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*, 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 extremely 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 increased 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 the biogeography of deep-sea Amphipoda (Barnard 1961; Thurston 1990) and other invertebrates.

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

125 experiment to compare the biodiversity of this mining impact proxy to the undisturbed

126 reference areas. We discuss the possible implications of our findings; aiming to use them to

127 formulate recommendations regarding the pending deep-sea mining of manganese nodule

128 activities in the NE Pacific ecosystem.

129 **Material and Methods**

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131 *Study area*

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133 We investigated the amphipod communities of two oceanic basins (Figure 1); (i) the Clarion-

134 Clipperton Fracture Zone (CCZ, six million km^2, 7000 km wide), an economically important

135 manganese nodule field in the NE Pacific, comprising several different contractor claim

136 areas, (who to date, have exploration licences only), and nine designated Areas of Particular

137 Ecological Interest (APEIs) as designated by the International Seabed Authority (ISA)

138 (Lodge et al, 2014) and (ii) the DISturbance and re-COLonisation (DisCOL) Experimental

139 Area (DEA, 11 km^2, 4 km wide), a mining disturbance proxy (also containing manganese

140 nodules) in the Peru Basin in the SE Pacific. In 1989, the DEA sediment bed was artificially

141 disturbed using a plough-harrow to create 78 track marks. These are supposed to simulate the

142 type of disruption which would be caused by a commercial mining operation (Appendix 1)

143 (Thiel, 1992). This baseline study was a new approach in deep-sea risk assessment and is still

144 ongoing today, providing us with crucial data from this long-term ecological experiment.

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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

175 - D5; Figure 1, Table 1). In the CCZ, we sampled within three different contractor claim

176 areas (Table 1) to obtain a pre-disturbance baseline, and to then compare it with one of the

177 nine protected APEIs around the CCZ. In contrast, in the DEA, sampling was conducted once

178 within the disturbed area (D1), twice 10 km away (D2, D3) and twice 40 km away (D4, D5)

179 from D1 in four surrounding reference areas (see Figure 1).

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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, primarily 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. Also, all pelagic amphipod specimens were omitted which were accidentally caught when the sampling equipment was retrieved to the sea surface. 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). Individual-based rarefaction 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)

250   plot. The ANOSIM function in the vegan package of R (R Core Team, 2013; Taguchi & Ono,

251   2005) was used to test the statistical significance of the differences in species compositions

252   between the two study areas.

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Results

Basin biodiversity

In total, 6916 scavenging amphipods (> 15 mm) were collected from the thirteen trap deployments in the two study areas, representing nineteen 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*, *A. gerulicorbis*, *Eurythenes sigmiferus*, *Paralicella caperesca* and *Valettietta tenuipes*. A further 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 eighteen morphotypes. Six of these have been identified to the species level: *Abyssorchomene distinctus*, *A. gerulicorbis*, *Eurythenes sigmiferus*, *Paralicella caperesca*, *Parandaniexis mirabilis* and *Tectovallopsis regelatus*. A further five which have been affiliated to a species: *Eurythenes* sp. 2. aff. *gryllus*, *Eurythenes* sp. 4. aff. *magellanicus*, *Paracallisoma* aff. *alberti*, *Stephonyx* sp. nov. aff. *arabiensis* and *Valettietta* cf. *gracilis* and the remaining seven identified to at least an affiliated genus (Tables 2a and 2b).

There are eight morphotypes shared between the basins: *Abyssorchomene distinctus, A. gerulicorbis*, *Abyssorchomene* spp., *Eurythenes sigmiferus*, *Eurythenes* spp., *Paracallisoma* aff. *alberti*, *Paralicella caperesca* and *Valettietta* cf. *gracilis* (Figure 2).

Two morphotypes were found only in the CCZ (*Hirondellea* sp. & *Valettietta tenuipes*), and eight morphotypes were found only in the DEA (*Eurythenes* sp. 1-4, gen. aff. *Cleonardo, Parandaniexis mirabilis, Stephonyx* sp. nov. aff. *arabiensis,* and *Tectovallopsis regelatus*) (Table 2).

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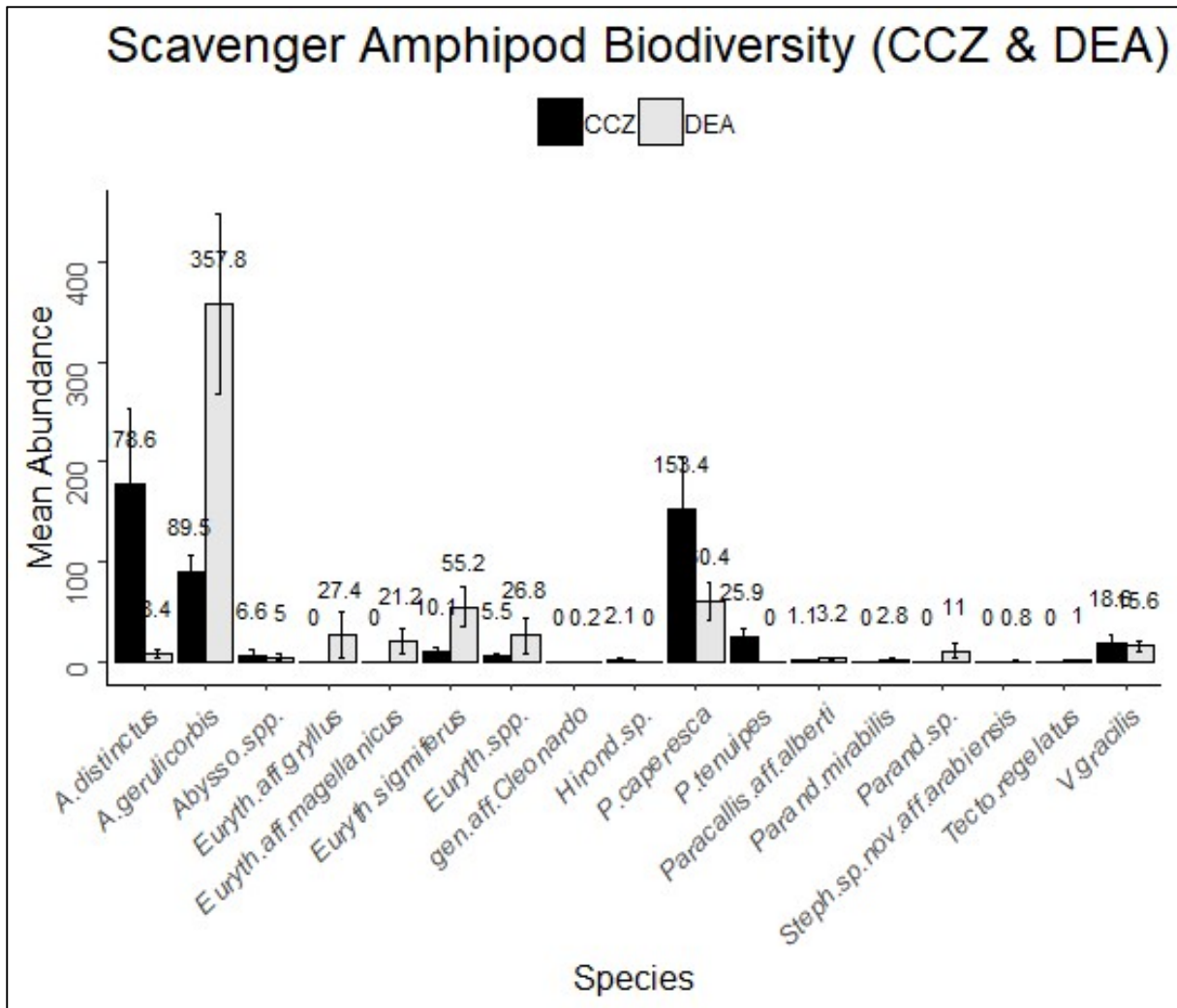
Figure 2: **Histogram showing the species assemblage for the scavenging community in the Clarion-Clipperton fracture Zone (CCZ) (black) and the DisCOL Experimental Area (DEA) (grey)**. The abundances of 17 morphotypes are shown.

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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, Yellow = 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
Tectovallopsis.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 normalise deployment times. The resulting Catch Per Unit Effort (CPUE) plot (Figure 3,) shows that, with the exception of C5, all stations in the DEA yielded higher abundances/hr than the CCZ. The highest numbers of individuals/hr were collected at station D3. Overall, there is a moderate negative exponential correlation with increasing deployment times (R = 0.67, p = 0.01).
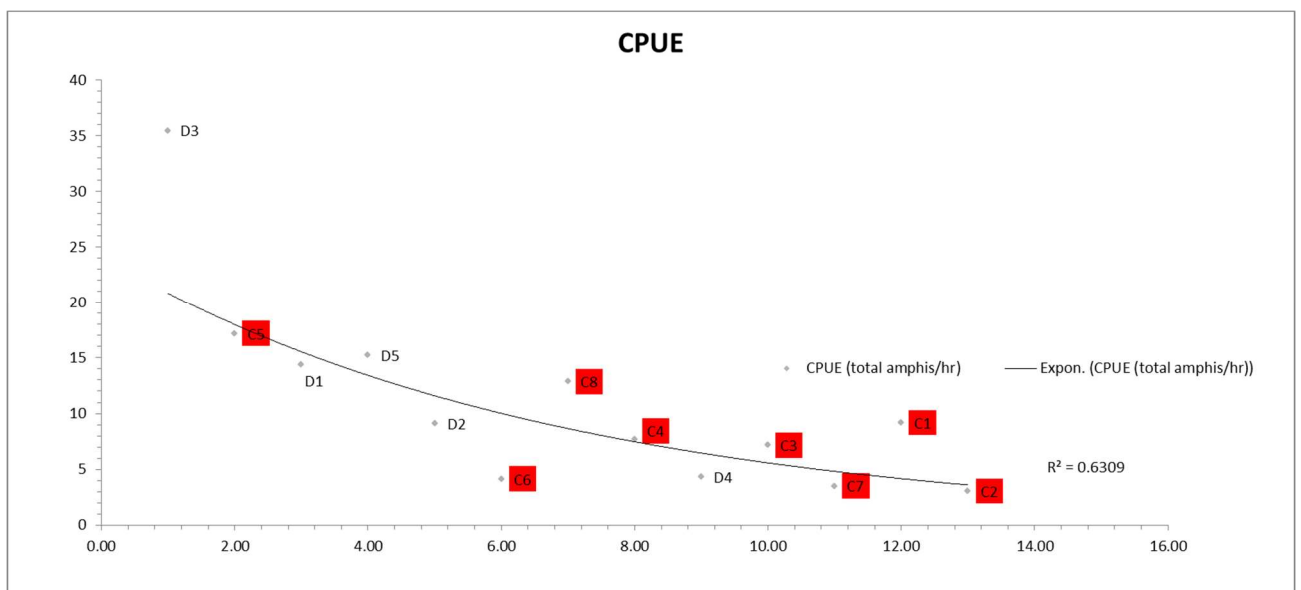
Figure 3: Catch Per Unit Effort (CPUE) illustrating the correlation between sampling time and number of individuals collected. Only the > 15mm fraction was included here to estimate number of collected individuals.

339   The rarefaction results (Figure 4) show that the curves for nine stations reach a plateau,

340   indicating that sampling effort was sufficient to assess diversity levels. These include all CCZ

341   stations except C7. In contrast, four of the five curves for the DEA (stations D1, D2, D4 and

342   D5) are unsaturated.

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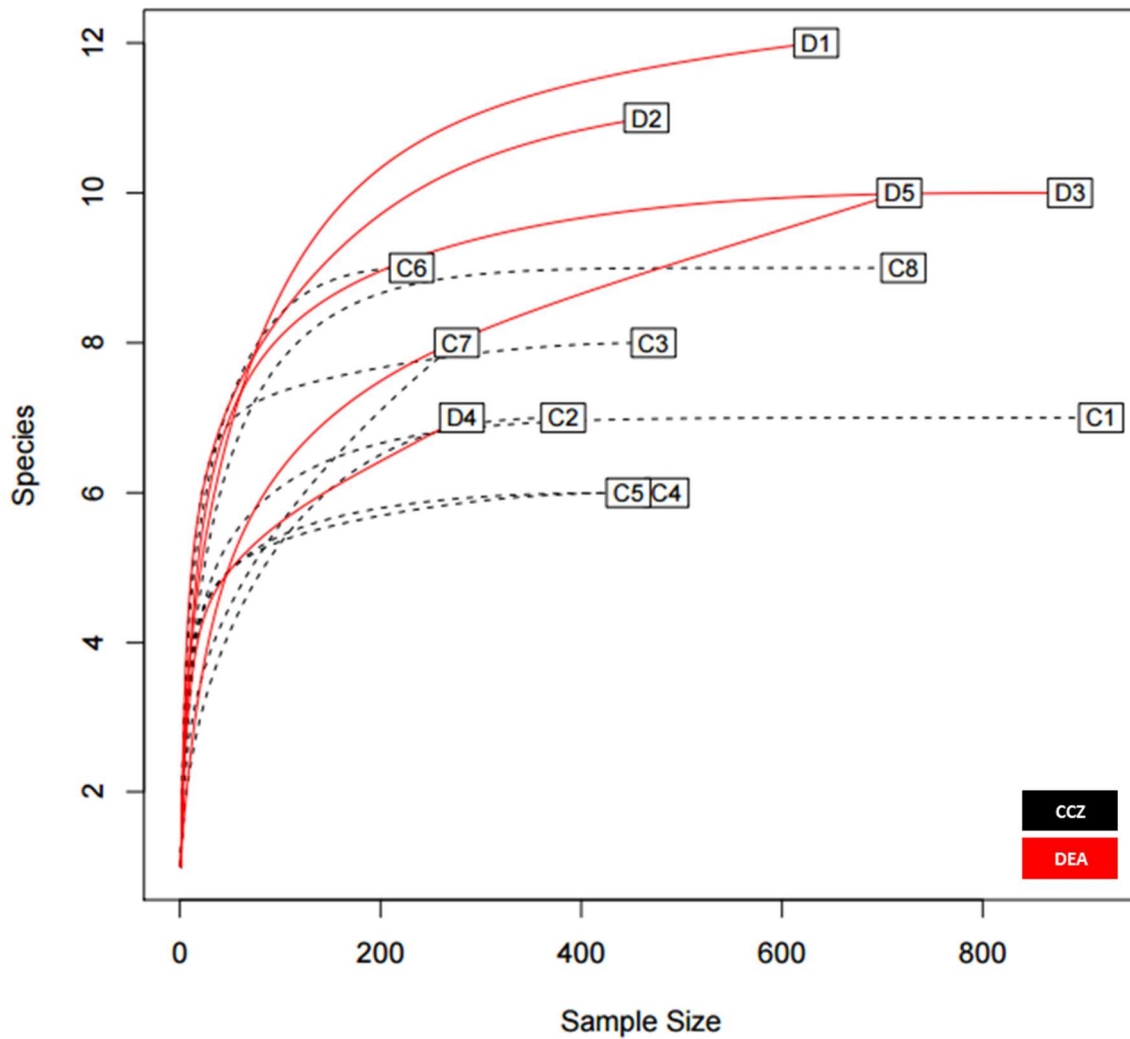
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345   **Figure 4: Species rarefaction curves for each of the 13 trap stations across both areas,**

346   **the Clarion-Clipperton fracture Zone and the DisCOL Experimental Area.** Only

347   individuals greater than 15 mm were considered here.

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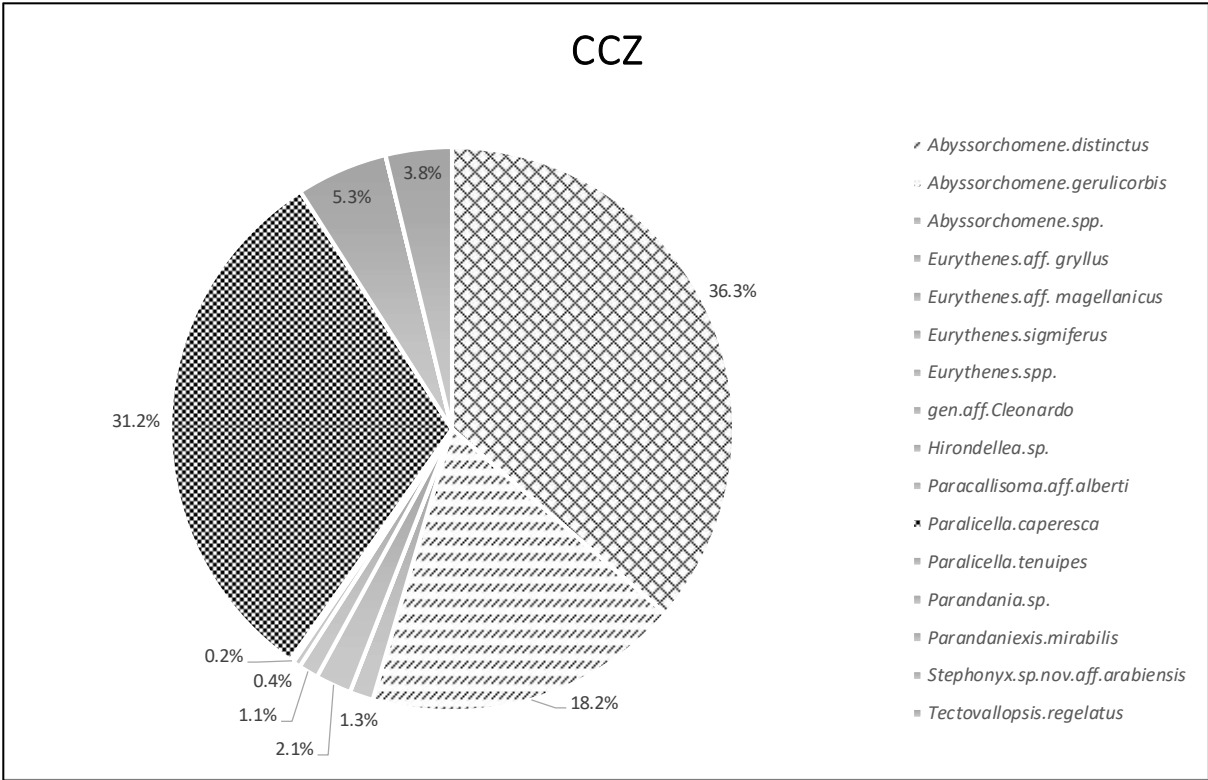
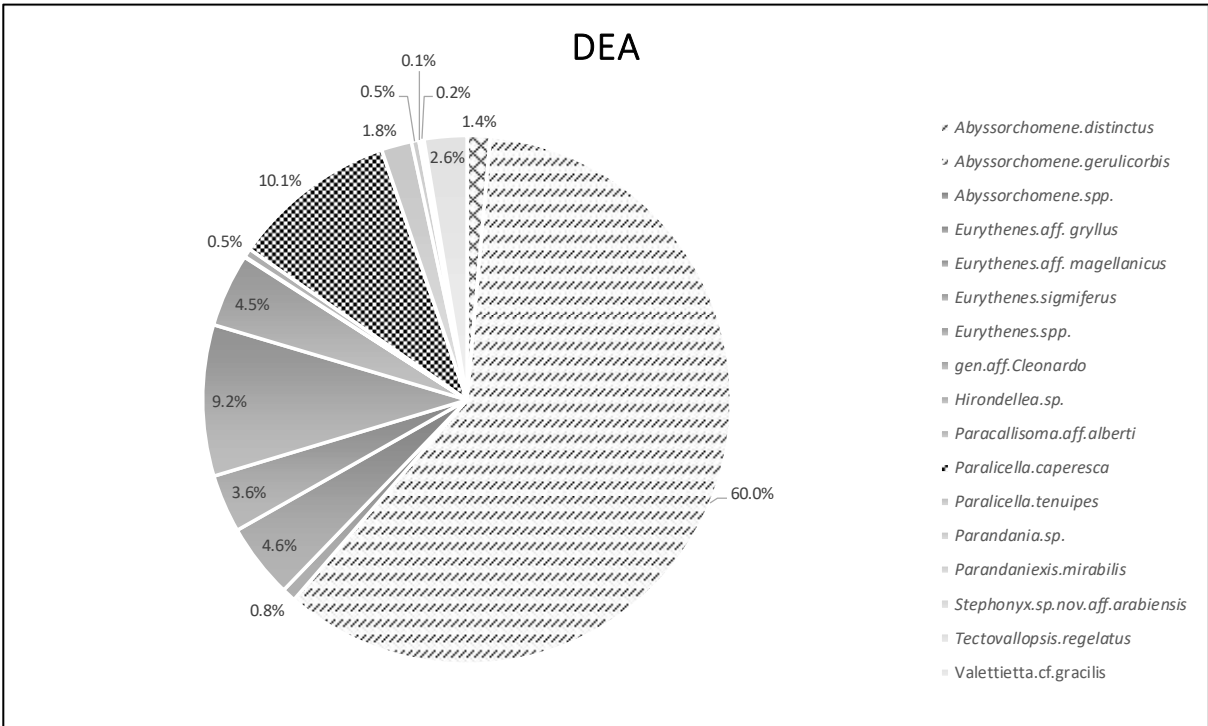
Biodiversity

Figures 5a and b show that the scavenging community in the CCZ is dominated by three species, *A. distinctus* (36%), *A. gerulicorbis* (18%) and *Paralicella caperesca* (31%), whereas, in contrast, the DEA scavenging community is dominated by a single species, *A. gerulicorbis*, accounting for almost 60% of all specimens. The Simpson Index (D) for the entire CCZ area is (with 0.73), higher than the 0.616 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).

394 **Figures 5a and b: Relative species abundances in the Clarion-Clipperton fracture Zone**

395 **and the DisCOL Experimental Area**. These abundances represent the greater than 15mm

396 fraction of the scavenging amphipod community only.

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398 **Table 3: Comparison of biodiversity calculated using the Simpson Index (D), for the**

399 **Clarion-Clipperton fracture Zone and DisCOL Experimental Area, and D for each**

400 **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

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406 *Species composition*

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408 The NMDS shows that the communities of the two basins are clearly separated (ANOSIM: p

409 = 0.002); Figure 6). The disturbed area in the DEA (D1) is showing a clear difference to the

410 four reference areas (D2 - 5). When the communities between the two basins are compared,

411 D2 appears to be most similar to the CCZ community, and more specifically to C6, C7 and

412 C8. The reliability of the data ranking is supported by a low stress value of 0.01.

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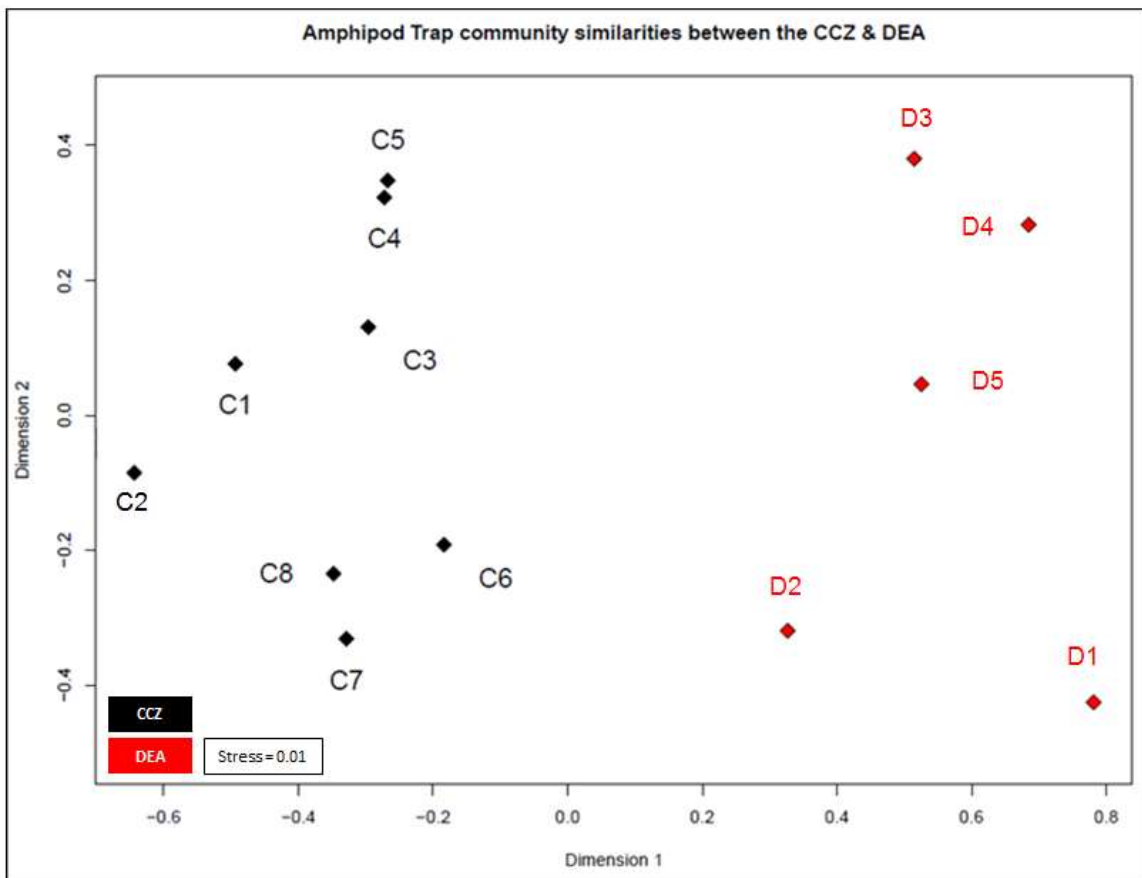
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415 **Figure 6 : NMDS plot** showing the beta biodiversity (dis/similarities) for each of the thirteen
416 amphipod trap sampling stations associated with the two basins, Clarion-Clipperton fracture
417 Zone (CCZ) (black) and the DisCOL Experimental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

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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 actually 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, Abyssorchomene gerulicorbis, Eurythenes sigmiferus, , Paralicella caperesca, Paralicella tenuipes Parandaniexis mirabilis & Tectovallopsis regelatus*) (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* in the CCZ, *Eurythenes* aff. *gryllus*, *Eurythenes* aff. *magellanicus*, *gen.* aff. *Cleonardo, *P*arandania sp., *Parandaniexis*

mirabilis, Stephonyx sp. nov. aff. *arabiensis,* and *Tectovallopsis regelatus* 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.

Catch Per Unit Effort

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 assume 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). This lack of correlation is supported by our findings for station C2 (with the shortest deployment time), which shows the highest Simpson Index of all thirteen stations ($D = 0.67$). Further evidence comes from the patterns visualised in Figure 3, which shows a correlation of R = 0.67 for Catch Per Unit Effort (CPUE) and deployment times.

Biodiversity within basins

Figures 5a & b show clearly that the DEA scavenging community has reduced abundances of all species including *A. distinctus* (1%) and *P. caperesca* (7%), and is now dominated by a single species, *A. gerulicorbis*, 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.*

Although the assemblage of the two basins has some overlap in its amphipod diversity (as is exemplified by the eight shared morphotypes), the sampling stations (and the two basins) are heterogeneous concerning species compositions of the subdominant and rarer species

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).

487 To explore whether this reduced diversity in the DEA was truly a result of the simulated

488 disturbance, D was also calculated for each sampling station within each basin (Table 3).

489 In the CCZ, the APEI (C8) shows a moderate level of biodiversity ($D = 0.44$), indicating that

490 it is not optimally-placed for representing the biodiversity of the scavenging amphipod

491 community of the CCZ. Additionally, this pre-existing lower biodiversity (in comparison to

492 the contractor claim areas), indicates that the APEI may not serve well as a refugium for

493 amphipods post-disturbance. However, due to the fact that only one of the eight APEIs have

494 been investigated thus far, this APEI along with the remaining eight APEIs would need to be

495 (re-) sampled.

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497 Within the DEA, the lowest biodiversities are observed at the site of the disturbance (D1) and

498 south of it (D2; Table 3), indicating that the reduced biodiversity in the DEA could indeed be

499 caused by the simulated disturbance in 1989 (Thiel, 1992).

500

501 The highest abundances in the DEA were collected from station D5 ($n = 717$); this station

502 also has the highest Simpson Index within the DEA ($D = 0.61$) (Table 3). Side-scan sonar

503 imaging shows a seamount range to the North West (NW) of the disturbed area (D1)

504 (Appendix 3). Although the relief change is only 150m, the range extends laterally for several

505 kilometres (SO242-1 Cruise report, 2016) hampering dispersal across barriers such as sills,

506 canyons and ridges (Smith, et al. 2006; Blankenship et al. 2006; Etter et al. 2011). However,

507 recent studies have shown that due to their mobile nature, geographic isolation alone would

508 not pose a true barrier to bentho-pelagic amphipod species (Havermans, 2016; Ritchie et al.

509 2017), and thus, cannot explain why such a high number of large scavenging individuals was

510 collected at station D5.

511

512 *Community similarities*

513

514 Scavenging amphipods are resilient and dispersive, but most importantly, they are highly

515 mobile (Ingram and Hessler 1983; Lörz et al. 2018). Often driven by their search for erratic

516 deposited feeding opportunities (Smith et al. 1989), they are probably less constrained by

517 local environmental abiotic conditions and seafloor topography.

518

519 Beta diversity can be regarded as the dissimilarities in species composition between spatially

520 different communities. As an indication for beta biodiversity, the NMDS (Figure 6) shows a

521   significant separation in the similarity index between the two basins (ANOSIM $p = 0.002$).

522   However, despite the dispersive and resilient nature of scavenging amphipods, their

523   biodiversity appears to have been affected by the disturbance experiment as evidenced by the

524   NMDS (Figure 6), where the disturbed area (D1) and the area closest to it (D2) are separated

525   from the remaining three reference sites (D3, D4 and D5).

526

527   In the CCZ, stations C1, C2, C3, C4 and C5 form one cluster in the NMDS (Figure 6), and

528   stations C6, C7 and C8 a second cluster. The CCZ is a geomorphologically very

529   heterogeneous region, with seamounts of 200 m altitude running from north-south. A barrier

530   of this height would be expected to affect sedimentation rates, nodule presence and currents.

531   Furthermore, the difference in depth from the eastern edge (3950m) and the western edge

532   (5150 m) is more than 1200 m. These combined factors very likely give rise to different

533   trends in species composition (Glover, et al. 2015). However, since it has been established

534   that bentho-pelagic amphipods are less sensitive to such barriers (Havermans, 2016), at this

535   stage, other biotic (e.g. the productivity gradient) and abiotic factors causing this separation

536   cannot be excluded as alternative explanations.

537

538   ***Dispersal and connectivity***

539

540   Whilst the NMDS (Figure 6) illustrates a visual separation of the two basins, there is also

541   some similarity in the amphipod fauna between the two areas, (as is obvious by the eight

542   shared species), indicating that the dispersal extent for these eight species might range up to

543   at least 3000 km. However, this hypothesis will need to be confirmed with subsequent

544   molecular analyses.

545

546

547   Abyssal amphipods have been shown to be able to travel actively at speeds of almost 4

548   cm/sec (Laver, 1985), even at temperatures as low as 3°C (Kankaanpää et al. 1995). It is

549   obvious that they are sufficiently strong to swim up weak currents since they can be found

550   several hundred meters above the seafloor searching pelagically for mates (e.g. *Eurythenes*

551   *gryllus* occurring up to 1800 m above the seafloor) (Thurston 1990; Havermans et al. 2013)

552   or following food-falls (Baldwin and Smith 1987).

553

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,* 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* 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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933

934

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 19 morphotypes are shown.

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

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 greater 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.

1028   **Appendix 2** – Photograph showing the baited free-fall lander trap designed and deployed by
1029   RBINS.

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1043   **Appendix 3** - Side-scan sonar image of site D5 showing possible seamount barriers. View
1044   from NW (top) to SE (bottom). Contours are every 25 m. (Source: GEOMAR, 2015).

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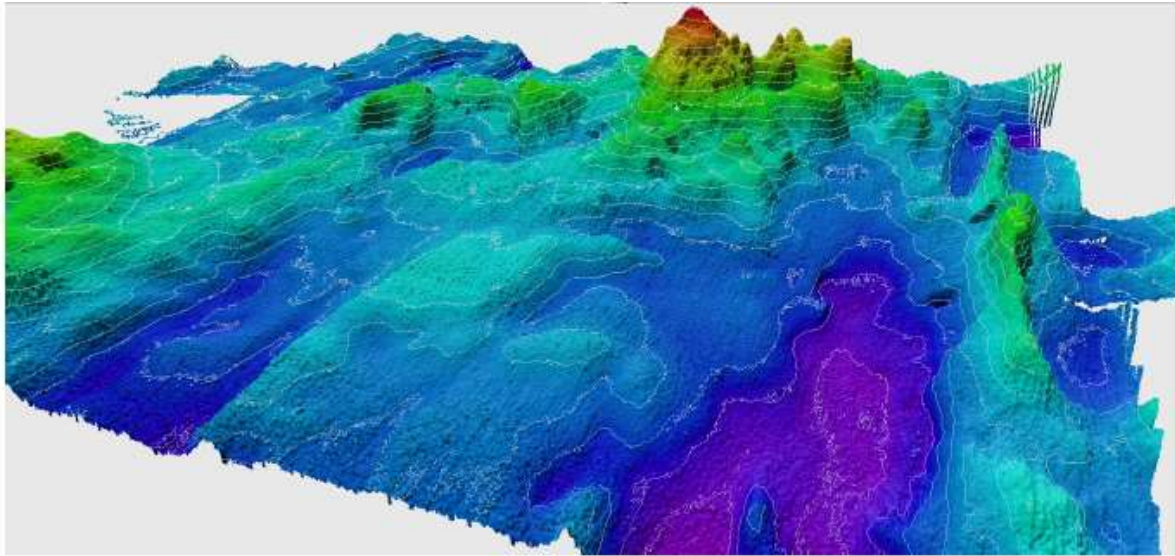
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1057   **Appendix 4** - Calculation of alpha biodiversity used in this manuscript.

1058   Simpson Index

1059 $$\text{Simpson Diversity Index (D)} = 1 - \frac{\sum n(n-1)}{N(N-1)} =$$

1060   D = Diversity Index

1061   n = number of individuals in each particular species

1062   N = Total number in community

1063   A high value of D indicates a high species diversity.

1064

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 very much for your useful feedback on our manuscript.

Below 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 blue, to indicate where the grammatical changes and suggested rephrasing of sentences, have been incorporated. Please be advised that all line numbers now refer to the revised PDF attached.

Finally, please see in table 1.0, a summary of comments which have been taken into account.

We thank both you and the journal for the useful comments and opportunity to submit a fully revised manuscript in the very near future.

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

Tasnim Patel.

Table 1.0 - Summary table showing the status of all comments addressed as of 18.12.18.

RC1	✓
RC2	✓
RC3(i)	✓
RC3(ii)	✓
RC4	✓

RC1: First, I miss details how individual statistical analyses were performed. Please, clarify them.

Our reply: The packages and functions used in R are stated for the rarefaction and NMDS analyses (e.g. Vegan, Bray-Curtis-index and testing of significance with ANOSIM). As suggested, we have now included the version of the Simpson Index equation used to calculate alpha diversity (D) in the appendix (4). If further clarification is required please let us know.

RC2: Second, I strongly encourage authors to revise their Catch Per Unit Effort analysis. My feeling is that dependent variable should not be divided by time, and that because of this modification authors miss an important result.

Our reply: We acknowledge your suggestion of "If you studied a simple relationship x = time, y = catched, I expect you will receive a 'log' shaped curve. From this curve, one could predict optimal time needed for setting the traps".

We looked into this, and from the literature we found that for this type of graph, the mass in (kg) should have been obtained at the time of collection. Our graph is based simply on the number of amphipods caught per hour, with the overall deployment time normalised. The purpose of the graph is to show that there is little to no relationship between sampling time and number of amphipods caught. We believe the optimal time for each deployment was identified, as in the field that after a certain time when the bait was finished, the amphipods began to eat each other.

RC3 (i): Third, I am not sure that D is best measure of alpha diversity. Please clarify / justify its use.

Our reply: We chose to use the Simpson Index (D) to measure alpha biodiversity over e.g. the Shannon-Wiener (H) or Sorenson measures, as they are similar, but D is more commonly used to express diversity in a location and is based on relative abundances of each species.

Secondly, we noted that we sampled many singletons/doubletons (species occurring at only 1 or 2 stations). The Shannon-Wiener index is more strongly influenced by the occurrence of these "rare" species. Therefore, these smaller numbers (e.g. in contrast to the > 500 individuals of *A. gerulicorbis*),

would mean that although they are similar methods, *H* is a less suitable method in this case. This is because *D* gives more weight to the evenness of species relative to the overall sample size. Therefore, it is looking at the basin and sites as a whole. Rather than being skewed by minute diversity changes (which we cannot rule out are an artefact of undersampling).

The Sorenson index was used, but not included as it is a similarity index which we have already shown using the Bray-Curtis similarity index in the NMDS plot.

RC3 (ii): All indices and relative numbers in this particular manuscript make the ms less intuitive and more complicated.

Our reply: We are unclear about this comment. If it is referring to the naming of sites as D1, D2, D3 etc, we considered to be clearer than SO241-1/33 etc. In figure 1.0, the geographical location of all sites mentioned are shown on a map.

If the comment refers to the Simspon Index as "*(D)*", we are happy to change this throughout to state the full name. We appreciate your clarification.

RC4: I found several small issues, that can be easily solved; they are labelled directly in the PDF.

Our reply: We attach with this reply a revised version of the manuscript. Please find highlighted in blue, the grammatical revisions suggested.

Other questions posed in the manuscript are answered below:

1. Please, state more details on procedure. At present, it is unclear how you constructed them.
   ➤ *Rarefaction curves were constructed using the "rarefy" function of the Vegan package in R.*

2. to compare the beta biodiversity, we estimated the variability of the community compositions per site.
   ➤ *Line 246 now reads – "to compare the beta biodiversity, we estimated the variability of the community compositions between sites."*

3. *I am not familiar with technique does it correct also for spatial nonindepndence?*

   ➤ *The NMDS ordination is a visualisation of a distance matrix based on the Bray-Curtis index, and correlations cannot be drawn between the points and community dis(similarities) e.g. between the CCZ and DEA. The subsequent ANOSIM is a*

statistical test to see if these species assemblages show a significant difference between the basins (which they do with p = 0.002).

We agree spatial autocorrelation is of importance in statistical tests in which you assume that your data are independent of each other (parametric tests). When they are spatially autocorrelated they are not independent. No such assumptions are made for NMDS, so spatial autocorrelation issues are not relevant here.

4. I would recommend that you simplify and unify the terminology species-morphospecies - morphotypes through the text.

  ➢ *All four instances of "morphospecies" have been changed to "morphotype".*

5. Please, label color codes (Table 2b)

  ➢ *This has been completed for Table 2b.*

6. Please clarfiy this plot. If I understand your work properly, two questions popup to me:
  1. Is relationship recovered when you analyze the two bioregions separately?
  2. I have concerns with dependent variable, which in your plot implicitly includes also independent variable. High values in the first hours of trapping may be result of low denominator. Levelling of after one day may mean that all individuals in vicinity were attracted, but the catch /hr is low because of high denominator. The number of cathced may increase, but slowly because individuals need time to reach the trap. Calculated per hour it means that time increases faster than individuals, and value is dropping. CPUE may change with respect to nominator and denominator..
  If you studied a simple relationship x = time, y = catched, I expect you will receive a 'log' shaped curve. From this curve, one could predict optimal time needed for setting the traps - and - in my opinion - this would be very useful information for future studies.

  ➢ *Please refer to our answer for your comment RC2.*

7. Perhaps state "negative exponential correlation"?

  ➢ *This has been changed. Line 322.*

8. This is likely related to previous plot. All D traps were set for a shorter period. If the traps were dropped for longer periods, they would reach the plateau. This is an application from my previous comment: one can expect saturated rarefaction if traps are set for roughly 6 hrs.

  ➢ *The rarefaction analysis is not related to the catch per unit effort. The rarefaction is an analysis of the number of species found (richness), relative to the total number of*

species found in the two basins. It is a visualisation of whether all species have been recovered or if more sampling would be required. The time of each deployment is not a factor in this analysis. Nonetheless, the residence time of each trap was standardised to approximately 48 hours (as is now shown in table 1).

9. The interpretation depends on which variation of D you used. It might be a good idea to define to which of many D you relay to. Only then you can state whether high D indicates high biodiversity.

   ➢ *The equation for the Simpson Index is now shown in appendix 4, in this version of the equation, a higher* D *is indicative of higher biodiversity.*

10. I find this division in 2 clusters partially arbitrary, other divisions into eg 3clusters are possible as well. I suggest you make a cluster analysis and check for the numebr of clusters if this is really needed. Otherwise simly cut this part of the sentence.

    ➢ *The authors agree. Line 408 now reads "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)."*

11. It seems a bit low number? Maybe write over 7000

    ➢ *Line 428 now reads "Over 7000 marine amphipod species have been found below 2000 m."*

12. I would be careful. Why you do not check also row numbers? Playing with indices (individuals / hr, D) is masking the real dynamics

    ➢ *The Simson Index was calculated based on the raw abundances which were simply transformed into relative abundances to show the percentages of each of the species in each basin. This was done, because some stations yielded far more individuals than others. The data was not manipulated to mask the raw numbers. Since the manuscript is not analysing biomass of each station, but biodiversity/assemblage patterns, the data was standardised from absolute abundances into relative abundances.*

13. Please consider also a recent paper in PeerJ, on predatrory amphipods: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5994337/

> *Thank you, the authors missed this paper. Line 514 now reads "Scavenging amphipods are resilient and dispersive, but most importantly, they are highly mobile (Ingram and Hessler 1983; Lörz et al. 2018)."*

14. As stated above, it is hard to directly compare DEA and CCZ because of different deployment times and different sampling efforts. Rearefactions would be needed for a thorugh comparisons of the two regions.

> *Clarifications of the deployment times are now provided in table 1. It shows that we tried to standardise the trap residence times, which again, are not related to the overall cruise length. Rarefaction is an estimate of the species richness and not related to the sampling effort and time.*

Response to Reviewer 2: Dr. Charlotte Havermans

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

Ref: bg-2018-347

Journal: BioGeosciences

Dear Dr Havermans,

We would like to thank you very much for your useful feedback on our manuscript.

Below you will find a point by point reply on how we have addressed the suggested comments in our manuscript. Attached also, is a copy of the manuscript with track changes highlighted in blue, to indicate where the grammatical changes and suggested rephrasing of sentences have been incorporated. Please be advised that all line numbers now refer to the revised PDF attached.

Finally, please see in table 1.0, a summary of comments which have been taken into account.

We thank both you and the journal for the useful comments and for the opportunity to submit a fully revised manuscript in the very near future.

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

Tasnim Patel.

Table 1.0 - Summary table showing the status of all comments addressed as of **18.12.18.**

RC1	✓
RC2(i)	✓
RC2(ii)	✓
RC3	✓
RC4	✓
RC5	✓
RC6	✓
RC7	✓
RC8(i)	✓
RC8(ii)	✓
RC9	✓
RC10	✓
RC11(i)	✓
RC11(ii)	✓
RC12	✓
RC13	✓
RC14	✓
RC15	✓
RC16(i)	✓
RC16(ii)	✓
RC17	✓
RC18	✓
RC19	✓
RC20	✓
RC21	✓
RC22	✓
RC23	✓
RC24	✓
RC25	✓
RC26	✓

RC1: Older views on dispersal and connectivity have been challenged by Havermans & Smetacek (2018), including updated discussions on biogeography and barriers to dispersal. In this context, I would also suggest to include, where possible, more information on the ecological roles of the species found. Of several species, an update on their feeding ecology has been given in the aforementioned review, and it would be of particular interest if the authors could compare also the type of scavengers between the different regions: e.g. omnivores with more specialized carrion feeders. This, combined with information on the productivity of the regions, would improve the discussion in view of the emphasis on bottom-up factors influencing species diversity of amphipod scavengers.

Our reply: Line 554 until 584 now includes a reference to the ecology and feeding adaptations of certain species. We cannot comment whether this follows the productivity gradient, but hope to take samples of POC during the next cruise in 2019. The paragraph now reads "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."

RC2(i): Recent molecular studies have also brought ample information on dispersal and connectivity of genera such as *Eurythenes* (Havermans et al.) and *Paralicella* (Ritchie et al.). These works would be better suited than the older works as they would place the discussion in a much more relevant framework.

Our reply: Line 554 until line 606 show an extended discussion including the studies of Havermans et al., 2013, Havermans, 2016, Ritchie, 2016, Ide et al. 2006 and Premke, 2003.

RC2(ii): Also recent work on shallow-water scavenger amphipods is interesting in this context (Seefeldt et al. 2017), where one particular species dominated an area impacted by sedimentation from glacier retreat. Hence, particular species may be more flexible and a comparison with the dominant species found here in the disturbed abyssal region may be particularly interesting.

Our reply: Line 471 now reads "Figures 5a & b show clearly that the DEA scavenging community has reduced abundances of all species including *A. distinctus* (1%) and *P. caperesca* (7%), and is now dominated by a single species, *A. gerulicorbis*, 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*."

RC3: Why not refer directly to the abyssal deep sea at the start of the introduction (as normally the deep sea includes also the bathyal zone).

Our reply: Line 59 now reads "The abyssal deep sea…"

RC4: Moreover, since this paper exclusively refers to depths below 3500m, I would leave out fishing as an exploitation of resources in the abyss (Line 98), as this is not relevant for abyssal fauna.

Our reply: Line 97 now reads "demand for exploitation of deep-sea resources e.g. rare earth element (REE) extraction"

RC5: I would rather extend in this paragraph on presenting the region studied as one of interest for deep-sea mining.

Our reply: Line 118 now reads "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."

RC6: For the CCZ this is mentioned in the methods, but it is not clear whether the DISCOL area is as well situated in an area where nodules occur, and potential deep-sea mining activities have been considered. This information is needed to grasp the context of your recommendations.

Our reply: Line 124 now reads "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."

RC7: Line 108: The authors state that knowledge on the biogeography of Amphipoda is still limited but to underpin this, references from the sixties and the nineties are cited that are no longer up to date. With molecular studies we have now a much better view on the actual species distributions. Therefore I suggest to consult recent literature and discuss these findings. For Eurythenes (the most species-rich genus found by the authors in this study), recent studies have shown that some species are unique to

particular habitats whilst others widespread. This already indicates the presence of a unique deep-sea fauna that may be impacted by anthropogenic activities.

Our reply: Line 110 onward has been updated to include references to Havermans, 2016 and Narahara-Nakano et al., 2017.

"Although recent morphological and molecular studies have shed important 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 data published 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 post-anthropogenic impact. Ultimately this impedes ecologists from providing advice on sustainable deep-sea mining practices, thus, underpinning the need for this dedicated deep-sea ecosystem research."

RC8 (i): Some information is missing. Was the bait mixture used exactly the same at the different localities?

Our reply: Line 168, now reads "baited for each station with an 800 g mixture of mackerel, squid and shrimp."

RC8 (ii): Which were the deployment times for each station? I suggest to add sampling dates and deployment times to Table 1. Particularly the latter are needed to interpret the subsequent results.

Our reply: We have added trap residence times to Table 1.0. We respectfully feel that adding the date of deployment will not allow us to draw any biological interpretations and clutter the table which is already quite text heavy.

RC9: The authors mention that all pelagic amphipods were omitted from their studies. These "pelagic" amphipods were certainly swarming or feeding on the bottom - many hyperiids are known to do so (Vinogradov) and to my belief several of those may spend a part of their life cycle near the seafloor. It would be interesting to present these findings of the remaining amphipods, as this fauna may well also be impacted by seafloor disturbances, and therefore I do not see the straight differentiation the authors make between the so-called "benthic" species (which actually are bentho-pelagic), and "pelagic" species. The species studied here are indeed not entirely benthic: Eurythenes can be found thousands of meters above the seafloor. I have recently deployed pelagic baited traps, attempting to catch scavengers in the water column, however as the traps could not be kept immobile due to wave action, it was impossible for scavengers to enter the traps. Therefore, I am convinced the pelagic species the authors refer to, were not caught on the way up to the surface, but entered the trap when it was still positioned on the seafloor and must be spending part of their life in this habitat.

Our reply: Thank you for this important distinction. As suggested, the terminology has been changed from "benthic" to "bentho-pelagic" throughout. The authors have omitted genera such as Pseudotiron, which is known up to 2250m. The authors have revisited the final species list, and no hyperiids were omitted.

RC10: Results and Figure 2. It is not clear to me which species are referred to under Eurythenes spp. nov, that were shared between the basins. E.sp 1 and 3 are not discussed in the text. I suggest to clarify this in the results part.

Our reply: For the 3 categories; *Eurythenes* sp. 1, 3 and spp. nov, determinations will require an extensive, in depth re-examination beyond the scope of this manuscript which is reporting on scavenger biogeography. Therefore, these 3 categories have been pooled as *Eurythenes* spp. Figure 2, Table 2 and Figures 5a and 5b have been updated.

RC11(i): Did you find consistent differences with the specimens of the so-called "aff." species (e.g, gryllus and sp. 2)?

Our reply: We did find consistent differences between *Eurythenes* aff. *gryllus* and aff. *magellanicus*. Please refer to our reply in RC10.

RC11(ii): As mentioned before, information on deployment times are missing (line 317).

Our reply: Line 317 now reads "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…"

RC12: Which trap station had the longest residence time? The only information given is that for the CCZ it was twice as long (only in line 478). It would help to interpret the results in the light of findings showing different species arriving at different times after deployment. Therefore this bias could have influenced the results not only in abundances but also the part of the scavenger guild that has been attracted.

Our reply: All trap residence times have been added to Table 1.0. The time of arrival at the bait after deployment is unfortunately not known, due to the fact that the deep-sea camera we intended to use was damaged during SO242-1.

RC13: What is known about the current speeds in the different areas? This could heavily influence the directionality and reach of the odour plume that attracted the scavengers to the bait and therefore current data may be needed to interpret the differences between sites.

Our reply: We agree that current speeds will impact the odour plume transport and extent. However, information on deep-sea currents is extremely limited. Data from the Sonne research cruise SO239 is still being analysed by colleagues at NIOZ. Therefore, we feel that speculations at this stage on the correlation between deep-sea currents, and scavenger attraction to bait are not prudent to the discussion, which is addressing the community assemblage.

A sensor array network to detect the directionality of deep-sea currents in the Eastern CCZ is planned for the Sonne cruise SO268 legs 1 & 2 (February - May 2019). We hope that the high-resolution data can be utilised for such a study in a follow-up manuscript.

RC14: In the discussion, references are limited to older studies (e.g. line 457) whereas we know so much more now about the true distributions deep-sea amphipods due to studies combining morphology and genetics. An updated discussion on biogeography in view of these recent works is needed. Both Eurythenes as well as Paralicella have been studied now with molecular markers which would be of relevance here.

Our reply: Cryptic diversity in these basins is most likely prevalent. The authors acknowledge the molecular studies of Havermans (2013;2016) and Ritchie (2016), and state that a manuscript is in preparation with genetic data. Line 572 now reads "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*, which they attributed to cryptic diversity. It is likely that the connectivity of the nine 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."

RC15: How can you update the bathymetry of species that are listed as aff. gryllus etc? I would leave these out until further morphological and molecular investigations allow to confirm the preliminary identification.

Our reply: Species listed as aff. have been removed from this statement. Line 445 now reads **"Here, we provide new data for the known bathymetric range of the seven amphipods which we have identified to species level (*Abyssorchomene distinctus, Abyssorchomene gerulicorbis, Eurythenes sigmiferus, Paralicella caperesca, Parandaniexis mirabilis, Tectovallopsis regelatus & Paralicella tenuipes*) (Table 2b)."**

RC16(i): Line 540-541: Not only in polychaetes (reference cited) but also for deep-sea amphipods this has been shown (e.g. vertical species segregation in deep-sea canyons, on seamounts and in trenches).

Our reply: Line 506 now reads "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, the resulting geographic isolation alone would not pose a true barrier to these bentho-pelagic species (Havermans, 2016), and thus, cannot explain why such a high number of large scavenging individuals was collected at station D5."

RC16(ii): The paragraph on dispersal and connectivity also lacks a comparison with recent studies.

Our reply: The authors have extended this section as suggested in RC2(i).

RC17: As shown in a population genetic study of *Paralicella*, species can be widespread over thousands of km but gene flow and hence dispersal may be restricted between particular geographic populations. Hence the statement in Line 549 that dispersal occurs over more than 3000 km cannot be confirmed here and this sentence needs to be rephrased.

Our reply: Line 542 now reads "indicating that the dispersal extent for these eight species might range up to at least 3000 km. However, this will need to be confirmed with subsequent molecular analyses."

RC18: Line 552-559: The likelihood of passive vs. active dispersal has now been revised by Havermans & Smetacek 2018. Arguments are given that amphipods being carried with currents is unlikely as they need to swim up current to detect an odour plume and locate food. Therefore, most amphipods (not only lysianassoids) are able to swim upcurrent.

Our reply: Line 554 onward has been revised to indicate that although passively dispersed, that active dispersal is likely the predominant method in play here.

"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).

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.

RC19: The reference cited here for swimming speeds does not refer to abyssal amphipods as mentioned, therefore I would refer to Ikeda et al. or Laver et al. who conducted ample work on swimming speeds of lysianassoids. This would be of more relevance here than the argument used by the authors in Line 556-557.

Our reply: Line 547 now reads "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)."

RC20: Bottom currents are horizontal and therefore swimming upwards into the water column does not prove that amphipods can swim upcurrent. In the same line, the extremely sluggish currents of the abyss, in particular in these regions, are very unlikely to carry along large dispersive amphipods, mentioned in line 565-566 (see also reference cited above for more discussion). This part could be omitted or rephrased.

Our reply: Lines 554 onward have been extensively rephrased in answer to your comment RC18.

RC21: I suggest to rather discuss the species diversity in view of the feeding resources available in the different regions as well as the different topographic features detected that could promote accumulation of sinking particles and food falls in certain regions more than others, and hence allow a more diverse scavenging guild.

Our reply: We feel that since sufficient data were not recorded on the topographic features at each of the 13 sampling stations (due to camera damage), we cannot confidently draw a link between topography and the community in this manuscript. In a follow-up cruise to the CCZ planned for 2019, we aim to use video guided sampling of scavenging amphipods.

RC22: Line 511-518. In the recent study of *Eurythenes*, sills and ridges have been shown not to be adequate barriers for dispersal of deep-sea amphipods. However, particular conditions linked to seamounts have been pointed out to have promoted differentiation, a link to these findings may be appropriate here.

Our reply: Line 533 now reads **"**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."

RC23: Line 524-525: confirming the findings of Havermans et al. 2016 and Ritchie et al. 2017 on abyssal and hadal amphipods respectively.

Our reply: Line 506 now reads "However, recent studies have shown that due to their mobile nature, the resulting geographic isolation alone would not pose a true barrier to these bentho-pelagic 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."

RC24: Line 614: cryptic amphipod species have also been found in the deep sea, which would be more relevant than comparing with terrestrial or freshwater studies mentioned here.

Our reply: This section has been extensively rephrased to include references to polymorphic amphipods in the deep sea, as per your RC20.

RC25: Line 523: the recent review mentioned before demonstrates that feeding opportunities may not be so erratic at all as previously emphasized. In this view also, there may well be a much wider scope of food for scavengers to thrive on than the POC or whale falls mentioned in line 599. Monitoring of different types of food falls could give much more information on the scavenger diversity.

Our reply: Line 617 onward has been extended to include the review by Haverman & Smetacek, 2018.

"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.

RC26: Finally, in line 608 as well as before the authors classify the scavengers studied as benthos but it is well known that several of the species here are bentho-pelagic. Therefore I would change this throughout the manuscript. This actually makes it even more interesting, because if mining activities

can impact bentho-pelagic species, not bound to the seafloor for food supply, it will be even more so for the true benthic amphipods, which would be less mobile and dispersive and could less easily recolonize affected habitats.

Our reply: References to benthic amphipods have been changed to "bentho-pelagic" as per your comment RC9.