on coastal areas, hydrothermal vents or chemosynthetic habitats, whereas open-ocean abyssal plains have been less extensively investigated (Ramirez-Llodra et al. 2010). This is unsurprising given the challenges of sampling this remote environment, which is impeded by several confounding factors. For example, deep-sea sampling is both financially expensive and labour intensive, and furthermore, constrained by the challenge of deploying equipment at low temperatures (0.01 - 4.0°C) and at high hydrostatic pressures (Sweetman et al. 2017). Therefore, to date very little of the deep sea has been sampled, and the oversampling in the North Atlantic basin has created a biased knowledge base (McClain & Hardy, 2010). Consequently, and owing to the low availability of data on deep-sea biodiversity, and with the inherent risk of under-sampling, it is difficult to estimate species richness in the deep sea.

In the traditional view of the deep sea, the abyss was considered to be homogeneous and many species were thought to have large biogeographical ranges, their dispersal aided by an apparent lack of barriers (Sanders, 1968). This hypothesis was challenged by the discovery of chemosynthetic habitats e.g. hydrothermal vents (Lonsdale, 1977), cold seeps (Paull et al. 1984), seasonal fluctuations in primary productivity (Billett et al. 1983) and erratic whale-falls (Smith et al. 1989). All of this research has demonstrated that the deep sea is an heterogeneous environment and is controlled by many factors, including: Particulate Organic Carbon (POC) flux, water depth, flow regime, current circulation, seafloor topography (Laver et al. 1985) and also historical factors e.g. the opening of ocean basins (i.e. rifting), sea-level rise and fall, and periods of deep-sea anoxia (Smith et al. 2006). All of these can result in a mosaic of different communities (Levin et al. 2001), many of which do not follow a latitudinal gradient (Brandt et al. 2007).

It has also been established that dispersal ability of species on the one hand, and their actual geographic and bathymetric distribution range on the other, are not always linked, and are often dependent on habitat suitability, fragmentation, and ecological flexibility (Lester et al.

2007; Liow 2007). Therefore, although the deep-seafloor includes some of the largest contiguous features on the planet, the populations of many deep-sea species are spatially fragmented, and may become increasingly so with continued human disturbance (Hilario et al. 2015).

In the last decade, there has been an bigger demand for exploitation of deep-sea resources e.g. rare earth element (REE) extraction (such as those concentrated in manganese nodule provinces) (Ramirez-Llodra et al. 2011). As a result, ecologists are increasingly asked to assess the ecological risks of these mining activities and to provide sustainable solutions for its mitigation, in order to prevent adverse changes to the deep-sea ecosystem (ISA, 2017).

Glover et al. (2001) showed that abyssal sediments can contain high biodiversity with more than 100 species of meiofaunal invertebrates (e.g. nematodes, copepods) and protists (e.g. foraminifers) found every square meter. In spite of this, our knowledge on the deep-sea ecosystem structure and functioning is still limited, and there is a paucity of data on the distribution, drivers and origins of deep-sea communities at global scales. This is especially true for deep-sea invertebrates, including Amphipoda (Barnard 1961; Thurston 1990).

Although recent morphological and molecular studies have shed new light on the distribution and habitat niches of certain bentho-pelagic amphipods (e.g. *Eurythenes*) (Havermans, 2016; Narahara-Nakano et al. 2017), there is little published so far on how widespread other amphipod species may be. This lack of information on species richness and ecological uniqueness hampers the answering of crucial questions on recoverability of anthropogenic impacts. Ultimately this impedes ecologists from providing advice on sustainable deep-sea mining practices, thus, underpinning the need for this dedicated deep-sea ecosystem research.

Here, we present distribution patterns of scavenging deep-sea amphipod communities, with the first comparisons of their biogeography and community structures in two oceanic basins. These two basins are the research areas for simulating/studying the anthropogenic impacts of deep-sea nodule mining.

We are investigating whether there are differences and similarities in the species compositions of the two basins (e.g. richness, abundances), and further exploit a disturbance experiment to compare the biodiversity of this mining impact proxy to the undisturbed reference areas. We discuss the possible implications of our findings; aiming to use them to formulate recommendations regarding the pending deep-sea mining of manganese nodule activities in the NE Pacific ecosystem.

Material and Methods

Study area

We investigated the amphipod communities of two oceanic basins (Figure 1); (i) the Clarion-Clipperton Fracture Zone (CCZ, six million km^2, 7000 km wide), an economically important manganese nodule field in the NE Pacific, comprising several different contractor claim areas, (who to date, have exploration licences only), and nine designated Areas of Particular Ecological Interest (APEIs) as designated by the International Seabed Authority (ISA) (Lodge et al, 2014) and (ii) the DISturbance and re-COLonisation (DisCOL) Experimental Area (DEA, 11 km^2, 4 km wide), a mining disturbance proxy (also containing manganese nodules) in the Peru Basin in the SE Pacific. In 1989, the DEA sediment bed was artificially disturbed using a plough-harrow to create 78 track marks (Appendix 1) (Thiel, 1992).. These are supposed to simulate the type of disruption which would be caused by a commercial mining operation This baseline study was a new approach in deep-sea risk assessment and is still ongoing today, providing us with crucial data from this long-term ecological experiment.

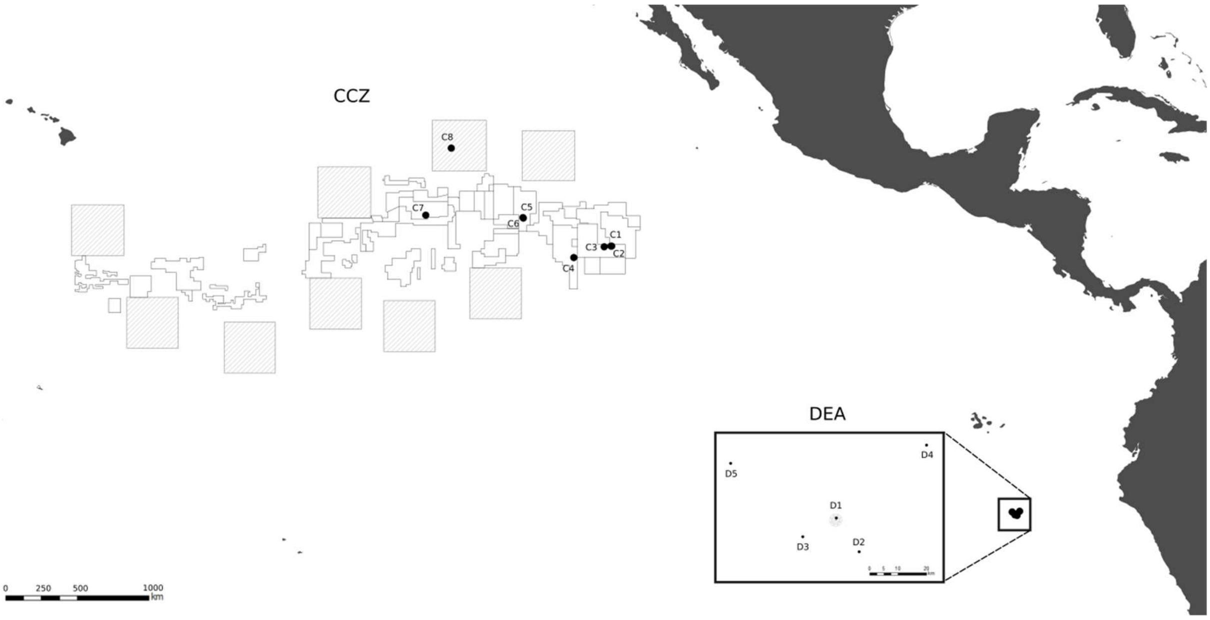


Figure 1: Geographic locations of the two study areas, the Clarion-Clipperton fracture Zone (CCZ) (Northeast Pacific) and the DISturbance and re-COLonisation (DisCOL) Experimental Area (DEA) (Peru Basin, Southeast Pacific). There are nine Areas of Particular Ecological Interest (APEIs) in the CCZ region, which are illustrated by 400 x 400 km^2 white boxes. Grey boxes indicate the various contractor claim areas in the CCZ. We deployed eight amphipod traps across the CCZ, which is 16,000 km^2 and 7000 km wide, and five in the DEA, which encompasses 11 km^2 with a width of 4 km.

Sampling

In 2015 (26 years after the first impact in the DEA in 1989), two research expeditions with the "RV Sonne" visited the CCZ (cruise SO239) and revisited the DEA (cruise SO242-1 & SO242-2), to assess if and how the deep-sea faunal communities had recovered within the DEA, and to attempt to quantify their recolonization potential.

Amphipod samples were taken from the CCZ and DEA using a free-fall lander (120 x 120 x 120 cm), to which four plastic traps were attached (two 20 x 25 x 40 cm traps with four cm openings and two 25 x 40 x 60 cm traps with eight cm openings), baited for each station with an 800 g mixture of mackerel, squid and shrimp. Using this specially designed deep-sea sampling equipment, more than 60,000 specimens of scavenging amphipods were collected from the CCZ and the DEA sites.

The baited trap was deployed eight times across the CCZ at a depth range of 4116 - 4932 m (samples C1 - C8), and five times in the DEA at a depth range of 4078 - 4307 m (samples D1

- D5; Figure 1, Table 1). In the CCZ, we sampled within three different contractor claim areas (Table 1) to obtain a pre-disturbance baseline, and to then compare it with one of the nine protected APEIs around the CCZ. In contrast, in the DEA, sampling was conducted once within the disturbed area (D1), twice 10 km away (D2, D3) and twice 40 km away (D4, D5) from D1 in four surrounding reference areas (see Figure 1).

Table 1: Station overview.

Codes refer to the codes used in this paper in figures 1, 3, 4, 6 and Table 3. The original station code represents the cruise codes from (SO239 and SO232-1). Depth refers to water depth (m) on deployment. Nodule presence/absence information is known only for stations D3 and D4.

Deployment Code	Original Station Code	Depth (m)	Known geological features	Remarks	Residence Time (h:m:s)
C1	SO239-33	4122	Plains	German claim	100:20:00
C2	SO239-37	4116	Plains	German claim	125:49:00
C3	SO239-63	4354	Plains	German claim	66:07:00
C4	SO239-96	4388	Seamount	Inter Ocean Metals (IOM) claim	63:02:00
C5	SO239-123	4529	Plains	Belgian claim	26:12:00
C6	SO239-139	4516	Plains North/South + seamount to west	Belgian claim	56:33:00
C7	SO239-173	4934	Plains	French claim	79:40:00
C8	SO239-205	4855	Plains	Area of Particular Ecological Interest (APEI)	55:59:00
D1	SO242/1-8	4146	Plains	Disturbed	44:26:33
D2	SO242/1-30	4307	Plains	Undisturbed Reference	51:11:18
D3	SO242/1-55	4043	Seamount No nodules	Undisturbed Reference	25:09:09
D4	SO242/1-68	4078	Seamount No nodules	Undisturbed Reference	65:20:46
D5	SO242/1-106	4269	Plains	Undisturbed Reference	47:00:50

Processing

On recovery of the lander, all traps were disconnected and placed in pre-cooled (4°C) buckets of filtered seawater. All specimens were washed on board in a cool-climate laboratory (4°C), morphologically pre-sorted and fixed in molecular grade (95%) ethanol, before being stored at -20°C.

Detailed sorting and identification was performed using the morphological species concept (Futuyama, 1998) and the keys of Lowry & Killagen (2014) and Schulenberger & Barnard (1976), to separate the samples into taxonomic "morphotypes". The larger fraction (> 15 mm length) has been identified to the lowest possible taxonomic resolution. Species not assigned with certainty are denoted here by as affiliated species (e.g. *genus* aff. *species*) or conferred species (e.g. *genus* cf. *species*).

Specimens with a size of less than 15 mm length were excluded from the analysis because these were mostly juveniles and their morphological differences were not sufficiently pronounced to allow an accurate identification to the species or even genus level. Some pelagic amphipods were collected accidentally and omitted. Finally, genera containing multiple (and as yet) unidentified species have been summarised as "spp."

Statistical analyses

Our null hypothesis (H_0) here is that there are no differences in the amphipod biodiversity of the two basins. To test this hypothesis, we firstly calculated the alpha biodiversity of the two basins was using the Simpson Index (*D*) (Simpson, 1949) (Appendix 4). Rarefaction was run on the longer than 15 mm fraction using default parameters. Individual-based curves were generated using the rarefy function of the vegan package in R 2.3.0 (R Core Team, 2013; Gotelli, 2001) to compare species richness across all thirteen sampling stations and to test for the completeness of sampling.

Secondly, to compare the beta biodiversity, we estimated the variability of the community compositions between sites. The Bray-Curtis dissimilarity metric (Bray & Curtis, 1957), was used to calculate differences between community compositions based on species densities, and the results were then visualised in 2D using a Non-Metric Dimensional Scaling (NMDS) plot. The ANOSIM function in the vegan package of R (R Core Team, 2013; Taguchi & Ono, 2005) was used to test the statistical significance of the differences in species compositions between the two study areas.

Results

Basin biodiversity

In total, 6916 scavenging amphipods (> 15 mm) were collected from the thirteen trap deployments in the two study areas, representing a total of seventeen morphotypes (Figure 2). In the CCZ, we collected 3932 individuals, which represent ten morphotypes. Five of these have been identified to the species level: *Abyssorchomene distinctus* (Birstein & Vinogradov, 1960), *Abyssorchomene gerulicorbis* (Shulenberger & Barnard, 1976), *Eurythenes sigmiferus* (d'Udekem d'Acoz & Havermans, 2015), *Paralicella caperesca* (Shulenberger & Barnard, 1976) and *Paralicella tenuipes* (Chevreux, 1908). Two are affiliated to a species (*Paracallisoma* aff. *alberti* and *Valettietta* cf. *gracilis*) and the remaining three are at least affiliated to a genus (Tables 2a and 2b). The 2984 individuals from the DEA represent fifteen morphotypes. Six of these have been identified to the species level: *Abyssorchomene distinctus* (Birstein & Vinogradov, 1960), *Abyssorchomene gerulicorbis* (Shulenberger & Barnard, 1976), *Eurythenes sigmiferus* (d'Udekem d'Acoz & Havermans, 2015), *Paralicella caperesca* (Shulenberger & Barnard, 1976), *Parandaniexis mirabilis* (Schellenberg, 1929) and *Tectovalopsis regelatus* (Barnard & Ingram, 1990). A further five which have been affiliated to a species: *Eurythenes* aff. *gryllus*, *Eurythenes* aff. *magellanicus*, *Paracallisoma* aff. *alberti*, *Stephonyx* sp. nov. aff. *arabiensis* and *Valettietta* cf. *gracilis* and the remaining four identified to at least an affiliated genus (Tables 2a and 2b).

There are eight morphotypes shared between the basins: *Abyssorchomene distinctus* (Birstein & Vinogradov, 1960)*, Abyssorchomene gerulicorbis* (Shulenberger & Barnard, 1976), *Abyssorchomene* spp., *Eurythenes sigmiferus* (d'Udekem d'Acoz & Havermans, 2015), *Eurythenes* spp., *Paracallisoma* aff. *alberti*, *Paralicella caperesca* (Shulenberger & Barnard, 1976) and *Valettietta* cf. *gracilis* (Figure 2).

Two morphotypes were found only in the CCZ (*Hirondellea* sp. & *Paralicella tenuipes* (Chevreux, 1908), and seven morphotypes were found only in the DEA (*Eurythenes* aff. *gryllus, Eurythenes* aff. *magellanicus*, gen. aff. *Cleonardo, Parandania* sp., *Parandaniexis mirabilis* (Schellenberg, 1929)*, Stephonyx* sp. nov. aff. *arabiensis,* and *Tectovalopsis regelatus* (Barnard & Ingram, 1990) (Table 2).

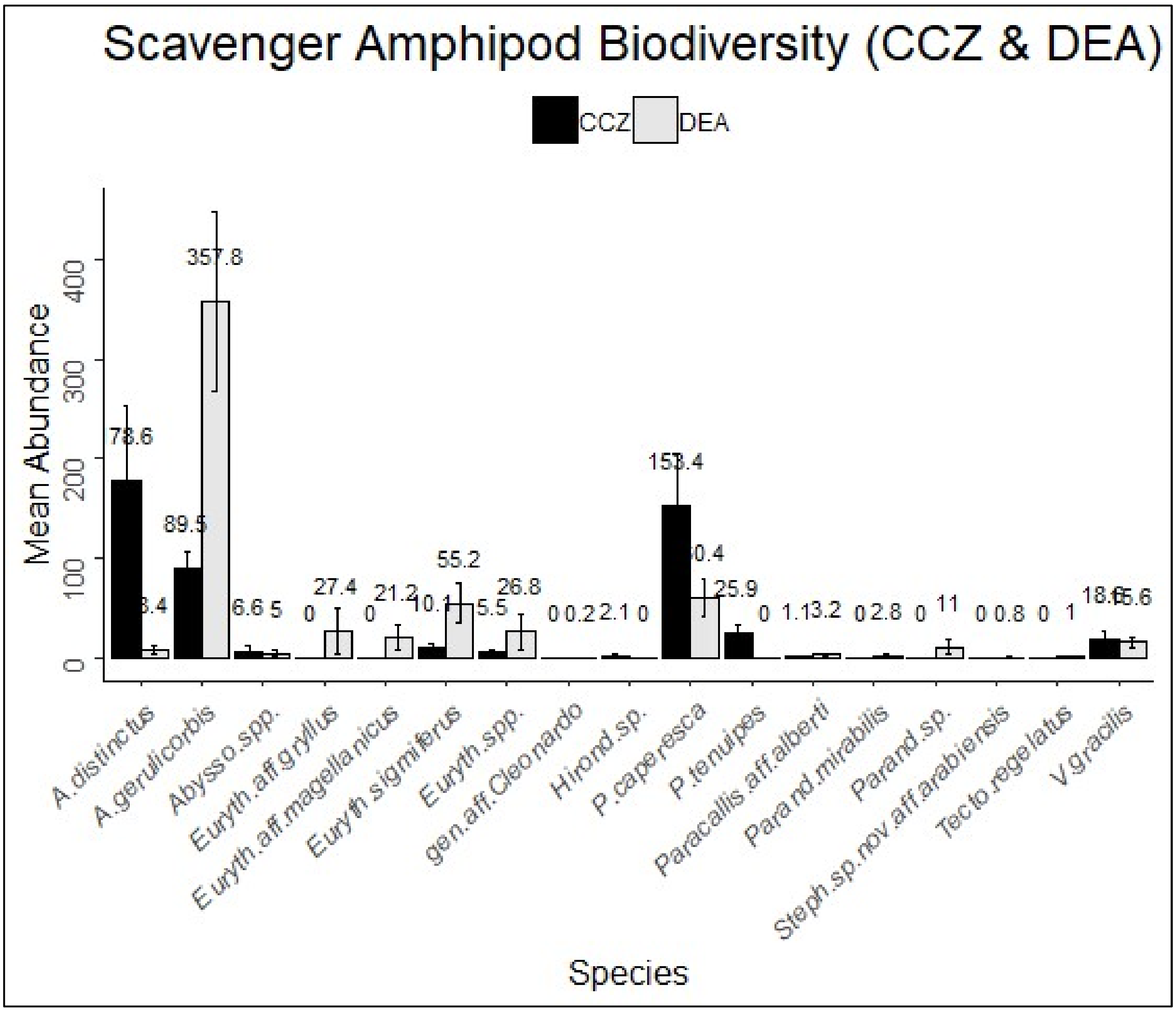
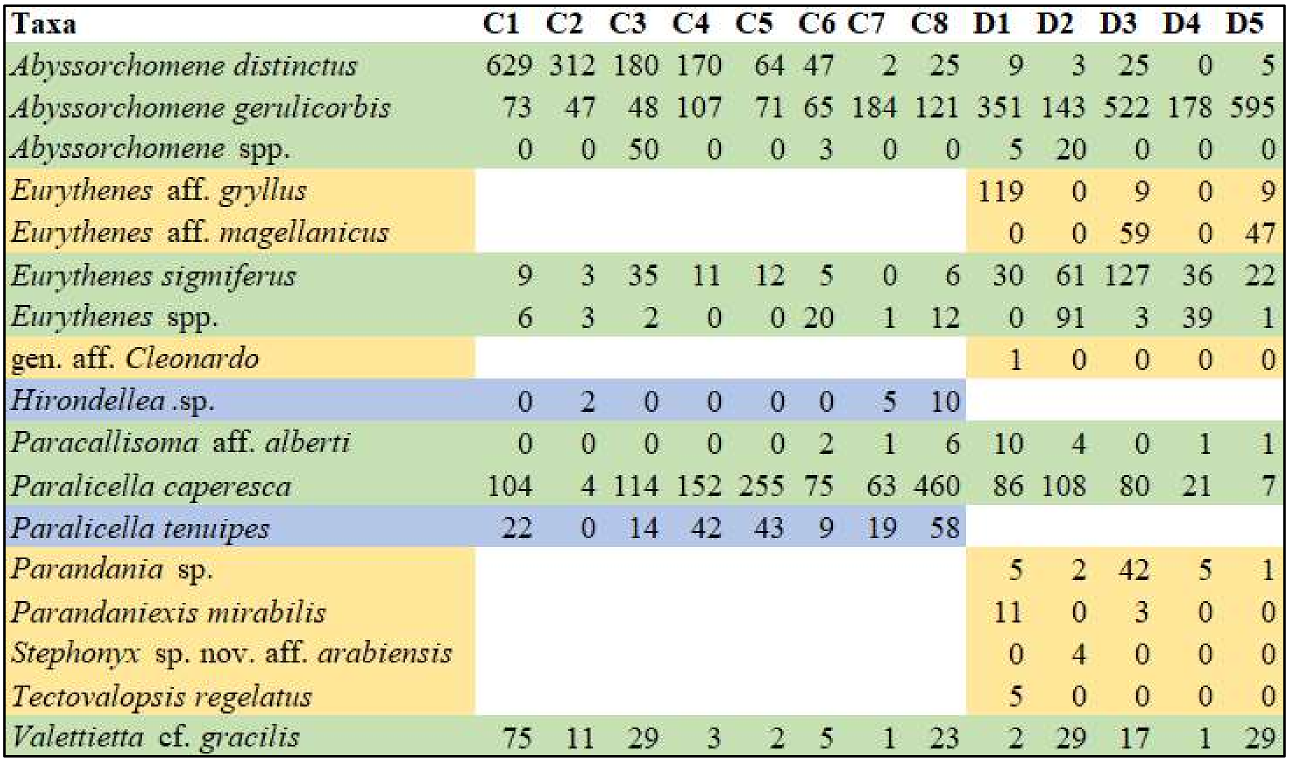


Figure 2: **Histogram showing the species assemblage for the scavenging community in the Clarion-Clipperton fracture Zone (CCZ) (black) and the DisCOL Experimental Area (DEA) (grey)**. The abundances of 17 morphotypes are shown.

Table 2a: Overview of morphotypes across the Clarion-Clipperton fracture Zone (CCZ) and DisCOL Experimental Area (DEA).

	Clarion-Clipperton fracture Zone	**DisCOL Experimental Area**
Total unique morphotypes collected	17 (10 found in the CCZ, 15 found in the DEA)	
Species possibly unique to this basin	2	7
Shared species between basins	8	

Table 2b: Distribution and abundances of morphotypes across the Clarion-Clipperton fracture Zone (CCZ) and DisCOL Experimental Area (DEA). Green = shared, Orange = DEA only, Blue = CCZ only.



Taxa	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**D1**	**D2**	**D3**	**D4**	**D5**
Abyssorchomene distinctus	629	312	180	170	64	47	2	25	9	3	25	0	5
Abyssorchomene gerulicorbis	73	47	48	107	71	65	184	121	351	143	522	178	595
Abyssorchomene spp.	0	0	50	0	0	3	0	0	5	20	0	0	0
Eurythenes aff. *gryllus*									119	0	9	0	9
Eurythenes aff. *magellanicus*									0	0	59	0	47
Eurythenes sigmiferus	9	3	35	11	12	5	0	6	30	61	127	36	22
Eurythenes spp.	6	3	2	0	0	20	1	12	0	91	3	39	1
gen. aff. *Cleonardo*									1	0	0	0	0
Hirondellea .sp.	0	2	0	0	0	0	5	10					
Paracallisoma aff. *alberti*	0	0	0	0	0	2	1	6	10	4	0	1	1
Paralicella caperesca	104	4	114	152	255	75	63	460	86	108	80	21	7
Paralicella tenuipes	22	0	14	42	43	9	19	58					
Parandania sp.									5	2	42	5	1
Parandaniexis mirabilis									11	0	3	0	0
Stephonyx sp. nov. aff. *arabiensis*									0	4	0	0	0
Tectovalopsis regelatus									5	0	0	0	0
Valettietta cf. *gracilis*	75	11	29	3	2	5	1	23	2	29	17	1	29

Sampling completeness

Due to differences in allocated ship-times (CCZ cruise being 52 days and the DEA cruise being 29 days), the trap deployments were not identical, making it necessary to check the effect of the different deployment times. The resulting Catch Per Unit Effort (CPUE) plot (Figure 3,) shows that there is no relationship between the length of time the trap was at the seafloor and total number of amphipods caught. There is a moderate but statistically insignificant negative correlation with increasing deployment times (R = - 0.05, *p* = 0.551).

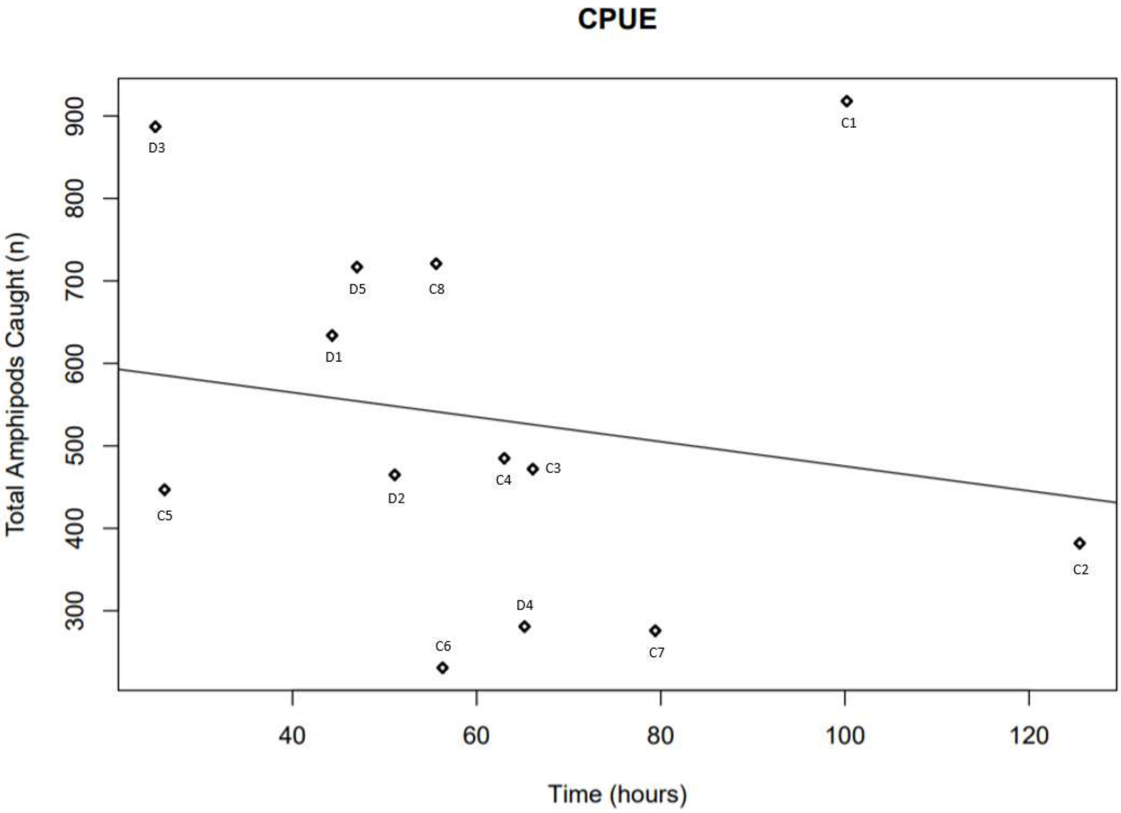


Figure 3: Catch Per Unit Effort (CPUE) illustrating the correlation between sampling time and number of individuals collected. Only the longer than 15mm fraction was included here.

The rarefaction results (Figure 4) show that the curves for nine stations reach a plateau, indicating that sampling effort was sufficient to assess diversity levels. These include all CCZ stations except C7. In contrast, four of the five curves for the DEA (stations D1, D2, D4 and D5) are unsaturated.

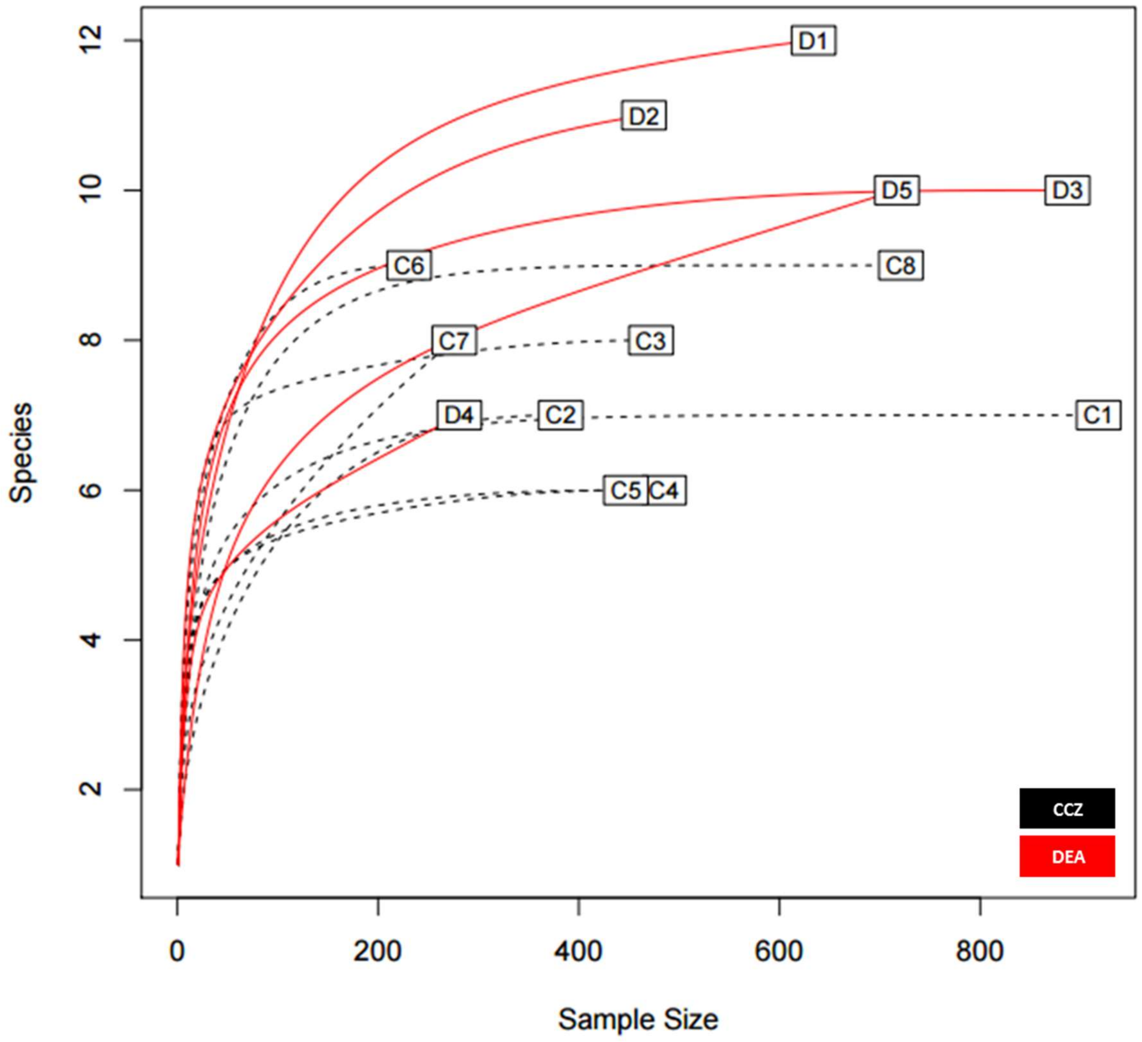
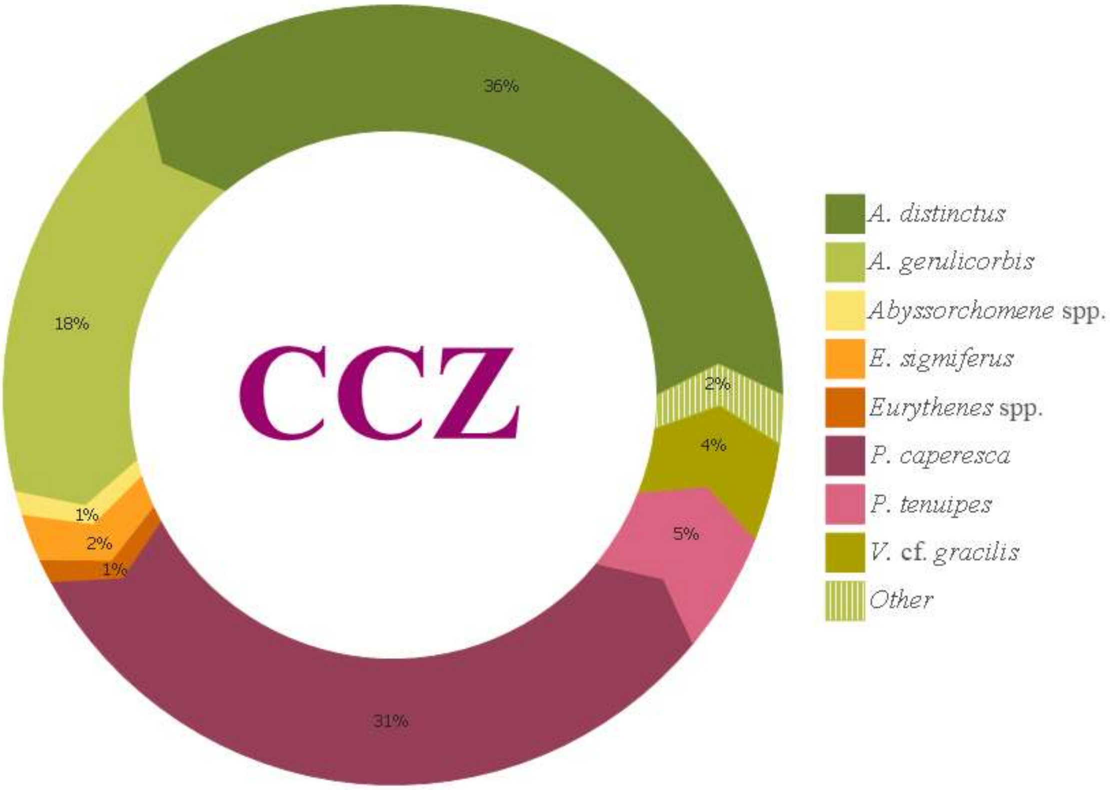
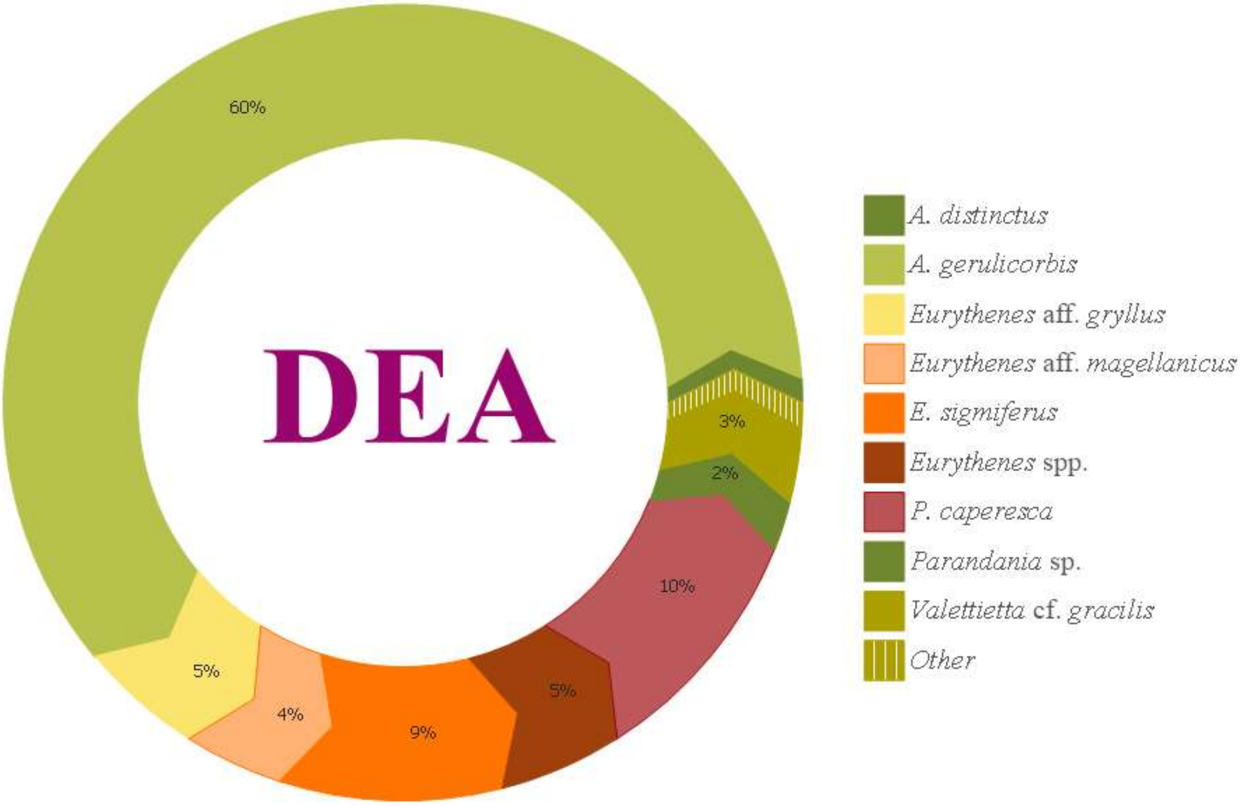


Figure 4: Species rarefaction curves for each of the 13 trap stations across both areas, the Clarion-Clipperton fracture Zone and the DisCOL Experimental Area. Only individuals longer than 15 mm were considered here.

Biodiversity

Figures 5a and b show that the scavenging community in the CCZ is dominated by three species, *A. distinctus* (Birstein & Vinogradov, 1960) (36%), *A. gerulicorbis* (Shulenberger & Barnard, 1976) (18%) and *Paralicella caperesca* (Shulenberger & Barnard, 1976) (31%), whereas, in contrast, the DEA scavenging community is dominated by a single species, *A. gerulicorbis* (Shulenberger & Barnard, 1976), accounting for almost 60% of all specimens. The Simpson Index (*D*) for the entire CCZ area is (with 0.73), higher than the 0.62 that was calculated for the whole of the DEA area (Table 3). The biodiversity of each individual station was further explored (Table 3). In the CCZ, the lowest biodiversity was found at C3 and C6 ($D = 0.23$) and the highest at C2 ($D = 0.67$), respectively. In the DEA, the lowest biodiversity of $D = 0.36$ was found at station D1 (the site of the actual disturbance) and just south of the disturbance site at D2 (0.21), while the highest biodiversity was observed at D5 ($D = 0.61$) (Table 3).


CCZ
36%
18%
1%
2%
1%
31%
5%
4%
2%
A. distinctus
A. gerulicorbis
Abyssorchomene spp.
E. sigmiferus
Eurythenes spp.
P. caperesca
P. tenuipes
V. cf. gracilis
Other


DEA
60%
3%
2%
10%
5%
9%
4%
5%
A. distinctus
A. gerulicorbis
Eurythenes aff. gryllus
Eurythenes aff. magellanicus
E. sigmiferus
Eurythenes spp.
P. caperesca
Parandania sp.
Valettietta cf. gracilis
Other

Figures 5a and b: Relative species abundances in the Clarion-Clipperton fracture Zone and the DisCOL Experimental Area. These abundances represent the longer than 15mm subsample of the scavenging amphipod community.

Table 3: Comparison of biodiversity calculated using the Simpson Index (*D*), for the Clarion-Clipperton fracture Zone and DisCOL Experimental Area, and *D* for each station is shown for further comparisons within these areas.

Simpson Index (*D*) whole Clarion-Clipperton Fracture Zone	0.73
Simpson Index (*D*) whole DisCOL Experimental Area	0.62
C1 (*D*)	0.41
C2 (*D*)	0.68
C3 (*D*)	0.23
C4 (*D*)	0.27
C5 (*D*)	0.38
C6 (*D*)	0.23
C7 (*D*)	0.45
C8 (*D*)	0.44
D1 disturbed (*D*)	0.36
D2 ref 1 (*D*)	0.21
D3 ref 2 (*D*)	0.38
D4 ref 3 (*D*)	0.44
D5 ref 4 (*D*)	0.61

Species composition

The NMDS shows that the communities of the two basins are clearly separated (ANOSIM: $p = 0.002$); Figure 6). The disturbed area in the DEA (D1) is showing a clear difference to the four reference areas (D2 - 5). When the communities between the two basins are compared, D2 appears to be most similar to the CCZ community, and more specifically to C6, C7 and C8. The reliability of the data ranking is supported by a low stress value of 0.01.

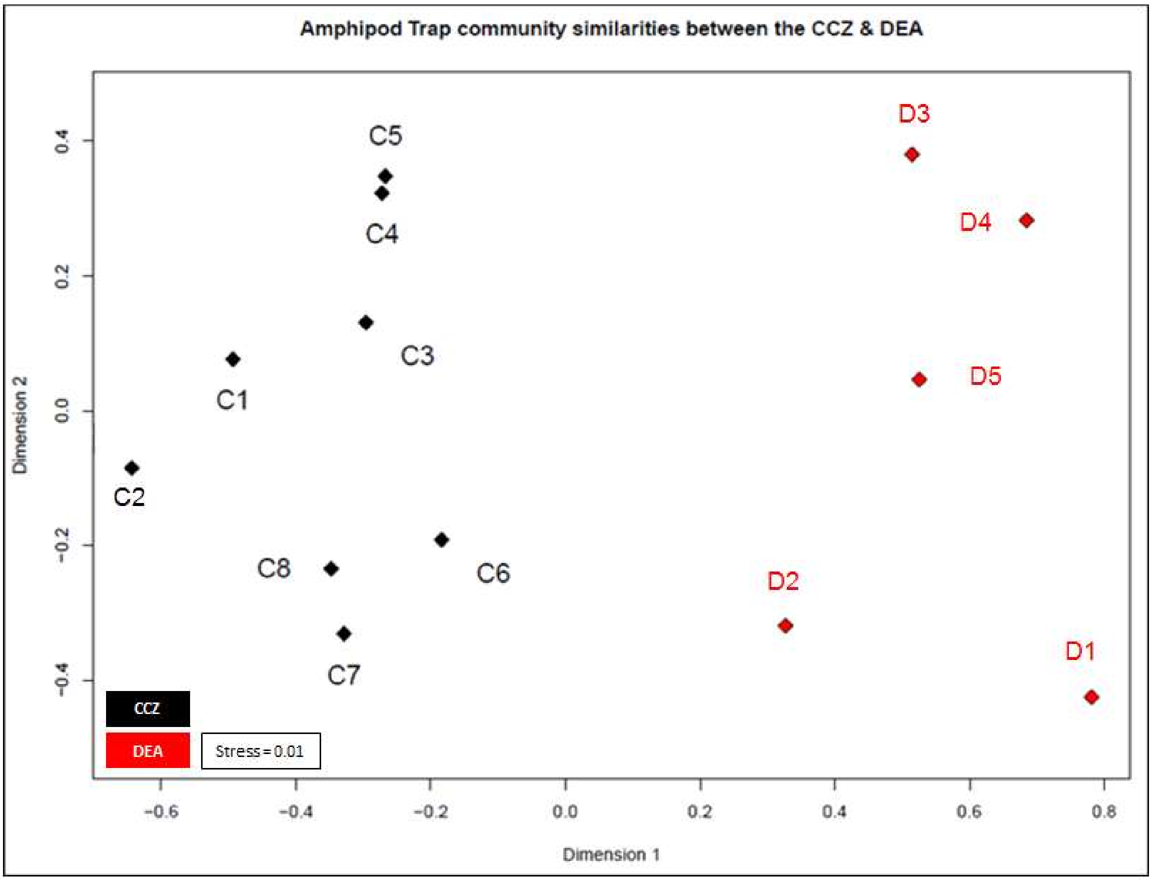


Figure 6 : NMDS plot showing the beta biodiversity (dis/similarities) for each of the thirteen amphipod trap sampling stations associated with the two basins, Clarion-Clipperton fracture Zone (CCZ) (black) and the DisCOL Experimental Area (DEA) (red). Data are supported by a low stress value of 0.01.

Discussion

An unexplored ecosystem

Although the most recent and comprehensive analysis of the Animal diversity of the World's oceans estimates a total of less than a million species over all depths (Appeltans et al. 2012), it is not currently known how many species inhabit the deep-sea. Regarding amphipods, only 328 benthic, demersal and benthopelagic species, belonging to 144 genera and 39 families. Over 7000 marine amphipod species have been found below 2000 m. These numbers are reduced to 173 known species, 87 genera and 37 families at depths below 3000 m, and 100 known species, 66 genera and 31 families are known to occur below 4000 m (Vader 2005; Brandt et al, 2012).

Lysianassoidea and their biogeography

The superfamily Lysianassoidea constitutes an important part of the abyssal amphipod fauna. Also, in our sampling, lysianassoid amphipods were collected in large numbers (99% of the samples taken in both basins). As a superfamily, they comprise 23% of all the species found below 2000 m, 35% of the species found below 3000 m and 31% of the species found below 4000 m (Brandt et al. 2012).

Many species in the Lysianassoidea occur in multiple abyssal basins, and some even have worldwide distributions (Thurston 1990). Despite the Ocean Biogeographic Information System (OBIS) database containing 615,650 records of Amphipoda, many of these are shelf or pelagic species, with very few records from the CCZ and DEA (OBIS, 2017). Here, we provide additional data for the known bathymetric range of the seven amphipods which we have identified to species level (*Abyssorchomene distinctus* (Birstein & Vinogradov, 1960), *Abyssorchomene gerulicorbis* (Shulenberger & Barnard, 1976), *Eurythenes sigmiferus* (d'Udekem d'Acoz & Havermans, 2015), *Paralicella caperesca* (Shulenberger & Barnard, 1976), *Paralicella tenuipes* (Chevreux, 1908), *Parandaniexis mirabilis* (Schellenberg, 1929) & *Tectovalopsis regelatus* (Barnard & Ingram, 1990) (Table 2b). In addition, we have found two possibly new species of *Eurythenes*, previously not known from these basins, which we plan to analyse further in the future.

While we only sampled *Hirondellea* sp. and *Paralicella tenuipes* (Chevreux, 1908) in the CCZ, *Eurythenes* aff. *gryllus*, *Eurythenes* aff. *magellanicus*, *gen.* aff. *Cleonardo,* Parandania sp., *Parandaniexis mirabilis* (Schellenberg, 1929)*, Stephonyx* sp. nov. aff. *arabiensis,* and *Tectovalopsis regelatus* (Barnard & Ingram, 1990) only in the DEA, we cannot conclude based on the current data only if these species are unique to their respective basins without confirming these distribution patterns with additional sampling campaigns.

Despite the sampling campaign in the CCZ being twice as long as the DEA, the number of individuals/species collected does not correlate positively with deployment effort. We posit that this is rather an effect of abiotic and organic factors, such as the productivity-driven gradients in the CCZ, which decrease from East-West and from North-South (Hannides & Smith, 2003).

Biodiversity within basins

Figures 5a & b show clearly that the DEA scavenging community has reduced abundances of all species including *A. distinctus* (Birstein & Vinogradov, 1960) (1%) and *P. caperesca* (Shulenberger & Barnard, 1976) (7%), and is now dominated by a single species, *A. gerulicorbis* (Shulenberger & Barnard, 1976), accounting for 60% of the DEA community. This indicates an interesting resilience and flexibility in the latter species. Similar patterns have been observed in Potter Cove (Seefeldt et al. 2017), where following glacial retreat, a change in sedimentation rates led to the dominance of a single amphipod scavenging species, *Cheirimedon femoratus* (Pfeffer, 1888).

The assemblages of the two basins have some overlap in their biodiversity (as is exemplified by the eight shared morphotypes). However, the sampling stations and the two basins as a whole are heterogeneous in their species compositions.

Thus, we can observe some negative influence (possibly attributed to the disturbance in the DEA) on the scavenging amphipod community. This reduced biodiversity is reflected in the higher Simpson Index (*D*) for the CCZ (0.73) as compared to the DEA (*D* = 0.62; Table 3).

To explore whether this reduced diversity in the DEA was truly a result of the simulated disturbance, *D* was also calculated for each sampling station within each basin (Table 3).

In the CCZ, the APEI (C8) shows a moderate level of biodiversity ($D = 0.44$), indicating that it is not optimally-placed for representing the biodiversity of the scavenging amphipod community of the CCZ. Additionally, this pre-existing lower biodiversity (in comparison to the contractor claim areas), indicates that the APEI may not serve well as a refugium for amphipods post-disturbance. However, due to the fact that only one of the eight APEIs have been investigated thus far, this APEI along with the remaining eight APEIs would need to be (re-) sampled.

Within the DEA, the lowest biodiversities are observed at the site of the disturbance (D1) and south of it (D2; Table 3), indicating that the reduced biodiversity in the DEA could indeed be caused by the simulated disturbance in 1989 (Thiel, 1992).

The highest abundances in the DEA were collected from station D5 ($n = 717$); this station also has the highest Simpson Index within the DEA ($D = 0.61$) (Table 3). Side-scan sonar imaging shows a seamount range to the North West (NW) of the disturbed area (D1) (Appendix 3). Although the relief change is only 150m, the range extends laterally for several kilometres (SO242-1 Cruise report, 2016) hampering dispersal across barriers such as sills, canyons and ridges (Smith, et al. 2006; Blankenship et al. 2006; Etter et al. 2011). However, recent studies have shown that due to their mobile nature, geographic isolation alone would not pose a true barrier to bentho-pelagic amphipod species (Havermans, 2016; Ritchie et al. 2017), and thus, cannot explain why such a high number of large scavenging individuals was collected at station D5.

Community similarities

Scavenging amphipods are resilient and dispersive, but most importantly, they are highly mobile (Ingram and Hessler 1983; Lörz et al. 2018). Often driven by their search for erratic deposited feeding opportunities (Smith et al. 1989), they are probably less constrained by local environmental abiotic conditions and seafloor topography.

Beta diversity can be regarded as the dissimilarities in species composition between spatially different communities. As an indication for beta biodiversity, the NMDS (Figure 6) shows a significant separation in the similarity index between the two basins (ANOSIM $p = 0.002$). However, despite the dispersive and resilient nature of scavenging amphipods, their biodiversity appears to have been affected by the disturbance experiment as evidenced by the NMDS (Figure 6), where the disturbed area (D1) and the area closest to it (D2) are separated from the remaining three reference sites (D3, D4 and D5).

In the CCZ, stations C1, C2, C3, C4 and C5 form one cluster in the NMDS (Figure 6), and stations C6, C7 and C8 a second cluster. The CCZ is a geomorphologically very heterogeneous region, with seamounts of 200 m altitude running from north-south. A barrier of this height would be expected to affect sedimentation rates, nodule presence and currents. Furthermore, the difference in depth from the eastern edge (3950m) and the western edge (5150 m) is more than 1200 m. These combined factors very likely give rise to different trends in species composition (Glover, et al. 2015). However, since it has been established that bentho-pelagic amphipods are less sensitive to such barriers (Havermans, 2016), at this stage, other biotic (e.g. the productivity gradient) and abiotic factors causing this separation cannot be excluded as alternative explanations.

Dispersal and connectivity

Whilst the NMDS (Figure 6) illustrates a visual separation of the two basins, there is also some similarity in the amphipod fauna between the two areas, (as is obvious by the eight shared species), indicating that the dispersal extent for these eight species might range up to at least 3000 km. However, this hypothesis will need to be confirmed with subsequent molecular analyses.

Abyssal amphipods have been shown to be able to travel actively at speeds of almost 4 cm/sec (Laver, 1985), even at temperatures as low as 3°C (Kankaanpää et al. 1995). It is obvious that they are sufficiently strong to swim up weak currents since they can be found several hundred meters above the seafloor searching pelagically for mates (e.g. *Eurythenes gryllus* occurring up to 1800 m above the seafloor) (Thurston 1990; Havermans et al. 2013) or following food-falls (Baldwin and Smith 1987).

However, it is apparent that the dispersal of abyssal amphipods is not always contingent on current direction, but also on passive dispersal. Amphipods can also be carried passively over long distances by stronger currents e.g. (the circumpolar current of the Southern Ocean)

(Laver et al. 1985), but even weaker deep-sea currents have been suggested as a mechanism for deep-sea dispersal of amphipods (e.g. *Eurythenes gryllus* (Schüller and Ebbe 2007)). This coupled with their ability to follow odour-plumes (Ide et al. 2006; Premke, 2003), significantly increases the probability and extent of their dispersal (Conlan 1991; Highsmith, 1985). Specialist feeding adaptations for several species in our assemblages have been reported in Havermans & Smetacek, 2018). For example, the semi-tubular flap-like molars of the genera *Hirondellea* and *Eurythenes*, and the distendable foregut (*Abyssorchomene*) and midgut (*Paralicella*). It is not clear from our study in the absence of POC data for the areas of the trap deployments whether the biogeography of these specialised feeders is linked to the productivity gradients in these two basins.

The lack of a clear dispersal pattern is obvious from Figure 6, where station D2 is the station clustering closest with the CCZ basin in terms of species composition despite the fact that station D5 is geographically the shortest distance away from the CCZ.

Recent research on *Eurythenes gryllus* has demonstrated that it thrives in every ocean with a wide (pelagic – hadal) depth range. However, assumptions that individual morphotypes of this species belong to the same genetic lineage have been challenged (Havermans et al. 2013 & Havermans, 2016). Ritchie et al. (2016) demonstrated with microsatellite markers heterozygote deficiency in *Paralicella tenuipes* (Chevreux, 1908), which they attributed to cryptic diversity. It is likely that the connectivity of the eight species we observed as being shared between the CCZ and DEA (based on traditional morphological methods), can be explained by the presence of one or more cryptic amphipod species, which are being tested in our future molecular research.

Unfortunately, in the absence of data on deep-sea currents in the study area, especially between the CCZ and DEA, it is not yet possible to fully explain the drivers and mechanisms of amphipod dispersal between these particular basins.

The DisCOL Experimental Area as a proxy

Higher abundances of scavenging amphipods were collected from the CCZ (3932 individuals) as opposed to the DEA (2984 individuals). Yet, we have identified more morphotypes in the DEA (18) than in the CCZ (10), indicating that the DEA is more speciose, and thus, more biodiverse.

Although the DEA is more speciose, many of its morphotypes were collected in low abundances, with several of these being singletons or doubletons (collected from one or two sampling stations only). This is reflected in the rarefaction curves (Figure 4), which indicate thorough sampling in the CCZ with all but station C7 reaching asymptotes. In contrast, four stations in the DEA (D1, D2, D4 and D5) are unsaturated. This pattern suggests firstly that the less abundant species which are present at fewer stations only may not necessarily be rare species and secondly, that there could be as yet undetected biodiversity in the DEA. Therefore, the effects of mining impact could be even more pronounced than we observed in this study. However, as the seafloor environment is subject to seasonal fluctuations (Billett et al. 1983), it is hard to predict exactly what the effects will be at this stage.

Our preliminary (basin-scale) comparison of the scavenging communities of the two study areas shows that even if the DEA is a small-scale disturbance experiment, it is a very diverse area. Thus, the DEA is a well-chosen site for monitoring the impacts of disturbance and instrumental in its role as a proxy to assess impending mining activities in the CCZ.

Future research

At several stations in both basins, we collected amphipods in very high abundances (C1, C8, D3 & D5) (Table 2b). Since biotic production is contingent on the sinking flux of particles from the euphotic zone (Sweetman, 2017), the biodiversity differences at each of the thirteen stations could be driven by Particulate Organic Carbon (POC) or erratic whale-falls (Smith et al. 1989). However, not all feeding behaviour of scavenging amphipods is based on opportunistic or erratic availability of nutrients (Havermans & Smetacek, 2018). During future sampling campaigns, the POC of these areas should be monitored, along with experiments on different types of food-fall in addition to obtaining side-scan sonar and abiotic data. This will provide a more comprehensive view of the food types required for these species to thrive in the deep sea.

It is not clear from our results whether substrate type (i.e. nodule/non-nodule) has any effect on the amphipod communities (Smith and Demopoulos, 2003) since this kind of data is only available for stations D3 and D4. To answer this question, resampling of the study areas in combination with an Ocean Floor Observation System (OFOBS) (video/camera) is required.

Although our study only addresses the scavenging amphipod species longer than 15 mm, we already find indications for a disturbance effect in the DEA. It is obvious that scavenging amphipods are only one of several bentho-pelagic indicator groups. Other truly benthic groups such as sponges or less dispersive amphipods (e.g. collected by epibenthic sledge (EBS)) may demonstrate an even more pronounced impact of mining activities and should be investigated in future studies.

With the application of molecular techniques to identify cryptic species (Delić et al, 2017), more realistic estimates of biodiversity can be obtained (Schön et al. 2012), improving our current knowledge of the biodiversity of this area. If these improved estimates of biodiversity also include cryptic species, it is possible that the biological impact of manganese nodule mining on amphipod and other deep-sea faunal communities may turn out to be even higher.

Conclusions

In summary, this study on the scavenging amphipod community of two abyssal oceanic basins has demonstrated that amphipods are present in high abundances across the CCZ and DEA, with eight shared species and some species possibly being unique to their respective basin.

Our results have indicated that the simulated mining experiment probably had an impact on the biodiversity of these scavenging amphipods, as demonstrated by the low *D* of the DEA overall, at the disturbance site itself (D1), and the 60% dominance of *A. gerulicorbis* (Shulenberger & Barnard, 1976) in this region.

Given the scarcity of sampling and industry experience of marine habitats at these depths, the formulation of effective regulations is challenging (International Seabed Authority, 2017).

Nonetheless, our study provides the first results on possible effects of disturbance activities on the abyssal amphipod biodiversity of deep-sea basins.

Sample and data availability

Biological samples pertaining to this manuscript are stored at the Royal Belgian Institute of Natural Sciences, and the data discussed in the manuscript are submitted to PANGEA.

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Figure captions

Figure 1: Geographic locations of the two study areas, the Clarion-Clipperton fracture Zone (CCZ) (Northeast Pacific) and the DISturbance and re-COLonisation (DisCOL) Experimental Area (DEA) (Peru Basin, Southeast Pacific). There are nine Areas of Particular Ecological Interest (APEIs) in the CCZ region, which are illustrated by 400 x 400 km^2 white boxes. Grey boxes indicate the various contractor claim areas in the CCZ. We deployed eight amphipod traps across the CCZ, which is 16,000 km^2 and 7000 km wide, and five in the DEA, which encompasses 11 km^2 with a width of 4 km.

Figure 2: **Histogram showing the species assemblage for the scavenging community in the Clarion-Clipperton fracture Zone (CCZ) (black) and the DisCOL Experimental Area (DEA) (grey)**. The abundances of 17 morphotypes are shown.

Figure 3: Catch Per Unit Effort (CPUE) illustrating the correlation between sampling time and number of individuals collected.

Figure 4: Species rarefaction curves for each of the 13 trap stations across both areas, the Clarion-Clipperton fracture Zone and the DisCOL Experimental Area. Only individuals longer than 15 mm were considered here.

Figures 5a and b: Relative species abundances in the Clarion-Clipperton fracture Zone and the DisCOL Experimental Area. These abundances represent the longer than 15mm fraction of the scavenging amphipod community only.

Figure 6: NMDS plot showing the beta biodiversity (dis/similarities) for each of the thirteen amphipod trap sampling stations associated with the two basins, Clarion-Clipperton fracture Zone (CCZ) (black) and the DisCOL Experimental Area (DEA) (red). Data are supported by a low stress value of 0.01.

Table captions

Table 1: Station overview. Codes refer to the codes used in this paper in figures 1, 3, 4, 6 and Table 3. The original station code represents the cruise codes from (SO239 and SO232-1). Depth refers to water depth (m) on deployment. Nodule presence/absence information is known only for stations D3 and D4.

Table 2a: Overview of morphotypes across the Clarion-Clipperton fracture Zone (CCZ) and DisCOL Experimental Area (DEA).

Table 2b: Overview of morphotypes across the Clarion-Clipperton fracture Zone (CCZ) and DisCOL Experimental Area (DEA).

Table 3: Comparison of biodiversity calculated using the Simpson Index (*D*), for the Clarion-Clipperton fracture Zone and DisCOL Experimental Area, and *D* for each station is shown for further comparisons within these areas.

Appendix/Electronic Supplementary Information (ESM) captions

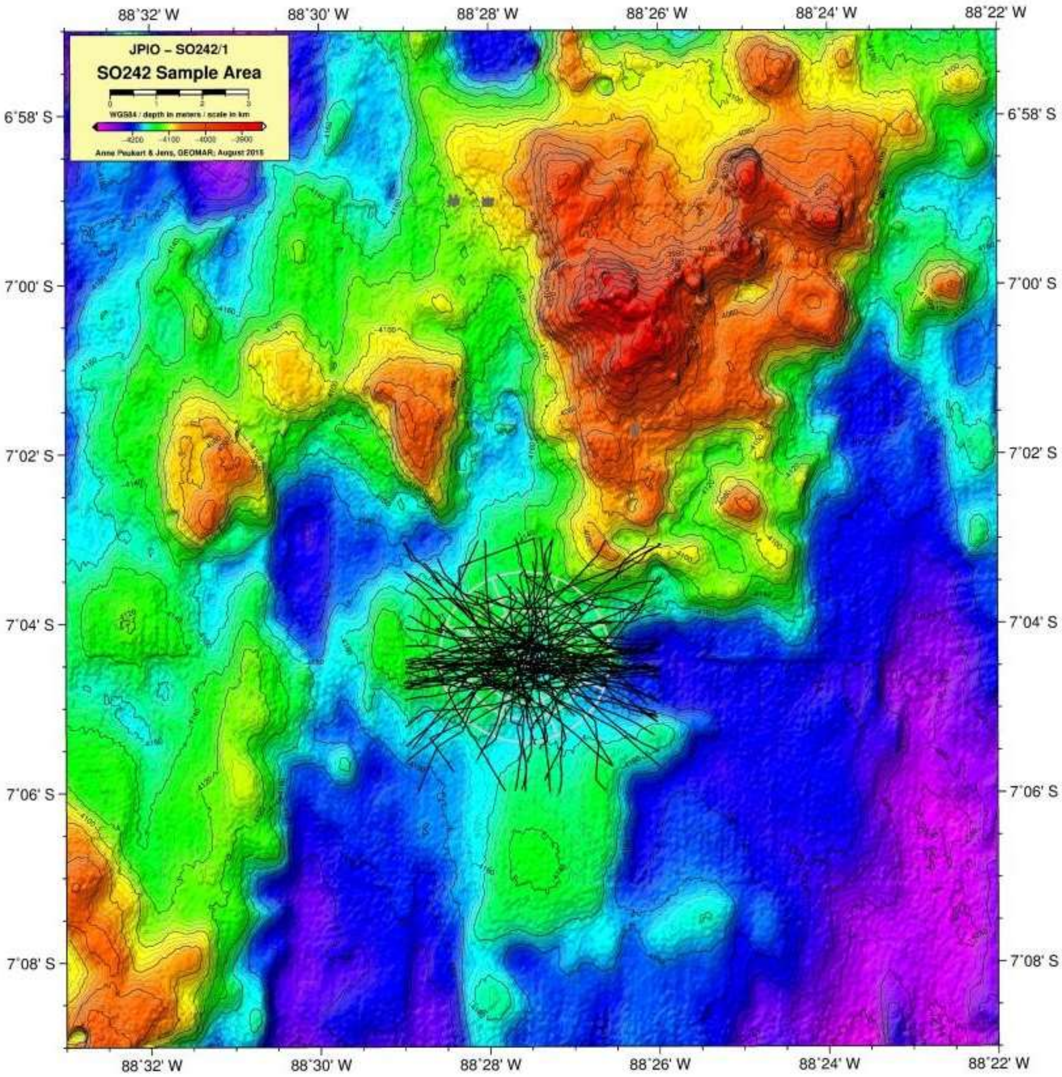
Appendix 1: Multibeam scan - Showing the location of the 78 track marks created by the plough harrow in the DisCOL Experimental Area to simulate manganese nodule extraction activity (D1)

Appendix 2 – Photograph showing the baited free-fall lander trap designed and deployed by RBINS.

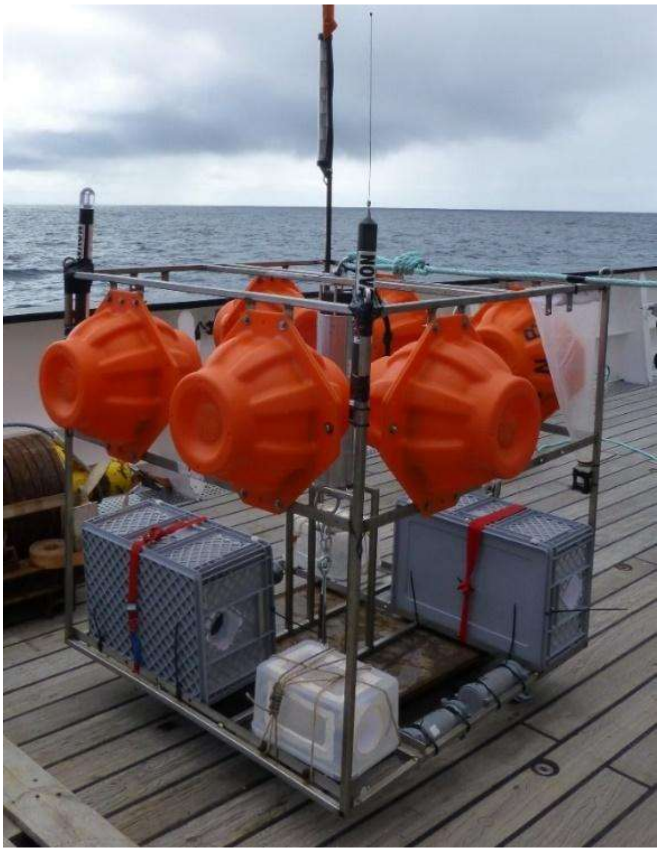
Appendix 3 - Side-scan sonar image of site D5 showing possible seamount barriers. View from NW (top) to SE (bottom). Contours are every 25 m. (Source: GEOMAR, 2015).

Appendix 4 – Calculation of alpha biodiversity used in this manuscript.

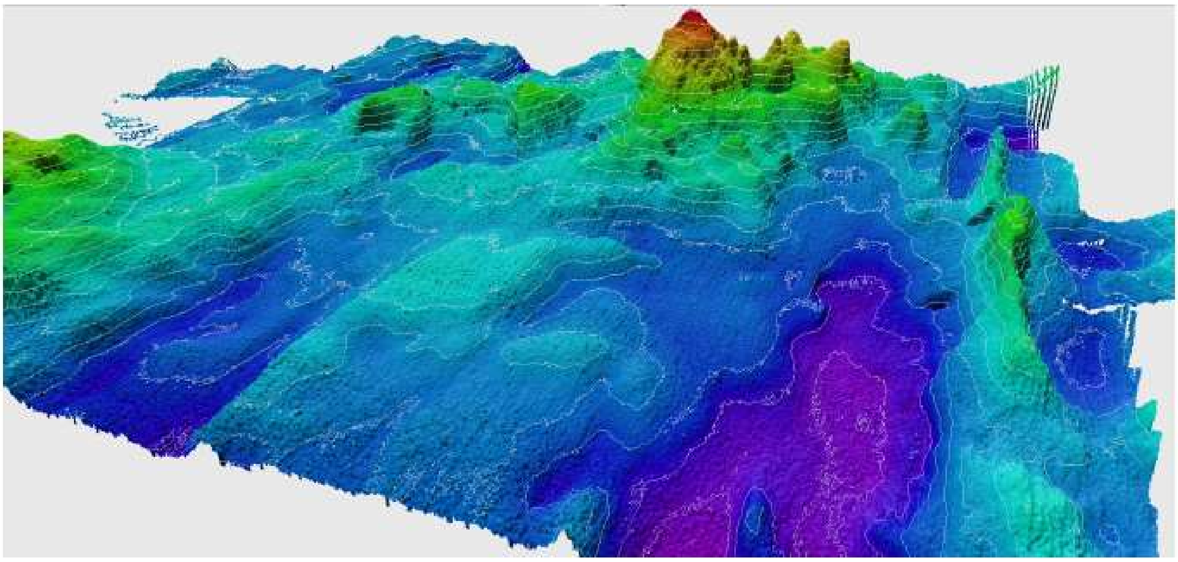
Appendix 1: Multibeam scan - Showing the location of the 78 track marks created by the plough harrow in the DisCOL Experimental Area to simulate manganese nodule extraction activity.



Appendix 2 – Photograph showing the baited free-fall lander trap designed and deployed by RBINS.



Appendix 3 - Side-scan sonar image of site D5 showing possible seamount barriers. View from NW (top) to SE (bottom). Contours are every 25 m. (Source: GEOMAR, 2015).



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Appendix 4 - Calculation of alpha biodiversity used in this manuscript.

Simpson Index

$$\text{Simpson Diversity Index (D)} = 1 - \frac{\sum n(n-1)}{N(N-1)} =$$

D = Diversity Index

n = number of individuals in each particular species

N = Total number in community

A high value of D indicates a high species diversity.