Response to Reviewer 2: Dr. Charlotte Havermans

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 already, and those requiring a significant extension of the discussion or re-examination of the specimens of *Eurythenes*, which will be completed by November 16th.

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, and those pending as of 19.10.18. Comments requiring an extension of the discussion and re-examination of material, etc (RC1, RC9, RC10, RC11(i), RC13 and RC14 will be completed by 16th November.

| RC1 | ✓ |
| RC2(i) | ✓ |
| RC2(ii) | ✓ |
| RC3 | ✓ |
| RC4 | ✓ |
| RC5 | ✓ |
| RC6 | ✓ |
| RC7 | ✓ |
| RC8(i) | ✓ |
| RC8(ii) | ✓ |
| RC9 | TBC |
| RC10 | TBC |
| RC11(i) | TBC |
| RC11(ii) | ✓ |
| RC12 | ✓ |
| RC13 | TBC |
| 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: An extension of the discussion using the papers suggested will be completed by mid-November.

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.


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 499 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 61 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 99 now reads “demand for exploitation of deep-sea resources e.g. hydrocarbon/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 121 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 127 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 115 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 171, 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. However, we plan to revisit the final species list, and recheck the known depth ranges of all omitted pelagic species by the middle of November.

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: We intend to re-examine the material of all Eurythenes species in the coming weeks. We aim to specify exactly how many possible new species we have and re-confirm that the shared species are indeed the same between the two basins. We thank you for your patience.
RC11(i): Did you find consistent differences with the specimens of the so-called “aff.” species (e.g., gryllus and sp. 2)?

Our reply: As mentioned in our reply to RC10, we would like to take this opportunity to re-examine all the material of Eurythenes.

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

Our reply: Line 330 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 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: An extension of the discussion using the papers suggested will be completed by mid-November.

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 473 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 signiferus, Paralicella caperesca, Parandaniexis mirabilis, Tectovallopsis regelatus & Valettietta 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 533 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 benthic-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 571 now reads “indicating that the dispersal extent for these nine 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 583 onward has been revised to indicate 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 576 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: Line 576 - 608 has 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 station (due to camera damage), we cannot draw a confident 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 562 now reads “However, since it has been established that benthic-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 534 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 benthic-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 637 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.