**Ecology of a polymetallic nodule occurrence gradient: implications for deep-sea mining**

| Journal: | *Limnology and Oceanography* |
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| Manuscript ID | LO-18-0366 |
| Wiley - Manuscript type: | Original Article |
| Date Submitted by the Author: | 12-Sep-2018 |
| Complete List of Authors: | Simon-Lledó, Erik; National Oceanography Centre Schoening, Timm; Helmholtz-Zentrum fur Ozeanforschung Kiel Benoist, Noemie; National Oceanography Centre, Bett, Brian; National Oceanography Centre, Ocean Biogeochemistry & Ecosystems Huveene, Veerle; National Oceanography Centre, Jones, Daniel; National Oceanography Center, Ocean Biogeochemistry and Ecosystems |
| Keywords: | biodiversity, abyssal plain, megafauna |

**Abstract:**

Abyssal polymetallic nodule fields constitute an unusual deep-sea habitat. The mix of soft sediment and the hard substratum provided by nodules increases the complexity of these environments. Hard substrata typically support a very distinct fauna to that of seabed sediments, and its presence can play a major role in the structuring of benthic assemblages. We assessed the influence of seafloor nodule cover on the megabenthic ecology of a marine conservation area (Area of Particular Environmental Interest 6, APEI6) in the Clarion Clipperton Zone (3950-4250 m water depth) using extensive photographic surveys from an autonomous underwater vehicle. Variations in nodule cover (1-20%) appeared to exert statistically significant differences in faunal standing stocks, some biological diversity attributes, faunal composition, functional group composition, and the distribution of individual species along the nodule cover gradient. The standing stock of both the metazoan fauna and the giant protists (xenophyophores) doubled with a very modest initial increase in nodule cover (from 1 to 3%). Notably, faunal density determined by sample-based rarefaction, was positively correlated with nodule cover, while taxon richness, determined by individual-based rarefaction, was invariant (c. 60 taxa among 500 individuals). Faunal composition varied continuously along the nodule cover gradient. We discuss these results in the context of potential seabed-mining operations and the associated sustainable management and conservation plans. We note in particular that successful conservation actions will likely require the preservation of areas comprising the full range of nodule cover and not just the low cover areas that are least attractive to mining.
Ecology of a polymetallic nodule occurrence gradient: implications for deep-sea mining

Erik Simon-Lledó¹,², Timm Schoening³, Noemie M. A. Benoist¹², Brian J. Bett¹, Veerle A. I. Huvenne¹, Daniel O. B. Jones¹

¹ National Oceanography Centre, University of Southampton Waterfront Campus, European Way, Southampton, UK
² Ocean and Earth Science, University of Southampton, National Oceanography Centre, University of Southampton Waterfront Campus, European Way, Southampton, UK
³ GEOMAR Helmholtz Centre for Ocean Research, Wischhofstraße 1-3, Kiel, Germany

Keywords: abyssal plains, conservation biology, megafauna, biodiversity, CCZ, NE Pacific
Abstract

Abyssal polymetallic nodule fields constitute an unusual deep-sea habitat. The mix of soft sediment and the hard substratum provided by nodules increases the complexity of these environments. Hard substrata typically support a very distinct fauna to that of seabed sediments, and its presence can play a major role in the structuring of benthic assemblages. We assessed the influence of seafloor nodule cover on the megabenthic ecology of a marine conservation area (Area of Particular Environmental Interest 6, APEI6) in the Clarion Clipperton Zone (3950-4250 m water depth) using extensive photographic surveys from an autonomous underwater vehicle. Variations in nodule cover (1-20%) appeared to exert statistically significant differences in faunal standing stocks, some biological diversity attributes, faunal composition, functional group composition, and the distribution of individual species along the nodule cover gradient. The standing stock of both the metazoan fauna and the giant protists (xenophyophores) doubled with a very modest initial increase in nodule cover (from 1 to 3%). Notably, faunal density determined by sample-based rarefaction, was positively correlated with nodule cover, while taxon richness, determined by individual-based rarefaction, was invariant (c. 60 taxa among 500 individuals). Faunal composition varied continuously along the nodule cover gradient. We discuss these results in the context of potential seabed-mining operations and the associated sustainable management and conservation plans. We note in particular that successful conservation actions will likely require the preservation of areas comprising the full range of nodule cover and not just the low cover areas that are least attractive to mining.
Introduction

Abyssal polymetallic nodule fields represent a unique deep-sea habitat (Radziejewska 2014). The hard substratum provided by the nodules combined with the background soft sediment seabed acts to increase habitat complexity, and is thought to promote the occurrence of some of the most biologically diverse seafloor assemblages in the abyss (Amon et al. 2016, Gooday et al. 2017). This unusual and diverse habitat is potentially subject to imminent large-scale human impacts in the form of seafloor mining (Gollner et al. 2017, Kuhn et al. 2017). Mining disturbances are likely to extend over extremely large seafloor areas (Aleynik et al. 2017) and have a clear potential to drive major changes in the resident fauna (Jones et al. 2017). Predicting the nature of such changes remains difficult; the ecology of this remote habitat is poorly understood, in particular, very little is known of the biodiversity associated with nodules (Veillette et al. 2007, Vanreusel et al. 2016).

The presence of hard substratum is thought to be a key factor in structuring heterogeneous deep-sea habitats (Buhl-Mortensen et al. 2010, Bell et al. 2016). For example, modest variations in the availability and the composition of hard surfaces can influence the larval settlement processes of the seafloor fauna (Van Dover et al. 1988, Roberts et al. 2006). Substratum selectivity is commonly exhibited by many deep-sea species, including soft corals (Sun et al. 2011), sponges (Lim et al. 2017), and foraminifera (Gooday et al. 2015). The presence and extent of hard substratum is therefore expected to exert a significant control on the composition of deep-sea benthic assemblages (Levin et al. 2001, Smith and Demopoulos 2003). Seafloor environments in the deep sea with extensive hard substratum range in nature from landscape-scale features such as seamounts (Clark et al. 2010) and canyons (De Leo et al. 2010), to widely dispersed pebbles, cobbles, and boulders referred to as iceberg drop-stones (Meyer et al. 2016), and the similar human artefact habitat produced by steamship clinker (Ramirez-Llodra et al. 2011). While individual polymetallic nodules are generally small, 1-20 cm in diameter, nodule fields can
extend over extremely large areas, many hundreds of km$^2$, as occurs in the Clarion Clipperton Zone (CCZ) of the central Pacific Ocean (Kuhn et al. 2017).

Polymetallic nodules in the CCZ are thought to support a specialised fauna that differs from that of nodule-free sediment areas (Thiel et al. 1993, Gooday et al. 2015). Nodule-dwelling meiofauna such as nematodes, tardigrades, harpacticoids, and foraminifera inhabit the crevices (Veillette et al. 2007, Miljutina et al. 2010), while sessile macro- and megafauna such as polychaetes, sponges, cnidarians and xenophyophores are commonly found attached to nodule surfaces (Gooday et al. 2015, Amon et al. 2016). Consequently, nodule occurrence has been linked with variations in faunal standing stocks and distributions (Amon et al. 2016, Vanreusel et al. 2016). However, logistic constrains have limited the detailed monitoring of nodule cover (Vanreusel et al. 2016, Tilot et al. 2018), restricting the assessment of seafloor ecology along nodule occurrence gradients.

Recent advances in large-scale seafloor visual imaging (Durden et al. 2016), coupled with automated nodule-detection algorithms (Schoening et al. 2016, Schoening et al. 2017) now make such studies possible. Here, we combine extensive nodule coverage and faunal data obtained by photography from an autonomous underwater vehicle (AUV) to examine the effect of nodule occurrence on the ecology of megafauna in the CCZ. We include protozoan, invertebrate, and fish species that can be distinguished in photographs, having body length-scales $> 1$ cm, as members of the megafauna. In particular, we consider variations in their standing stock, biological diversity, and faunal composition along a nodule cover gradient. This work is carried out within an ‘Area of Particular Environmental Interest’ (APEI), a form of marine protected area designed as a conservation measure in response to potential future seabed mining in the region (ISA 2012). Consequently, we also cast our results in the context of the sustainable management and conservation of this unusual abyssal habitat.
Methods

Study area

Our initial study area was a 5500 km$^2$ rectangular region of seafloor centred on 122° 55' W 17° 16' N within the APEI6 region (Fig. 1). This location was selected to have similar topographic relief to mining contract areas in the central CCZ. Water depth ranged 3950-4250 m, and the seafloor landscape comprised a succession of crenulated ridges and shallow troughs oriented north-south between dispersed level-bottom (<3° slope) areas. General seafloor conditions were described by Simon-Lledo et al. (submitted) and are only briefly summarised here. Surface sediments (0-1 cm) were homogenous across the study area, dominated by very fine silt and clay particles (58-68% <7.8 µm diameter), and having a very low content of total organic carbon (TOC, 0.44 ± SD 0.05 %). The polymetallic nodules present were of a flattened, ellipsoidal form with smooth surfaces. The seafloor exposed mean individual nodule area was 2.5 cm$^2$, with most nodules <5 cm$^2$ (90%), and very few >10 cm$^2$ (1%). In individual seafloor photographs, average nodule cover was 6.4% and ranged from nodule-free to 37%. Nodule cover was patchy, with extremes of variation occurring at metre-scales (Fig. 1). All results reported here were acquired April-May 2015, during RRS James Cook cruise JC120; additional supporting technical detail is provided by Jones (2015).

Data collection and processing

Seafloor images were collected using a digital camera (FLIR Integrated Imaging Solutions Inc. Grasshopper2; 2448 x 2048 pixels) mounted vertically beneath the AUV Autosub6000 (Morris et al. 2014). The AUV was programmed for a target altitude of 3 m above the seafloor, a speed of 1.2 m s$^{-1}$, and a photographic interval of 850 milliseconds. At the target altitude, individual vertical photographs imaged 1.71 m$^2$ of seabed. Three landscape types (Ridge, Flat, and Trough), delimited by objective analysis of bathymetric data, were surveyed using zig-
zag designs with random start points (Strindberg and Buckland 2004) as detailed by Simon-Lledó et al. (submitted). A total of 40 individual image transects were surveyed in each landscape-type. Images taken as the vehicle changed course, i.e. junctions between transects, were removed. In the remaining straight-line sections, every second image was removed to avoid overlap between consecutive images and to prevent double counting. To ensure consistency in specimen and nodule detection, images outside the altitude range 2-4 m were also removed. Four transects were randomly selected from each landscape-type for subsequent analysis. The full resultant dataset was composed of data from 10052 non-overlapping images, representing a seafloor area of 18580 m².

All images were colour corrected, as described by Morris et al. (2014), before manual and automated analyses were performed to obtain biological and environmental data. Nodule cover (%) was quantified using the Compact-Morphology-based poly-metallic Nodule Delineation method (CoMoNoD, Schoening et al. 2017). The CoMoNoD algorithm calculates the size of each nodule (i.e. seafloor exposed area size) detected in an image, enabling the calculation of descriptive nodule statistics. Megafauna specimens were identified to the lowest taxonomic level possible, and their physical dimension measured, using BIIGLE 2.0 (Langenkämper et al. 2017). Each specimen was assigned to a ‘nodule-attached’ (NA) or ‘nodule-free-living’ (NFL) life-habit category. The biovolume of individual metazoan specimen was estimated as a proxy for biomass, using the generalised volumetric method described by Benoist et al. (submitted).

To ensure consistency in specimen identification, a CCZ-standardised megafauna morphospecies catalogue was developed upon the taxonomic compilation developed by the International Seabed Authority (available online: http://ccfzatlas.com), which we further expanded in consultation with international taxonomic experts and by reference to existing literature (Dahlgren et al. 2016, Glover et al. 2016, Amon et al. 2017, Kersken et al. 2018). The likely feeding behaviour of each morphospecies was inferred from similar organisms described in
the literature (Iken et al. 2001). The full dataset comprised 7837 metazoan specimens across 133
morphospecies, and 47133 giant foraminifera (xenophyophores) specimens across 22
morphospecies.

Data analysis
To perform an initial broad assessment of the potential influence of seafloor nodule cover on the
ecological characteristics of the megafauna, all images from the three landscape types were
pooled. This total image set was ordered by estimated nodule cover, and then divided into ten
subsets at nodule-cover breakpoints chosen to yield approximately equal numbers of megafaunal
observations in each image subset. Metazoan and xenophyophore data were processed separately
on the basis that it was not possible to determine whether the latter were living from the images
(Hughes and Gooday 2004). Across the ten resultant nodule-cover classes, metazoan megafauna
counts ranged 784-787, and xenophyophore counts 4714-4719. To establish measures of
variability in ecological characteristics within the nodule-cover classes, the corresponding image
subsets were resampled using a modified form of bootstrapping (Davison and Hinkley 1997).
Each image subset was randomly resampled with replacement until a minimum of 500 specimens
were encountered, and that process was repeated 1000 times for each nodule-cover class. This
resampling process yielded bootstrap-like samples that ranged in metazoan specimen counts 500-
565, and xenophyophore counts 500-587. We adopted these specimen-count based methods to
recognise and control the impact of specimen number on the estimation of biological diversity
and faunal composition parameters (Sanders 1968, Forcino et al. 2015, Simon-Lledo et al.
submitted).

A range of ecological parameters was calculated for each of the 10 × 1000 bootstrap-like
samples, including metazoan and xenophyophore numerical density (ind m⁻²) and metazoan
biovolume density (ml m⁻² ≈ g fresh wet weight m⁻²). To examine the range of diversity
characteristics, Hill’s diversity numbers of order 0, 1, and 2 (Jost 2006) were calculated as metazoan morphospecies richness ($S_N$), the exponential form of the Shannon index ($\text{Exp } H^'$), and the inverse form of Simpson’s index (1/D). We also calculated morphospecies density ($S_A$), based on an additional set of bootstrap-like samples generated following the same procedure, but with a controlled minimum seabed area encompassed by each sample, that was set to the smallest seabed area (c. >700 m$^2$) obtained in the specimen-controlled set of bootstrap-like samples used to calculate the rest of parameters. Variation in metazoan community composition was assessed by 2d non-metric multidimensional scaling (nMDS) ordination of all 10000 bootstrap-like samples, based on square-root transformed faunal density and use of the Bray-Curtis dissimilarity measure (Clarke 1993). The resultant dimension 1 scores (MDS-d1) were used as a univariate measure of faunal composition.

Mean (median in the case of biovolume assessment) values of these various parameters were calculated from each bootstrap-like sample set, together with corresponding 95 % confidence intervals based on the simple percentile method (Davison and Hinkley 1997). Data processing and analyses described above were performed using a custom R (R Core Team, 2014) script incorporating multiple functions of the ‘vegan’ package (Oksanen et al. 2018).

In addition to the general analyses of ecological responses to the nodule cover gradient, we considered landscape-type-related variations in those responses by undertaking a separate analysis within each landscape-type. This material is provided in Appendix 1.
Results

Standing stocks

Metazoan and xenophyophore density were significantly and substantially lower in the lowest nodule-cover class (Fig. 2a). We found no significant correlation between density and nodule availability (Table 1); density variation of both groups across the nodule gradient described a rapid asymptote, stabilising in cover levels >2-3%. In contrast, metazoan biomass density showed a high dispersion rate and no significant variations along the nodule cover gradient (Fig. 2a).

Biological diversity

Diversity measures calculated with controlled number of individuals exhibited no significant correlation with nodule cover (Table 1). Morphospecies richness ($S_N$) was near constant across nodule-cover classes with no indication of any significant difference between any pair of classes (Fig 2b). $H'$ was more variable across classes, but exhibited no coherent substantive change across the nodule gradient. In contrast, $1/D$ showed a significantly lower value in the lowest nodule class. On the other hand, morphospecies density ($S_A$; calculated with controlled seabed area) was significantly correlated with nodule cover (Table 1). $S_A$ was consistently lower than $S_N$ across the nodule gradient, though marginally (confidence intervals overlapped), except in the lowest nodule class, where $S_A$ was significantly and substantially lower than $S_N$.

Faunal composition

Assemblage

Two-dimensional MDS ordination of bootstrap-like samples showed that metazoan assemblages progressively differed across the nodule gradient (Fig. 3a); the lowest and the highest nodule-cover classes yield the largest dissimilarity rates. MDS-d1 was strongly and
significantly correlated with nodule cover (Table 1). MDS-d1 score in the lowest nodule class was substantially and significantly different from all other cover classes (Fig. 3b).

**Functional groups**

Neither nodule-attached (NA) nor nodule-free-living (NFL) faunal density was significantly correlated with nodule cover (Table 1). However, in both cases density in the lowest nodule-cover class was significantly lower than in any other class (Fig. 4a). Both deposit-feeder and suspension-feeder faunal density was significantly and substantially lower in the lowest nodule-cover class, while predator and scavenger density showed no significant variations across the nodule cover gradient (Appendix 2; Fig. B1). Variation in suspension and deposit-feeder density across the nodule gradient described a rapid asymptote, yet none of the three functional groups densities exhibited a significant correlation with nodule cover (Appendix 2; Table B1).

**Taxonomic groups**

Among the 15 most abundant morphospecies (Appendix 2: Fig B3) a graded series of distributions across nodule-cover classes was apparent (Appendix 2: Fig B4; Table B1). For example (Fig. 4b): (i) negative monotonic, Porifera msp-5, strong and statistically significant correlation with nodule cover (Table 1); (ii) unimodal, *C. cf bayeri*, statistically significant difference between tails (classes 1, 8-10) and centre (classes 2-6) of the distribution; (iii) positive unimodal, *Lepidisis* msp, strong and statistically significant correlation with nodule cover (Table 1). The density of Polychaete msp-5 and Actinia msp-18 was significantly and substantially lower in the lowest nodule-cover class, while density of *Ophiosphalma* sp., *Columnella* msp, and *Irregularia* msp-1 was also lower in the lowest nodule-cover class, though marginally (Appendix 2: Fig B4). Among major taxa levels (i.e. most dominant phyla) a graded series of distributions across nodule-cover classes was also apparent (Appendix 2: Fig B2 and Table B1).
Discussion

We found substantial and statistically significant variations in megafaunal standing stock, biological diversity, and faunal composition along a gradient of seafloor nodule cover. These responses were generally graded with nodule cover. However, in many cases the magnitude of change between the first two cover classes was particularly marked. Both of these observations are of direct relevance to sustainable management and conservation concerns in relation to seabed mining in the CCZ and similar environments elsewhere.

Standings stocks

Differences in metazoan density across the nodule cover gradient were predominately driven by variations in suspension feeder abundance, particularly anthozoans living attached to nodules; the abundance of which was substantially and statistically significantly reduced in the lowest nodule class (Appendix 2: Fig. B1; Fig. B2b). Hard substrata provide a stable anchor point for suspension feeders and enable the placement of food-catching structures into faster off-bottom currents (Wildish and Kristmanson 2005). Enhanced densities of hard substratum attached fauna has been observed on bedrock in seamounts or canyons (Clark et al. 2010, Baker et al. 2012, Jones et al. 2013), in areas with drop-stones (Jones et al. 2007, Meyer et al. 2016), and in polymetallic nodule fields (Amon et al. 2016, Vanreusel et al. 2016). Our results provide additional detail that suggests a non-linear, asymptotic relationship between standing stock and nodule cover (Fig. 2a). This response may be simply explained by resource limitation (Tilman 1982), i.e. hard substratum is initially limiting, but food resource (i.e. advecting organic particles) becomes limiting as attached suspension feeder density increases (Jeffreys et al. 2009). Variation in suspension-feeder density at the landscape-type scale sustains this hypothesis and suggest that the transition between limiting resources (i.e. from nodules to food) occurs at nodule cover > 2-3% (Fig. 5a).
Xenophyophore density showed a rapid asymptotic relationship with nodule cover in the broad assessment but a different pattern in each area when investigated at the landscape-level, with a clearly higher abundance in the Ridge (Fig. 5b). Other studies have documented enhanced xenophyophore density on elevated terrain, e.g. seamounts (Levin and Thomas 1988, Wishner et al. 1990) and abyssal hills (Stefanoudis et al. 2016), and their dominance in the megafauna and high taxonomic diversity in the CCZ (Amon et al. 2016, Gooday et al. 2017). Although sediment-dwelling species are well-known, nodules clearly represent a very important habitat for xenophyophores (Gooday et al. 2015, Kamenskaya et al. 2015). While the specific feeding modes of xenophyophores remain uncertain (Gooday et al. 1993, Laureillard et al. 2004), the nodule-attached forms are most likely suspension feeders, and the sediment-dwellers most likely deposit feeders (Gooday et al. 2017). Yet our results suggest that, although nodule resource may limit the development of a part of the xenophyophore fraction (i.e. suspension feeder forms), geomorphological variations are a stronger control on the overall xenophyophore standing stock.

**Biological diversity**

Variation between morphospecies richness and morphospecies density was evident in the lowest nodule class (Fig. 2b), suggesting either a lower faunal density and/or a lower evenness between taxa abundances where nodule resource is limiting, yet no reduced taxa richness, as previous CCZ megafauna assessments suggested (Amon et al. 2016, Vanreusel et al. 2016, Tilot et al. 2018). However, previous studies typically used fixed-area samples, in fact reporting taxa density. For instance, Tilot et al. (2018) compared richness between areas with varying nodule abundance based on subsample units with fixed seabed areal cover, yet ranging in size from ~150 to ~450 individuals, which possibly generated strong biases in richness estimations as these are highly sensitive to the number of individuals surveyed (Gotelli and Colwell 2001). Distinction between morphospecies richness and density becomes particularly relevant in the assessment of
nodule-field communities, as the lower megafaunal density characteristic of areas with low
nodule cover can lead to the underestimation of taxonomic richness. In turn, if richness appears to
be essentially invariant with respect to nodule cover, indices more sensitive to the variation in
taxa evenness (i.e. heterogeneity diversity) may consequently be more appropriate monitoring
targets.

Heterogeneity diversity measures indicated a clearly reduced diversity in the lowest nodule class,
markedly so in the case of 1/D index (Fig. 2b). Our results concur with Amon et al. (2016) that
nodule availability does not need to be high to promote higher megafauna diversity (although not
necessarily richness), and with Vanreusel et al. (2016) that suspension feeder abundance
distribution appears to lead (most) of this variation. Lower diversity in the lowest nodule class
was predominantly generated by two combined factors: (i) general reduction in the abundance of
almost all suspension feeder taxa, and (ii) extremely high numerical dominance of one taxon
(Porifera msp-5), possibly better adapted to the environmental conditions in the lowest nodule
class. On the other hand, landscape-type level analyses showed a clearly higher diversity in the
Ridge compared to the Trough in areas with low nodule cover (2-3%; Appendix 1: Fig. A2e-f),
possibly resulting from a more balanced taxa evenness, generated by the higher deposit feeder
taxa abundance within the Ridge (Simon-Lledo et al. submitted). Structurally more complex
habitats can provide a wider range of niches and diverse ways of exploiting the environmental
resources, promoting species coexistence in the deep-sea benthos (Levin et al. 2001). Hence, our
results suggest that nodules may act as ‘keystone structures’ (Tews et al. 2004) in the regulation
of habitat complexity at fine scales (tens of meters), while geomorphological variations
presumably modulating bottom water flows and deposition patterns (Mewes et al. 2014, Peukert
et al. 2018), may play an important role at larger scales (few kilometres) (Simon-Lledó et al.
submitted).


**Faunal composition**

Our data suggest that faunal composition changes continuously with nodule cover across the full spectrum of the gradient studied. The first step on that gradient (from nodule class 1 to 2) was, however, substantially greater than those that followed (Fig. 3). This initial ‘jump’ is consistent with the change from an overwhelmingly background sedimentary habitat to a mosaic habitat with a varying admixture of nodule hard substrata to that sediment background. A higher dissimilarity of the lowest nodule-class assemblage was somewhat expected, since most of the APEI6 megafaunal community (70% of taxa richness) were nodule-dwelling taxa (Simon-Lledo et al. submitted) with reduced abundance in the lowest nodule class (Appendix B: Fig. B4). These populations may simply not find enough suitable substratum to develop where nodules are limited, as typically occurs in the smaller-sized meio- and macrofaunal communities (Mullineaux 1987, Veillette et al. 2007). This first, sharply defined, faunal composition change numerically supports that even subtle increases in nodule availability can drive substantial variations in megafaunal communities (Amon et al. 2016). Yet the following, rather continuous variations, suggest a potential diversification of habitats along the nodule gradient beyond the simple presence or absence of a minimum nodule resource level.

We found a clear shift in dominance from sponges (predominantly Porifera msp-5) in the lowest nodule class to cnidarians in the remaining classes, and within the latter, an alternation of dominance between primnoid soft corals, anemones, and bamboo corals with increasing nodule cover. This suggests that other environmental drivers may potentially co-vary along the nodule cover gradient. For instance, nodule size was positively linearly correlated with nodule cover ($r_p=0.72$, $p<0.001$), with mean surface areas of nodules found in the lowest cover class (median: 1.66 cm$^2$; IQR: 0.44) being almost half the size of those in areas with the highest coverage (median: 2.87 cm$^2$; IQR: 0.42). Such comparably larger nodule sizes are commonly found in areas with lower sediment accumulation rates and relatively stronger bottom-current speeds (Skornyakova and Murdmaa 1992, Mewes et al. 2014). Variable development of particular deep-
sea suspension feeder populations can be regulated by bottom current speeds (Thistle et al. 1985, 339
Smith and Demopoulos 2003), and also by the size of the available hard structures (Meyer et al. 2016), especially in soft corals (Watanabe et al. 2009). Areas with larger and hence potentially more physically stable nodules possibly provide a more suitable long-term anchoring point for bamboo coral taxa, enabling their greater final colony height compared to, for example, primnoid soft corals (Lapointe and Watling 2015, Cairns 2016). In turn, the presumably stronger bottom current speeds in areas with large nodule size perhaps limits the development of primnoids, which appear to find a suitable habitat in areas with comparably lower nodule availability (4-6%). Therefore, we hypothesise that factors interrelated with nodule availability, like nodule size or bottom current speeds possibly act as environmental filters, ultimately controlling population recruitment rates.

Conclusions

Sustainable management and conservation

Our results suggest that areas less likely to be exploited by deep-sea mining (i.e. low to intermediate nodule-cover classes) would not serve the preservation of the full range of taxa that live in polymetallic nodule fields. Although these may act as source populations of taxa that also live in high nodule abundance areas (i.e. actinians or bryozoans), our results show that these cannot support abundant populations of the fauna found in high nodule cover areas (i.e. bamboo corals). Moreover, the potential deposition of sediment plumes in non-directly exploited areas (Aleynik et al. 2017) may also compromise the preservation of source populations for most suspension feeder taxa (Bluhm 2001), that represent the vast majority of the metazoan standing stock at the CCZ (Amon et al. 2016, Vanreusel et al. 2016), and appear to be the most sensitive fauna to variations in nodule cover (i.e. this study). This suggests that the combined effects of nodule removal and sediment plume deposition are likely to generate biodiversity and standing
stock losses at the landscape scale, with the corresponding loss in rate processes and ecosystem services provided by the megafauna.

Simplistically, a nodule field could be considered as two habitats: (a) the background sedimentary habitat, and (b) the hard substratum environment of the nodules. More realistically, and certainly at the physical scales inhabited by megafauna, the nodule field is likely better considered as a mosaic habitat comprising those two components. However, our results make clear that the mosaic habitat does not support a single biotope, nor indeed two biotopes; within the limits of the nodule cover gradient that we have been able to study, faunal composition exhibits continuous variation. Equally, it is also clear that we do not yet fully understand the drivers of ecological variation along the nodule cover gradient. Consequently, sustainable management and conservation plans (Levin et al. 2016, Durden et al. 2017), together with the monitoring programmes that support them, must recognise this complexity and uncertainty if they are to be effective.

In closing, we should note that our primary analyses have concerned a broad assessment of nodule cover using data drawn from three distinct abyssal landscape types. These landscape-scale variations in environmental and ecological characteristics (Simon-Lledo et al. submitted, Supplementary material Appendix 1) add an additional layer of complexity that can be expected to operate at the physical scale of individual conservation areas (Area of Particular Environmental Interest in the CCZ) and potential mining operation areas.
Acknowledgements

We thank the captain and crew of RRS James Cook, and the Autosub6000 technical team for their assistance during cruise JC120. We grateful to all the taxonomic experts consulted (directly or indirectly) during the generation of our megafauna catalogue. This work forms part of the Managing Impacts of Deep-seA reSource exploitation (MIDAS) project of the European Union Seventh Framework Programme (FP7/2007-2013; grant agreement no. 603418), and was additionally funded by the UK Natural Environment Research Council (NERC). VH was also funded through the European Research Council Starting Grant project CODEMAP (grant no. 258482). BB, VH, and DJ received additional support from the NERC Climate Linked Atlantic Sector Science programme.
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**Figures (Legends)**

**Figure 1.** Study area location and sampling operations within the APEI6 of the CCZ (North Pacific Ocean). (a) Bathymetric survey chart of the study location. Landscape types depicted in dark lines (left to right: Flat, Ridge, and Trough). White rectangles indicate AUV sampling areas targeted within each landscape. (b) Map of the eastern CCZ showing contractor areas, Areas of Particular Environmental Interest, and study location. (c) to (e) Full AUV imagery dataset collected at each landscape type. Colour of survey tracks represents the nodule coverage level of the seabed, obtained from automatic detection in survey images using the CoMoNoD algorithm (Schoening et al. 2017). (c) Flat survey. (d) Ridge survey. (e) Trough survey.

**Figure 2.** Variation in (a) standing stock and (b) diversity with nodule cover at the APEI6 seafloor. Points indicate mean (median for metazoan biomass) values of each parameter calculated from each nodule-cover class bootstrap-like sample set. Error bars represent 95% confidence intervals. (a) Density of metazoans and xenophyophores (left y-axis), metazoan biomass density (right y-axis). (b) Metazoan diversity: morphospecies richness (SN), morphospecies density ($S_A$), Exponential Shannon index ($\text{Exp } H'$), and Inverse Simpson index ($1/D$).

**Figure 3.** Variation in community composition with nodule cover at the APEI6 seafloor. (a) nMDS plot describing 2D ordination of dissimilarity (distance) between the assemblages of each bootstrap-like sample. Ellipses represent 95% confidence intervals for each nodule-cover class bootstrap-like sample set. (b) Variation of nMDS dimension-1 with nodule cover. Data are mean values of the parameter as calculated from each nodule-cover class bootstrap-like sample set. Error bars represent 95% confidence intervals.
Figure 4. Variation in metazoan density (a) life modes and (b) selected morphospecies (sponge: Porifera msp-5; primnoid soft-coral: *Callozostron cf. bayeri*; bamboo soft-coral: *Lepidisis* msp), with nodule cover. Data are mean values of the parameter as calculated from each nodule-cover class bootstrap-like sample set. Error bars represent 95% confidence intervals.

Figure 5. Example of landscape-type variation in faunal response to nodule cover. Variation in the density of (a) suspension-feeder metazoans and (b) xenophyophores with nodule cover. Data are mean density values as calculated from each nodule-cover class bootstrap-like sample set for each separate landscape-type analysis (Flat, Ridge, Trough). Error bars represent 95% confidence intervals.
Tables

Table 1. Spearman’s rank correlations of ecological parameters with nodule cover. Summary results of tests performed between mean (median for metazoan biomass density) values of each parameter calculated from each nodule-cover class bootstrap-like sample set, with detail of significant differences between nodule class 1 (mean cover = 1.1%) and the rest of classes (cover >2%). Distinct class 1: no overlap of the confidence interval of the lowest nodule-cover class with any other class. Note that correlation approach fails to detect significance in the variation of unimodal responses.

| Distinct class 1 | Correlations | p-value |
|------------------|--------------|---------|
| **Standing stock** |              |         |
| Xenophyophores (ind m$^{-2}$) | yes | -0.297 | 0.404 |
| Metazoans (ind m$^{-2}$) | yes | 0.345 | 0.328 |
| Metazoan biomass (g fwwt m$^{-2}$) | no | 0.624 | 0.053 |
| NA metazoa (ind m$^{-2}$) | yes | 0.466 | 0.174 |
| NFL metazoa (ind m$^{-2}$) | yes | 0.224 | 0.533 |
| Porifera msp-5 (ind m$^{-2}$) | no | -0.976 | <0.001*** |
| C. cf bayeri (ind m$^{-2}$) | no | -0.6 | 0.067 |
| Lepidisis msp (ind m$^{-2}$) | no | 0.952 | <0.001*** |
| **Diversity and composition** |              |         |
| Morphospecies richness ($S_N$) | no | 0.248 | 0.405 |
| Morphospecies density ($S_A$) | no | 0.721 | 0.018* |
| Exponential Shannon (Exp $H'$) | no | 0.478 | 0.161 |
| Inverse Simpson (1/D) | yes | 0.345 | 0.328 |
| MDS-dimension 1 | yes | 0.891 | 0.001** |
(a) 

(b) 

Nodule cover (%) 

Density (ind m\(^{-2}\))
Supplementary material

Appendix 1: Additional analyses within landscape type

The dataset was collected in three landscape types (LT), ‘Flat’, ‘Ridge’, and ‘Trough’ (main text, Fig. 1). To assess the potential influence of LT on ecological responses to the nodule cover gradient, we additionally carried out separate analyses within each LT. As in our broad analysis, images were ordered by nodule cover and divided into nine cover classes at breakpoints to yield an approximately equal number of megafauna specimens in each class. Megafauna data from each cover class, in each LT, was then subjected to a bootstrap-like resampling procedure to produce 1000 targeting a minimum of 250 specimens per sample. Faunal density and diversity measures (as in main text) were calculated for each bootstrap-like subsample, and 95% confidence intervals derived by the simple percentile method (see main text).

Figure A1. Areal distribution of nodule cover within each landscape type.
Figure A2. Variation of different ecological parameters across the nodule coverage gradient of each different APEI6 landscape type. Data are mean values of each parameter as calculated from each nodule cover class bootstrap-like sample set. Error bars represent 95% confidence. (a) Metazoan density. (b) Xenophyophore test density. (c) Morphospecies richness. (d) Morphospecies density. (e) Exponential Shannon index. (f) Inverse-Simpson index.
Appendix 2: Additional results of broad ecological assessment

Figure B1. Variation in the density of three functional groups with nodule cover at the APEI6 seafloor. Data are mean density values of different metazoan types (SF: suspension feeders; PSC: predators and scavengers; DF: deposit feeders) calculated from each nodule cover class bootstrap-like sample set. Error bars represent 95% confidence intervals.
Figure B2. Variation in the density of taxonomical groups with nodule cover at the APEI6 seafloor. Data are mean density values of the six most dominant metazoan phyla as calculated from each nodule-cover class bootstrap-like sample set. Error bars represent 95% confidence intervals. (a) Sponges. (b) Cnidarians. (c) Bryozoans. (d) Annelids. (e) Echinoderms. (f) Arthropods: crustaceans.
Figure B3. Top-15 most abundant metazoan morphospecies recorded at the APEI6 seafloor during JC120 AUV survey. Scale bars indicate 50 mm. (a) Porifera msp-5. (b) Cladorhiza cf mexicana. (c) Polychaete msp-5. (d) Irregularia msp-1. (e) Mastigoteuthis sp. (f) Ophiosphalma sp. (g) Columnella msp (Bryozoa). (h) Smithsonius msp (Bryozoa). (i) Aspidodiadematidae msp. (j) Actinia msp-18. (k) Actinia msp-22. (l) Callozostron cf bayeri. (m) Calyptrophora cf persephone. (n) Bathygorgia cf profunda. (o) Lepidisis msp.
Figure B4. Variation in morphospecies density with nodule cover at the APEI6 seafloor. Data are mean density values of top-15 most abundant metazoan morphospecies as calculated from each nodule cover class bootstrap-like sample set. Error bars represent 95% confidence intervals. (a) Porifera msp-5. (b) Cladorhiza cf mexicana. (c) Polychaete msp-5. (d) Irregularia msp-1. (e) Mastigoteuthis sp. (f) Ophiosphalma sp. (g) Columnella msp (Bryozoa). (h) Smithsonius msp
(Bryozoa). (i) Aspidodiadematidae msp. (j) Actinia msp-18. (k) Actinia msp-22. (l) Callozostron cf bayeri. (m) Calyptrophora cf persephone. (n) Bathygorgia cf profunda. (o) Lepidisis msp.

**Figure B5.** Variation in the density of selected metazoan taxonomic groups with nodule cover. Lines represent mean density values of each group as calculated from each nodule-cover class bootstrap-like sample set fitted by weighted least-squares, using a local polynomial regression.
Table B1. Spearman’s rank correlations of all ecological parameters with nodule cover. Summary results of tests performed between mean density (ind m$^{-2}$) values of different metazoan groups as calculated from each nodule cover class bootstrap-like sample set and nodule cover variation, with detail of significant differences between nodule class 1 (mean cover = 1.1%) and the rest of classes (cover >2%). Distinct class 1: no overlap of class 1 confidence interval with any other class.

| Functional group                     | Distinct class 1 | Correlations |   r  | p-value |
|--------------------------------------|------------------|--------------|------|---------|
| Deposit feeders                      | yes              | -0.15        | 0.676|
| Predators and scavengers             | no               | 0.28         | 0.425|
| Suspension feeders                   | yes              | 0.50         | 0.138|
| **Taxonomic Phylum**                 |                  |              |      |         |
| Annelida                             | yes              | 0.10         | 0.777|
| Bryozoa                              | yes              | 0.78         | **0.008***|
| Cnidaria                             | yes              | 0.49         | 0.150|
| Arthropods: crustaceans              | no               | 0.83         | **0.003***|
| Echinodermata                        | yes              | -0.25        | 0.489|
| Porifera                             | no               | -0.84        | **0.002***|
| **Morphospecies**                    |                  |              |      |         |
| Polychaete msp-5                     | yes              | 0.18         | 0.627|
| Columnella msp                       | yes              | 0.76         | **0.011***|
| Smithsonius msp                      | no               | 0.50         | 0.138|
| Actinia msp-18                       | yes              | 0.47         | 0.174|
| Actinia msp-22                       | no               | 0.83         | **0.003***|
| C. cf persephone                     | no               | -0.36        | 0.310|
| B. cf profunda                       | no               | 0.55         | 0.098|
| Lepidisis msp                        | no               | 0.95         | 0*** |
| C. cf bayeri                         | no               | -0.60        | 0.067|
| Irregularia msp-1                    | no               | -0.03        | 0.934|
| Aspidodiadematidae msp               | no               | -0.54        | 0.108|
| Ophiosphalma sp                      | no               | -0.03        | 0.934|
| Porifera msp-5                       | no               | -0.93        | 0*** |
| C.cf mexicana                        | no               | -0.24        | 0.511|
| Mastigoteuthis sp                    | no               | 0.44         | 0.200|
Manuscript Details

**Manuscript number** PROOCE_2018_141
**Title** Megafaunal variation in the abyssal landscape of the Clarion Clipperton Zone
**Article type** Full Length Article

**Abstract**
The potential for imminent polymetallic nodule mining in the Clarion Clipperton Fracture Zone (CCZ) has attracted considerable scientific and public attention. This concern stems from both the extremely large seafloor areas that may be impacted by mining, and the very limited knowledge of the faunistics and ecology of this region. The key environmental factors regulating local seafloor ecology are still very poorly understood. In this study, we focus on megafaunal ecology in the proposed conservation zone ‘Area of Particular Environmental Interest 6’. We employ swath bathymetric survey data to objectively define three key landscape types in the area (Flat, Ridge, Trough; water depth: 3950-4250 m) that are generally characteristic of the wider CCZ environment. We use direct seabed sampling to further characterise the sedimentary environment in each landscape type, detecting no statistically significant differences in particles size distributions or organic matter content. Additional seafloor environmental characteristics and data on both the metazoan and xenophyophore components of the megafauna were derived by extensive photographic survey from an autonomous underwater vehicle. Image data revealed that there were statistically significant differences in seafloor cover by nodules and in the occurrence of other hard substrata habitat between landscape types. Statistically significant differences in megafauna standing stock, functional structuring, diversity, and faunal composition were found between landscape types. Geomorphological variations presumably regulating local bottom water flows and the availability of nodule and xenophyophore test substrata between study areas may be the mechanism driving these assemblage differences. We also used these data to assess the influence of the sampling unit size choice on the estimation of ecological parameters. Sampling unit size evaluation supported our results, although each parameter exhibited a different sensitivity to this factor. All of these results are important to the appropriate management of potential mining activities in the CCZ and elsewhere in the deep ocean.

**Keywords** polymetallic nodules; geomorphology; biodiversity; deep-sea mining; abyssal plain; AUV survey; NE Pacific; CCZ; APEI

**Manuscript category** Interdisciplinary
**Corresponding Author** Erik Simon-Lledo
**Corresponding Author's Institution** National Oceanography Centre
**Order of Authors** Erik Simon-Lledo, Jennifer Durden, Noemie Benoist, Timm Schoening, Rachel Jeffreys, Brian Bett, Veerle Huvenne, Daniel O.B. Jones
**Suggested reviewers** Diva Amon, Lenaick Menot, Ann Vanreusel

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Dear Sir/Madam,

Would you please consider the attached manuscript "Megafaunal variation in the abyssal landscapes of the Clarion Clipperton Zone (CCZ)" for submission to Progress in Oceanography. We believe that the attached research paper addresses an important topic that is highly appropriate for PIO.

Our paper explores the influence of seafloor landscape on benthic megafauna communities in the abyssal Pacific. We targeted one of the proposed marine protected areas (Area of Particular Environmental Interest 6) set up by the International Seabed Authority to protect the environment from the harmful effects of potentially imminent polymetallic nodule mining. There is an urgent need for baseline ecological analyses such as the present study, since knowledge of the drivers structuring biological communities in the area is scarce, yet essential for effective regulation.

We analysed a large image dataset, covering ~15,000 m$^2$ of seafloor, which we demonstrate, through analysis of sampling effort, to be enough to provide good quality and stable estimates of key biological metrics. Our analysis detected clear differences in community structure, biodiversity levels, and fauna standing stock between characteristic landscape types, showing that local geomorphological variations at the landscape scale can play an important role in the structuring of the CCZ megabenthos. Changes in terrain have long been hypothesised to be important in the ecology of the abyss, but these effects have primarily been assessed in topographically prominent environments, such as canyons, ridges, and seamounts. Very few studies have assessed such patterns in abyssal plains, and this is the first to demonstrate that biodiversity can be modulated by terrain variations at the CCZ. The results have highly relevant conservational value. Differences found between formerly assumed homogenous abyssal landscape types flag the importance of taking geomorphological variations into consideration during the development of environmental policies at the CCZ.

Possible referees:

Dr. Lenaick Menot. Ifremer, Plouzané, France: lenaick.menot@ifremer.fr
Dr. Diva Amon, Natural History Museum, London, UK: divaamon@gmail.com
Prof. Dr. Ann Vanreusel, Ghent University, Belgium: ann.vanreusel@ugent.be
Authors remarks:

This is the first time that this manuscript has been submitted to any journal. All authors participated in the research and/or article preparation and have approved the final article. Authors declarations of interest: none. The funding sources of this article, listed in the main text, had no involvement in the study design, nor in the collection, analysis, or interpretation of data. We would like to use colour for figures in print.

Yours faithfully

Erik Simon-Lledó

Telephone: +44 7596414311

Email: esl1n13@soton.ac.uk
Highlights

- Used objective landscape classification based on AUV surveys to explore the influence of geomorphology in the structuring of abyssal megafauna

- Statistically significant differences in megafauna standing stock, functional structuring, diversity, and faunal composition were found between landscape types.

- Lower megafauna density and diversity were found in a bathymetric valley (Trough area), which can have important implications for mining exploitation

- Evaluation of the effect of the sample unit size supported our results and highlighted the importance of the choice of sampling unit in abyssal sampling, particularly at the CCZ.
1. Introduction

The likelihood of polymetallic nodule mining in the Clarion Clipperton Fracture Zone (CCZ) has attracted considerable scientific attention (Levin et al., 2016; Van Dover et al., 2017; Wedding et al., 2015). The potential impacts of mining are likely to extend over extremely large seafloor areas (Aleynik et al., 2017; Glover & Smith, 2003). Such disturbance may lead to major change in the benthic fauna (Jones et al., 2017) and full recovery might take thousands of years (Glasby et al., 1982). Sixteen nodule mining exploration contract areas (75,000 km$^2$ each) were granted in the CCZ between 2001 and 2014 by the International Seabed Authority (ISA) (Wedding et al., 2015). The ISA also allocated a series of nine Areas of Particular Environmental Interest (APEIs) beyond these claim areas, where exploitation is prohibited (ISA, 2012). The APEIs were designated to preserve source populations of species for future recolonization of disturbed areas (Lodge et al., 2014). However, the majority of these APEIs remain unstudied; it is not clear if their environmental conditions and faunas are similar to those of the mining claims (Glover et al., 2016a). As a result, improved knowledge of the drivers structuring biological communities in the CCZ is urgently needed to test the presumed functionality and current spatial arrangement of the APEIs system, and to re-assess the regional environmental plan (ISA, 2012).

The CCZ is generally considered as an extensive abyssal plain delimited by the topography of two WSW-ENE trending fracture zones, Clarion and Clipperton. There is a gradual increase in water depth from east (4000 m) to west (5000 m) owing to the sinking of older, cooler oceanic crust to the west (Pushcharovsky, 2006). However, slight variations in spreading rate appear to have shaped the CCZ seafloor into a series of bathymetric highs and lows with a characteristic spacing of 1 to 10 km, elongated perpendicular to fracture zones (Klitgord & Mammerickx, 1982; Olive et al., 2015). These horst and graben structures shape the CCZ seafloor as a succession of ridges, valleys, and intervening ‘flat’ zones. This topographic variation is thought to be generally characteristic of the abyssal environment worldwide (Harris et al., 2014). The very low influx of terrigenous sediments to the CCZ prevents the blanketing of this topography, as may occur on abyssal plains adjacent to continental margins (Smith & Demopoulos, 2003).

Abyssal plains represent some 70% of the world’s seafloor (Harris et al., 2014) and are considered the largest ecosystems on Earth (Ramirez-Llodra et al., 2010). They are poorly explored but appear to have high species richness, including very many undescribed taxa (Smith et al., 2006). Despite their name, abyssal plains can have significant topography that influences the diversity and composition of deep-sea fauna (Durden et al., 2015; Leitner et al., 2017; Stefanoudis et al., 2016). This ecological variation appears to result from the interconnected effects of topographically-driven variation of local current dynamics (Thistle et al., 1991), sediment composition (Durden et al., 2015), and food supply (Smith and Demopoulos, 2003; Morris et al., 2016). However, habitat complexity derived from abyssal landscape geomorphology may have been
underappreciated in global estimations of ecological heterogeneity at the deep-sea floor (Durden et al., 2015; Morris et al., 2016); a factor that might be particularly significant to the ecology of the CCZ.

The CCZ appears to have one of the highest levels of deep-sea megafaunal (>1 cm length) species richness (Kamenskaya et al., 2013; Tilot et al., 2018). Morphospecies richness estimations from imagery data can rise above 200 taxa in local assessments (Amon et al., 2016). True species diversity and genetic biodiversity is expected to be much higher (Glover et al., 2015). Given their smaller body size, even higher local diversity is to be expected in the meio- and macrofaunal assemblages of the CCZ (De Smet et al., 2017; Pape et al., 2017). Epifauna, particularly suspension feeders, appear to have higher numerical densities in locations with higher nodule coverage (Vanreusel et al., 2016), with nodule-free areas having an higher proportion of deposit feeders, such as holothurians (Stoyanova, 2012). However, the precise role of nodules, and other local environmental factors, in the ecology of CCZ megafauna at the CCZ is still poorly understood. Faunal composition analyses are scarce, and most quantitative studies have been based on relatively small sampling unit areas (<1000 m²) and low replication levels. Meaningful comparison across the CCZ is also hampered by a lack of standardization between studies.

Reliable estimation of ecological parameters relies on appropriate sampling of the populations under investigation. It is often these parameters that serve as the sole basis for conservation management decisions (Andrew & Mapstone, 1987; Magurran, 2004). Investigation of the pros and cons of different sampling strategies is commonplace in terrestrial and shallow-water marine ecology (Andrew & Mapstone, 1987; Buckland et al., 2001; Heck Jr et al., 1975) but rarely tackled in deep-sea studies, except for diversity estimators (Etter & Mullineaux, 2001; Grassle & Maciolek, 1992; Soetaert & Heip, 1990). In part, this lack of research stems from logistic constraints, however, the need is no less. In the CCZ, a key factor may be the very low numerical density of the megafauna, such that identifying an appropriate sampling unit size may be a particular issue (Benoist et al., submitted; Durden et al., 2016). Studies that demonstrate appropriate sampling to support their conclusions are key in ecology, not least those concerned with the regulation of mining activities (Durden et al., 2017a; Levin et al., 2016).

Our study assesses the ecology of the megafauna in the dominant landscape types of APEI6 in the eastern CCZ. We define the landscape types by objective analysis of the bathymetry, establish corresponding sedimentary environmental conditions by direct sampling, and further environmental characteristics and faunal data by extensive seafloor photography from an autonomous underwater vehicle (AUV). In this contribution we examine landscape-type-related variations in standing stock, diversity, and faunal composition and how these parameters, and their interpretation, might vary with the choice of sampling unit size.

2. Materials and methods

2.1. Study area

The CCZ basin floor is covered by extensive polymetallic nodule fields that add to the seabed heterogeneity and constitute a unique deep-sea habitat (Radziejewska, 2014). Seafloor nodule coverage can be extremely patchy and change drastically over tens of metres (Peukert et al., 2018). Surface sediment is mainly composed of Cenozoic pelagic clays and radiolarian oozes (ISA, 2010). The average carbonate compensation depth (CCD) is around 4500 m (Mewes et al. 2014), although much shallower to the east (~3500 m) than the
west (~5000 m) (Radziejewska, 2014). Bottom currents are generally weak (<10 cm s\(^{-1}\)), but direction shifts and periods of stronger flows are not infrequent (Aleynik et al., 2017). The supply of sinking food particles to the seafloor is extremely low (Lutz et al., 2007), although higher in the APEI6 area than in western areas (Veillette et al., 2007).

All results reported here relate to the APEI6 area, and were acquired during RRS James Cook cruise 120 (Jones, 2015). The survey represented a 5,500 km\(^2\) rectangle of seafloor centred on 122° 55' W, 17° 16' N (Fig. 1), chosen to have similar topographic relief to mining contract areas in the central CCZ. Water depth ranged 3950-4250 m, and the seafloor landscape comprised a succession of crenulated ridges and shallow troughs oriented north-south between dispersed level-bottom (<3° slope) areas.

**Fig. 1.** Bathymetric survey chart of the study location within the APEI6 of the CCZ (North Pacific Ocean). Depth (in metres) is indicated by the colour bar. Landscape types mapped using objective classification depicted in dark lines. Yellow dashed line shows seafloor bathymetric profile depicted in Figure 2. A map of the eastern CCZ is inset, showing exploration licenced areas (black polygons), Areas of Particular Environmental Interest (green polygons), and study location (red square).

2.2. Survey design
2.2.1. Bathymetric mapping and landscape characterisation

Multibeam data were collected with the shipboard Simrad EM120 system (191 beams) and processed using CARIS HIPS and SIPS software (TeledyneCARIS; v8.0). The resultant digital elevation model (~100 m horizontal resolution) was used to calculate broad bathymetric position Index (bBPI) (Weiss, 2001) and terrain ruggedness index (TRI) (Wilson et al., 2007) using SAGA v. 2.1.4 software (Conrad et al., 2015). BPI was calculated using an inner radius of 500 m and an outer radius of 10,000 m, and TRI was calculated with a 500 m radius circular neighbourhood. These areas were selected to be representative of the landscape-scale geomorphological variation that was the target of this study. After visual inspection of the generated datasets, classification thresholds were set to map ridge (bBPI: 50 to 100; TRI: 0 to 150), trough (bBPI: -100 to -50; TRI: 0 to 150), and flat (bBPI: -100 to 50; TRI: 0 to 50) areas. Contours were drawn using ArcGIS v10 (ESRI, 2011) along threshold values of each dataset, and used to delimit representative polygons. Three polygons each representing a characteristic landscape type were chosen for stratified-random sampling: Flat area, Ridge area, and Trough area (Fig. 2). Data were projected in Universal Transverse Mercator projection, Zone 10N, using the World Geodetic System 1984 datum.

![Survey Landscape type study areas investigated at the APEI6. A) Seafloor bathymetric profile depicted as yellow-dashed line in Fig 1. B to D: Detail of sampling operations: grey lines indicate full AUV image survey tracks, thick white contours were drawn using ArcGIS v10 along threshold values of each dataset, and used to delimit representative polygons. Three polygons each representing a characteristic landscape type were chosen for stratified-random sampling: Flat area, Ridge area, and Trough area (Fig. 2). Data were projected in Universal Transverse Mercator projection, Zone 10N, using the World Geodetic System 1984 datum.](image)
2.2.2. Direct sampling

Five sediment sampling stations, with a minimum separation of 100 m, were randomly selected within each study area (Fig. 2.B-D). Two Megacore (Gage and Bett, 2005; 10cm internal diameter) samples were collected per station. Each sample was initially sliced and split by sediment depth. Sediment grain-size distributions were assessed from one core in 0-5 and 5-10 mm depth horizons, by laser diffraction using a Malvern Mastersizer 2000 after homogenisation (grains >2 mm removed), dispersal in 0.05% (NaPO$_3$)$_6$ solution, and mechanical agitation. Grain-size distributions obtained for the two horizons were averaged for presentation. The 0-10 mm horizon from the second core were assessed for sediment chemistry. Total carbon (TC) and total nitrogen (TN) contents were measured in duplicate (reproducibility <±5%) using a Carlo Erba NC 2500 CHN Elemental Analyser. Total organic carbon (TOC) was determined after de-carbonation of the samples using the acid HCl vapour method of (Yamamuro & Kayanne, 1995).

2.2.3. Photographic survey

Seafloor photographic images were collected using two digital cameras (FLIR Grasshopper2; 2448 x 2048 pixels), one mounted vertically, and one forward oblique facing on the autonomous underwater vehicle (AUV) Autosub6000 (Morris et al., 2014). The camera layout and the underwater navigation system were set as described in Morris et al. (2014). The AUV was programmed for a target altitude of 3 m above the seafloor, a speed of 1.2 m s$^{-1}$, and a photographic interval of 850 milliseconds. At the target altitude, individual vertical photographs imaged 1.71 m$^2$ of seabed.

In each area, a zig-zag survey design (Fig. 2.B-D), with random start point, was chosen to maximise sampling efficiency while minimising design-based bias in the spatial distribution of the replicate sampling units (Buckland et al., 2001; Strindberg & Buckland, 2004). A total of 40 sampling units, the straight line zig and zag sections, were surveyed in each area. Four sampling units were randomly selected in each area for subsequent analysis. Images taken as the vehicle changed course, i.e. junctions between sampling units, were discounted. In the remaining straight line sections, every second image was discounted to avoid overlap between consecutive images and the risk of double counting. To ensure consistency in specimen detection, images outside the altitude range 2-4 m were also discounted. The total seabed area analysed from each of the randomly selected sampling units was then standardised to c. 1320 m$^2$ (range 1321-1324 m$^2$) by random selection from the remaining constituent images, typically 715 photographs (range 555-781; SM, Table A.1). All images used for data generation were colour corrected as described by Morris et al. (2014).

2.3. Data analysis

2.3.1. Environmental assessment

Sediment grain size statistics were calculated using Gradistat v.8 software (Blott & Pye, 2001), applying the geometric method of moments (Krumbein, 1936). Mud content was calculated as the proportion of particles <63 μm. Carbonate content wet weight (% wt) was calculated from the difference between TC and TOC.
(assuming all carbonate was CaCO$_3$). The ratio of total organic carbon to total nitrogen (C:N) was calculated as the molar ratio.

Nodule seafloor coverage (% cover) and total surface covered by nodules ($m^2$) were quantified from AUV imagery using the Compact-Morphology-based poly-metallic Nodule Delineation (CoMoNoD) method (Schoening et al., 2017). CoMoNoD attempts to detect all polymetallic nodules present in an image and calculates their areal extent ($cm^2$) based on an ellipsoidal shape projection, to correct for potential underestimation resulting from sediment cover. Only nodules ranging from 0.5 to 60 cm$^2$ (i.e. with maximum diameters of ~1 to ~10 cm) were considered for analysis to avoid inclusion of large non-nodule formations. Angular-shaped cobbles to large rocks and whale bones (min. diameter >10 cm) coated in ferromanganese crust were manually counted and measured. Average nodule cover (%) and total nodule area extent ($m^2$) were calculated across the selected images of each sampling unit.

2.3.2. Megafauna assessment

Images used for megafauna data generation were reviewed in random order to minimise time or sequence-related bias (Durden et al., 2016). Specimens (>10 mm) were identified to the lowest taxonomic level possible (morphospecies: msp), measured using the BIIGLE-DIAS software (Bielefeld Image Graphical Labeller and Explorer: Deep-sea Image Annotation System; (Langenkämper et al., 2017), and assigned to an "attached to hard substrata (i.e. nodules or rocks)" or "attached to sediment/unattached" life habit. To ensure consistency in identification, a megafauna morphospecies catalogue was developed and maintained in consultation with international taxonomic experts and by reference to the existing literature (Amon et al., 2017; Cummings et al., 2014; Dahlgren et al., 2016; Glover et al., 2016b). The likely feeding behaviour of each morphospecies was inferred from similar organisms described in the literature (i.e. Cummings et al., 2014; Iken et al., 2001). Individual metazoan specimen biovolume was estimated, as a proxy for biomass, from two body measurements using the generalised volumetric method described of Benoist et al. (submitted). Despite being comparable in size to metazoan morphospecies, xenophyophores were analysed separately since it is not possible to determine whether they are living from images (Hughes & Gooday, 2004).

A range of ecological parameters were calculated for each replicate sampling unit, including numerical density (ind $m^{-2}$) and proxy biomass density (ml $m^{-2} = g$ fresh wet weight $m^{-2}$). To examine the range of diversity characteristics, Hill’s diversity numbers of order 0, 1, and 2 (Jost, 2006) were calculated as morphospecies richness ($S$), the exponential form of the Shannon index (exp $H'$), and the inverse form of Simpson’s index (1/D), using the ‘vegan’ package implemented in R (Oksanen et al., 2007). Additionally, sample-based morphospecies rarefaction curves were fitted using the analytical method proposed by Colwell et al. (2012), using Estimate S v.9.1 software (Colwell, 2013), by randomly resampling sample data of each study area without replacement, while exp $H'$ and 1/D accumulation curves were calculated with replacement. K-dominance curves were also generated to explore dominance patterns (Clarke, 1990).

2.3.3. Statistical analyses

Generalized linear models (GLM) (Dobson & Barnett, 2008) were built to test whether statistically significant variation in environmental or biological parameters was apparent between study areas, using the ‘car’ package (Fox et al., 2016) implemented in R (R Core Team, 2017). Models were fitted with quasi-Poisson errors in non-negative integer metrics (i.e. density, $S$) with over-dispersion (Gardner et al., 1995), and with
normal errors applied to non-integer variables (i.e. mean grain size, exp H’, 1/D) (Freund & Littell, 1981). Differences in proportional metrics (i.e. nodule coverage, mud content, or functional group percentages) were tested with beta-regression models (Ferrari & Cribari-Neto, 2004) using the ‘betareg’ package (Cribari-Neto & Zeileis, 2010). When statistically significant effects were detected in these global tests, simultaneous tests were applied to make multiple comparisons between individual study areas, using the ‘multcomp’ package in R (Hothorn et al., 2008). Homogeneity of variance and normality assumptions were verified by visual inspection of model histograms and QQ plots. Statistical significance was reported for p < 0.05.

Variations in community composition between study areas were explored using a range of abundance-based multivariate approaches. The Bray-Curtis dissimilarity measure, based on square-root transformed faunal density, as calculated using the ‘vegan’ package in R, was used throughout these analyses. Non-metric multidimensional scaling (nMDS) ordination was used to visualise variations (‘vegan’ package in R). A one-way permutational MANOVA (PERMANOVA) analysis (Anderson, 2001), with follow-up pair-wise tests, was used to test for statistically significant variations in assemblage composition between study areas, using PRIMER v.7 (Clarke & Gorley, 2015). A SIMPER (“similarity percentages”) analysis was performed to assess morphospecies contribution to between-group dissimilarity (‘vegan’ package in R).

2.3.4. Megafauna sampling effort evaluation

To assess the reliability of the biological survey developed in the present study, we investigated the effect of varying sampling unit size (seabed area or individuals covered per sample unit) on the accuracy (i.e. stabilization of mean value) and precision (i.e. coefficient of variation: CV) of different ecological parameters. Image data were first pooled within study area (i.e. across sampling units) and then randomly resampled 1000 times with or without replacement (depending on the target parameter and approach used: see below) into new sampling unit sets of increasing image number size. The mean (or median), the precision (CV), and the confidence intervals (95%) of each parameter were calculated at each sample unit size increase, together with the mean total seabed area and individuals represented by the images composing each subset.

Morphospecies rarefaction curves were fitted using the analytical method proposed by Colwell et al. (2012), using Estimate S v.9.1 software (Colwell, 2013), by randomly resampling image sets of increasing size without replacement. Accumulation curves were interpolated and extrapolated up to 3000 individuals sampled, to balance for differences in fauna densities. Additionally, curves were extrapolated up to 15,000 m² per study area (see SM-Fig S3). The autosimilarity approach proposed by Schneck and Melo (2010), as implemented in the seabed image case by Durden et al. (2016a), was applied to evaluate precision in assemblage description. At each sample size, Bray-Curtis dissimilarity was computed between two groups of images, each randomly selected without replacement and composed by half the total number of images of each set. Metazoan density, biomass density, and exp H’ and 1/D indexes were computed by bootstrapping image subsets resampled with replacement (Buckland et al., 2001). Custom R scripts and the ‘vegan’ package were used to process image data and calculate all ecological indices.

3. Results

3.1. Environmental assessment
Surface sediments (0-10 mm horizon) were dominated by radiolarian-bearing pelagic clay to fine silt particles (diameter <7.8 μm; 58-68% of particles), and medium to very coarse silt grains (diameter = 7.8-63 μm; 28-39% of particles). Mean and median particle size, and mud proportion showed no statistically significant variation between areas, though larger value ranges were evident among the Ridge area samples (Table 1). Subsurface sediments (>50 mm horizon) in the Ridge and Trough showed much greater variability in grain size distributions than those in the Flat area (SM, Fig. A.1; Table A.2). Relative proportions of TOC, TN, and CaCO₃ were almost homogenous across the study areas; no statistically significant differences were detected between study areas (Table 1).

The polymetallic nodules observed during the present study were of an ellipsoidal-flat shape with smooth surfaces. Mean nodule surface area was 2.5 cm², with most nodules <5 cm² (90%), and very few >10 cm² (1%). Nodules in the Flat were larger than in the other areas, though not significantly (Table 1). Average nodule cover was 6.4% and ranged from nodule-free to 38%. The highest mean nodule coverage was recorded in the Flat area (Table 1), although both the within-sampling unit and within-area deviations for this metric were high (SM, Table A.1). Nodule coverage did exhibit a statistically significantly difference between study areas (Table 1), with a statistically significant pair-wise difference between the Flat and Trough areas (Tukey, p < 0.05). Larger (>60 cm² in surface) hard substratum formations coated in ferromanganese crust were especially common in the Ridge area, where angular shaped cobbles, boulders, and whale bones were about ten times more abundant than in the other study areas (Table 1). However, the inclusion of these structures (total survey area surface <6 m²) to the total hard-substratum availability of each sample unit was negligible, even in Ridge samples.
Table 1. Environmental and biological features assessed for each landscape type of the APEI6 with detail on the general linear models (GLM) applied to explore variations of these parameters between study areas. **Sediment parameters:** measured from surface sediment (0-10 mm) and shown as: mean (minimum - maximum) obtained amongst all replicate Megacore samples (n=5) collected in each area. **Parameters:** particle size; mud content (particles <63 μm) percentage; percentages of total organic carbon (TOC) and CaCO$_3$; and molar C$_{org}$/Total nitrogen ratio. **Image parameters:** measured from seafloor imagery data and shown as: mean (95% confidence intervals: lower – upper) calculated amongst all replicate image samples (n=4) collected in each area. **Parameters:** seafloor percentage cover and total nodule area calculated using the CoMoNoD algorithm on seabed imagery (see text); density of non-nodule (>10 cm) hard substrata (boulders and whale bones); total density and proportion of metazoan and xenophyophore individuals (>10mm) split in different functional (SF: suspension feeders; DF: deposit feeders) and attachment-type (OHS: on hard substratum) categories; biomass (grams of fresh wet weight) density inferred using the generalised volumetric method (see text); and diversity: richness, exponential Shannon (exp H'), and inverse Simpson (1/D) indices.
Fig 3. Examples of metazoan megafauna photographed at the APEI6 seafloor during AUV survey. Scale bars representing 50 mm. A) Actiniaria msp-6. B) Actiniaria msp-13. C) Bathygorgia cf. profunda. D) Abyssopathes cf lyra. E) Left: Chonelasma sp.; right: Hyalonema sp. F) Cladorhiza cf kensmithi. G) Bathystylodactylus cf echinus. H) Nematocarcinus sp. I) Sabellida msp-1 (polychaete). J) Left: Freyastera sp.; right: Caulophacus sp. K) Psychropotes cf longicauda L) Benthodytes cf. typica. M) Coryphaenoides sp. N) Typhlonus nasus O and P: probable new Mastigoteuthis sp. same specimen photographed with different cameras. O) Vertical view P) Oblique view. Image taken ~1" prior to the vertical shot.
| Phylum/Class | Group | Morphospecies | Flat | Ridge | Trough |
|-------------|-------|---------------|------|-------|--------|
|             | (*)   | (n)           | OSS  | OHS   | OSS    |
| Ctenophora  | Tentaculata | 2 | 1 | 1 |
| Porifera    | Porifera | 10 | 26 | 45 | 33 | 40 | 52 | 35 |
|             | Desmospongiae | 7 | 42 | 126 | 53 | 119 | 174 | 342 |
|             | Hexactinelidae | 9 | 8 | 19 | 19 | 4 | 17 | 9 |
| Cnidaria    | Scyphozoa | 2 | 5 | |
|             | Aff. Anthozoa | 1 | 4 | 7 | 1 | 5 |
|             | Actiniaria | 13 | 49 | 306 | 39 | 242 | 36 | 93 |
|             | Alcyonacea | 6 | 107 | 821 | 125 | 633 | 52 | 252 |
|             | Antipatharia | 1 | 1 | 1 |
|             | Ceriantharia | 2 | 8 | 3 | 2 | 1 | 5 | 1 |
| Bryozoa     | Cheilostomatida | 4 | 19 | 251 | 44 | 226 | 25 | 95 |
| Annelida    | Echiura | 3 | 21 | 20 | 10 |
|             | Polychaeta | 5 | 63 | 152 | 60 | 173 | 34 | 104 |
| Mollusca    | Bivalvia | 1 | 74 | 140 | 66 |
|             | Gastropoda | 2 | 8 | 1 | 3 |
|             | Octopoda | 1 | 1 | 1 |
|             | Scaphopoda | 1 | 19 | 7 | 8 |
|             | Teuthoidea | 1 | 29 | 29 | 22 |
| Arthropoda  | Aff. Crustacea | - | 33 | 36 | 38 |
|             | Amphipoda | 3 | 12 | 11 | 11 |
|             | Cirripeda | 2 | 2 | 23 | 2 | 14 | 3 | 7 |
|             | Copepoda | 2 | 12 | 2 | 8 |
|             | Decapoda | 8 | 43 | 20 | 30 |
|             | Isopoda | 1 | 16 | 17 | 14 |
|             | Peracarida | 1 | 7 | 8 | 3 |
| Echinodermata | Asteroidea | 5 | 14 | 4 | 4 |
|             | Crinoidea | 6 | 1 | 12 | 4 | 20 | 5 | 19 |
|             | Echinoidea | 5 | 60 | 79 | 45 |
|             | Holothuroidea | 11 | 32 | 19 | 16 |
|             | Ophiuroidea | 4 | 78 | 161 | 38 |
| Chordata    | Urochordata | 2 | 3 | 6 | 1 | 1 | 3 | 7 |
|             | Osteichthyes | 7 | 23 | 18 | 15 |

**Table 2.** Total abundance and taxonomical classification of metazoan morphospecies groups sampled at each APEI6 study area. Abundances show how specimens were found: sessile attached to hard-substratum (OHS); sessile on sediment or mobile fauna (OSS). (*) “Group” level taxonomical classification is not hierarchical, ranges from Class to Family level, to simplify tabulation.
3.2 Megafauna assessment

3.2.1 Metazoan fauna

A total of 6740 megafauna individuals (>10 mm) were recorded in the 15,840 m² of seabed examined during the present study (Table 2). Megafauna were classified into 129 morphspecies and 11 higher taxonomic categories (i.e. Order, Family; Table 2). Rare taxa (≤3 records) represented a third of the total morphspecies richness. The fauna observed (Fig. 3) were predominantly cnidarians (25 msp; 0.18 ind m⁻², ~70% of which were Alcyonacea bamboo corals), sponges (27 msp; 0.07 ind m⁻²), annelids (9 msp; 0.04 ind m⁻²), bryozoans (4 msp; 0.04 ind m⁻²), and echinoderms (32 msp; 0.04 ind m⁻²). Mollusc, crustacean, fish, tunicate, and ctenophore morphspecies were also recorded at lower densities (<0.03 ind m⁻²; Table 2). The metazoan fauna was primarily composed of suspension feeders (78%) and deposit feeders (16%), while predators and scavengers were scarce (4%). Almost 80% of suspension feeding individuals were found attached to polymetallic nodules or other hard substrata. The proportion of nodule-attached individuals was >70% of the total abundance in 37 morphspecies. These "nodule-dwelling" taxa constituted 70% of the total abundance, and 30% of the total richness recorded.

3.2.1.1 Patterns in faunal distribution

Mean metazoan density exhibited a statistically significantly difference between study areas (Table 1), with densities in Flat and Ridge areas higher than those in the Trough (Tukey, p < 0.05). We detected statistically significantly higher densities of suspension feeders in the Flat area compared to the Trough, and statistically significantly higher densities of deposit feeders in the Ridge than in the other study areas (Tukey, p < 0.05). Mean density and proportion of predators and scavengers was similar in all study areas (Table 1). Although the proportion of the fauna attached to nodules was not statistically significantly different between study areas (Table 1), the densities of nodule-attached individuals were statistically significantly higher in the Flat than in the Trough (Tukey, p < 0.01). The mean biomass density recorded across all sampling units was 1.22 g fwtt m⁻² (in c. 1320 m² observed), with no statistically significant difference detected between study areas (Table 1).

Mean morphospecies richness (S) was higher in the Flat, though we found no statistically significant difference between study areas (Table 1). Sample-based morphspecies accumulation curves showed that this pattern was consistent at whole study areas sampling level (Fig. 4.A), and extrapolation of image-based curves predicted the same scenario even when triplicating the total sampling performed per study area (SM-Fig. A.2). Variations in diversity between study areas were more evident at progressively higher Hill’s orders (q > 0). Mean exp H’ and 1/D indices were higher in the Flat and the Ridge areas compared to the Trough, although these differences were statistically significant only in 1/D index (Table 1). These patterns were consistent at whole study areas sampling level (Fig. 4.B-C). We also detected a higher morphospecies dominance in the Trough area, and more even abundances in the Flat and Ridge areas (Fig. 5.A).
Fig. 4. Sample-based diversity accumulation curves calculated for each APEI6 study area. Fauna occurrences of each replicate sample were randomly resampled (with or without replacement) 1000 times at each sampling effort level (n=1-4). A) Species rarefaction calculated without replacement. B) Exponential Shannon index, calculated with replacement. C) Inverse Simpson index, calculated without replacement. Error bars represent 95% confidence intervals between runs.
3.2.1.2. Variations in community composition

Cnidarians, sponges, bryozoans, and echinoderms showed the clearest variations in density between study areas (Fig. 6). In total, 54% of the morphospecies recorded were present in all three study areas, 22% were noted in only two areas, and 24% were detected in one area only area. Most (70%) of the single area records were singletons (SM, Fig. A.3) and the rest rare morphospecies (≤ 5 occurrences). Nevertheless, a statistically significant difference in faunal composition was detected between the study areas (PERMANOVA, $R^2 = 0.39$, $p < 0.001$) (Fig. 7.A), with statistically significant differences apparent in paired comparisons between the Trough and the other study areas (pair-wise PERMANOVA, $R^2=0.36-0.37$, $p < 0.05$). SIMPER analysis showed that variations in the density of 10-15 morphospecies were responsible for 70% of the dissimilarity between study areas, but three morphospecies, a sponge (Porifera msp-5) and two soft corals (Lepidisis msp and Callozostron cf. bayeri), contributed most to the significant dissimilarities. Total density of Porifera msp-5 in the Trough (8.7 ind 100$^{-1}$ m$^{-2}$) was four times higher than in the Ridge and Flat areas; total density of Lepidisis msp in the Flat (3.8 ind 100$^{-1}$ m$^{-2}$) was four times higher than in the Ridge and 20 times higher than in the Trough areas; while total density of C. cf bayeri in the Ridge and the Flat (~2.5 ind 100$^{-1}$ m$^{-2}$) was four times higher than in the Trough area.
Fig. 6. Density variations of different metazoan taxonomic groups between APEI6 study areas. Points represent the mean density of each group calculated amongst the four replicate samples analysed for each area. Error bars represent 95% confidence intervals.

Fig. 7. Interpreted megafauna morphospecies composition nMDS for APEI6 samples. Two-dimensional representations of nMDS developed on Bray-Curtis resemblance matrix calculated from square-root transformed megafauna composition by abundance data. A) nMDS plot developed including only metazoan fauna. B) nMDS plot developed including metazoans and xenophyophores. Arrows indicating the (non-linear) trend in water depth and bathymetric derivatives suggested for each axis.
3.2.1.3. Sample unit size evaluation

Estimates of most of the ecological parameters assessed were consistent at the sample unit size used in the present study (c. 1320 m$^2$ of seabed) (Figs. 8-9). The maximum precision (CV) reached by each parameter with increasing sample unit size ranged from 0.02 to 0.30 (SM, Fig. A.4), yet increases in precision were relatively minor for most parameters with unit sizes >300 individuals (700-900 m$^2$), except for autosimilarity, which required much smaller sizes (>150 individuals; 300-450 m$^2$) to reach an almost constant precision rate (SM, Fig. A.5). Analysis of accuracy yield more variable results. Estimation of mean taxa richness required the largest unit size to stabilise (>500 individuals; 1000-1500 m$^2$) (Fig. 8.A-B), while fauna density required the smallest (>30 individuals; 50-100 m$^2$) (Fig. 9.A-B). Mean autosimilarity required unit sizes >500 individuals (1000-1500 m$^2$) to stabilise (Fig 9.C-F). At this size, mean within-sample similarity was >70% (i.e. two sub-samples of 250 individuals randomly generated from 500 individuals yield an average similarity >70%). Accuracy of biomass density estimates differed between study areas: sample unit sizes >500 individuals were required for stabilisation of median values in the Flat and Trough samples, while stabilisation in the Ridge occurred >250 individuals. Mean exp $H'$ stabilized with unit sizes >350 individuals (700-1000 m$^2$) (Fig. 8.C-D), while mean $1/D$ stabilised with >200 individuals (400-600 m$^2$) (Fig. 8.E-F).
Fig. 8. Variation of the different metazoan community diversity indices used in the present study, as a function of the seabed area or number of individuals encompassed by the sample unit size. Lines represent mean values across the 1000 randomisations performed at each sample unit size increase, for each study area collated sample (n=3) (see methods). Shadowing representing 95% confidence intervals. Ticks on x-axis indicate the sampling unit size used in the present study (replicate sample areas = 1320 m²).

A and B: Rarefied metazoan morphospecies accumulation curves. A) Area-based accumulation curves. B) Individual-based accumulation curves. Dashed lines represent sample extrapolation.

C and D: Variation of metazoan exp H' diversity index. E) Area-based mean exp H'. F) Individual-based mean exp H'.
mean exp $H'$. **E and F:** Variation of metazoan $1/D$ diversity index. **I)** Area-based mean $1/D$. **J)** Individual-based mean $1/D$.

**Fig. 9.** Variation of the different metazoan community parameters used in the present study as a function of the seabed area or number of individuals encompassed by the sample unit size. Lines represent mean or median values across the 1000 randomisations performed at each sample unit size increase, for each study area collated sample ($n=3$) (see methods). Shadowing representing 95% confidence intervals. Ticks on x-axis indicate the sampling unit size used in the present study (replicate sample areas = 1320 m$^2$). **A and B:** Variation of mean metazoan density. **A)** Area-based mean
density. B) Individual-based mean density. C and D: variation of median metazoan biovolume concentration. A) Area-based median biovolume. H) Individual-based mean biovolume. E and F: autosimilarity curves showing mean Bray-Curtis dissimilarity index calculated amongst pairs of metazoan samples. E) Area-based autosimilarity curves. F) Individual-based autosimilarity curves.

3.2.2. Xenophyophore fauna

Xenophyophore tests (Fig. 10) numerically dominated the megafauna recorded during the present study; being overall, six times more abundant than metazoans, and reaching a peak density of 17 ind m\(^{-2}\) in an image from the Ridge area. Mean xenophyophore density exhibited a statistically significantly difference between study areas (Table 1), with densities in the Ridge higher than those in the Trough (Tukey, p < 0.01). The recently described species *Aschemonella monile* (Gooday et al., 2017a) (Fig. 10.B) dominated the fauna, having mean densities of 3.27, 1.51, and 0.85 ind m\(^{-2}\) in the Ridge, Flat, and Trough areas respectively. The numerical dominance of xenophyophores has substantial impact on the perception of relative faunal diversity among the study areas (Fig. 5.B), inclusion of these foram taxa markedly increased rank 1 dominance (Berger-Parker index) in the Flat and Ridge areas, indicating a very substantial reduction in diversity in the Ridge area particularly.

Xenophyophores were classified in 23 morphospecies. Xenophyophore faunal composition exhibited statistically significant variation between study areas (PERMANOVA, \(R^2 = 0.55, p < 0.001\)), and statistically significant differences detected in all paired comparisons (pairwise PERMANOVA, \(R^2 = 0.39-0.61, p < 0.05\)). Joint analysis of xenophyophore and metazoan faunal composition yielded comparable results (Fig 7.B) to those obtained from the analysis of metazoan taxa only (Fig 7.A); statistically significant variations between study areas (PERMANOVA, \(R^2 = 0.48, p < 0.001\)) were led by statistically significant differences between the Trough and the other study areas (pairwise PERMANOVA, \(R^2 = 0.37-0.45, p < 0.01\)).

![Fig. 10. Examples of xenophyophore megafauna photographed at the APEI6 seafloor during AUV survey. Scale bars representing 50 mm. A) Reticulammina msp. B) Aschemonella monile. C) Fan-shaped Psammina msp. D) Indeterminate Psamminid msp, possibly Shinkaiya or Syringammina. E) Syringammina cf limosa. F) Triradiate Psammina msp, possibly P. multiloculata.](image-url)
4. Discussion

4.1. Environmental setting at the APEI6

The high homogeneity in particle size and nutrient availability found across the APEI6 study areas suggests that these factors may be consistent over scales broader than the tens of kilometres between areas studied here. Our results were somewhat unexpected since variations in sediment grain-size distributions and particulate organic matter have commonly been reported between landscape types in previous assessments in the north Atlantic abyss (Durden et al., 2015; Morris et al., 2016), where bottom current speed ranges (Vangriesheim et al., 2001) are comparable to those expected at the APEI6, but sediments were coarser and more heterogeneous. Surface sediment particle sizes at the APEI6 were comparable in range to those found in eastern CCZ contract areas (Khripounoff et al., 2006; Mewes et al., 2014; Pape et al., 2017). Although sediments in these more southerly areas exhibit bimodal particle size distributions, being primarily composed of clays and fine silts (<6.3 μm), but with higher proportions of sands (>63 μm) than at the APEI6. Ranges of TOC (0.41-0.44%) and C:N ratios (3.8-4.1) were also comparable to those reported in eastern CCZ contract areas (Khripounoff et al., 2006; Mewes et al., 2014; Pape et al., 2017). This suggests that the sedimentary environment of the APEI6 may be generally representative of the environment found at a larger scale (i.e. eastern CCZ), although further exploration in other contract areas would be required to draw more precise conclusions in this regard.

Variations in nodule abundance could be indicative of environmental change between study areas. Locally stronger bottom-water currents reducing deposition rates are presumed to enhance nodule formation (Mewes et al., 2014; Skornyakova & Murdmaa, 1992). Higher nodule abundances in mild slopes and elevated seafloors, such as the Flat and the Ridge areas, have commonly been linked with low sedimentation rates (Frazer & Fisk, 1981; Mewes et al., 2014). Yet convergent channelling of bottom currents in bathymetric valleys, such as the Trough area, has also been suggested to limit deposition enhancing nodule growth (Peukert et al., 2018). The more irregular nodule coverage we observed in the Ridge (SM, Table 1) concurs with previous descriptions of hilltop environments at the CCZ (Jung et al., 2001; Margolis & Burns, 1976; Skornyakova & Murdmaa, 1992). In these, current circulation over rugged seafloor can generate scattered redistribution of surface materials (Jung et al., 2001; Nasr-Azadani & Meiburg, 2014; Peukert et al., 2018), which may have reduced the sediment blanketing of hard structures (i.e. rock fragments, whale bones) and trace fossils (Durden et al., 2017b) within the Ridge.

4.2. Sample unit size evaluation

Narrowing of the precision range with increasing sample unit size was apparent in all parameters (SM, Fig. A.5), as was expected from previous image-based assessments (Durden et al., 2016b), but the accuracy of each parameter (Figs. 8-9) showed a different sensitivity to this factor. The sample unit size we used in this study (c. 1320 m² of seafloor) was therefore sufficiently large for reliable estimation of fauna density, diversity of higher orders, and community dissimilarity, but was arguably too small for the assessment of taxa richness and biomass density patterns, as not all samples collected (SM, Table A.1) contained the minimum of 500 individuals suggested by our analysis for a reliable characterisation of these two parameters.
It is conceivable that the higher sensitivity to sample size was a “rarity-driven” effect. On the one hand, the low density combined with the high taxa richness we found at the APEI6 yield high rates of taxon rarity in our assessment. This is commonplace in abyssal sampling (Smith & Demopoulos, 2003), but has a negative effect on the accuracy of those diversity indices more sensitive to rare taxa, such as richness (Magurran, 2004; Soetaert & Heip, 1990). On the other hand, the high rarity of particularly large individuals appeared to restrict the accuracy of biomass density assessment, especially within the Flat and the Trough areas, where larger-sized fauna were even rarer. Predominance of the smaller taxa is common in low-productivity abyssal habitats (Rex et al., 2006; Smith et al., 2008a), yet large megafaunal species have an important ecological role in these environments (Billett et al., 2001; Ruhl et al., 2008), and these appear to require rather large sample unit sizes to be best characterised (i.e. 250-500 ind: this study). Higher rarity rates are therefore expected in abyssal megafauna surveys as an artefact of lower sample unit sizes, which can influence other parameters such as diversity or community composition analysis.

Our results underline that sampling unit evaluation is important for assessing the reliability of ecological patterns inferred from abyssal sampling. Minimum sample sizes for accurate estimation exhibited by different parameters were extremely variable (range: 30-500 individuals; 100-1500 m² of seafloor per sample unit). This means that with sampling units <400 m², most biological parameters estimated here would have been largely inaccurate and imprecise. For instance, it is likely that no variation in diversity nor community composition between areas might have been detected if transect size of this study had been set below 600 m², which would have biased the overall conclusions. This underlines the importance of appropriate tuning of the sampling unit size in abyssal ecology, especially at the CCZ, where these may have a paramount influence on conservation policy (Durden et al., 2017a; Levin et al., 2016). However, sample unit analyses have been commonly ignored in most assessments of megafauna at the CCZ (Stoyanova, 2012; Tilot et al., 2018; Vanreusel et al., 2016; Wang & Lu, 2002). This adds a level of difficulty to the already constrained comparability between studies in the region (Amon et al., 2016), and bounds the study of ecological patterns at the regional scale.

The use of different sampling devices and methods (i.e. definition of megafauna size, camera altitude, sampling unit size), is an ongoing issue for the comparability of image-based analyses (Durden et al., 2016b), especially at the CCZ (Amon et al., 2016). For example, megafauna assessments performed by Tilot et al. (2018) and Stoyanova (2012) using a different camera set-up reported densities ten times lower than those reported by Vanreusel et al. (2016) at the same contractor areas (IFREMER-2 and IOM-2, respectively). The application of improved imaging systems may have increased the apparent megafauna densities, influencing diversity estimations. This stresses the need for a standardization of both assessment method and morphotype taxonomy across the CCZ, to enable more reliable comparisons between the various APEI and claim areas, and simplify the detection of possible biogeographical boundaries across the CCZ.

4.3. Landscape ecology of metazoan megabenthos

Differences in megafauna density across the landscape types studied were predominately driven by variations in suspension feeder abundance (Table 1), particularly sessile cnidarians (Fig. 6). Potential topographically-enhanced bottom water current speeds have previously been suggested to promote the development of suspension feeding fauna in the abyss (Durden et al., 2015; Smith & Demopoulos, 2003; Thistle et al., 1985). Suspension feeders usually dominate the megabenthos in the CCZ and show higher abundances in areas with higher nodule density (Amon et al., 2016; Stoyanova, 2012; Vanreusel et al., 2016). Factors promoting higher nodule densities also enhance the development of suspension feeders (Vanreusel
et al., 2016); for example, in the present study most suspension feeders (80%) were attached to nodules. Suspension feeder density, and relative abundance, may therefore be related to both the availability of hard substrata and local enhancements in bottom water currents, and that the latter two factors may themselves be related. These factors suggest that low slopes or elevated topographies, as found at the Flat and Ridge areas, enhance suspension feeder densities increasing the overall metazoan standing stock of these areas, as compared to depressions, like the Trough area.

Variations in functional composition between study areas were driven by the distribution of deposit feeder fauna, suggesting enhanced resource availability for this group in the Ridge. This could indicate a higher food supply at the more elevated seafloor of the Ridge, owing to less particulate organic carbon loss during sinking (Smith et al., 2008a), but this is likely a small effect at abyssal depths for changes of few hundred meters (Lutz et al., 2007). Moreover, sediment TOC exhibited no statistically difference between study areas, nor was there a statistically significant difference in the C:N ratio. This suggests that, if there were variations in food supply for deposit feeders, these may either have occurred at a finer spatial scale (i.e. patch accumulations: Lampitt, 1985; Smith et al., 1996), or be related with the quality rather than the quantity of the available resource (Ginger et al., 2001).

Deposit feeder abundance was predominantly composed by ophiuroids (Table 2), and the density of these was both positively correlated with xenophyophore test abundance ($r_s = 0.77-0.79$, $p < 0.01$), as was the density of predator and scavenger fauna, although at a weaker level ($r_s = 0.65$, $p < 0.05$). Biological structures can be important in the generation of habitats in the deep-sea (Buhl-Mortensen et al., 2010). Such associations are common in the in the north-eastern Pacific abyss, for instance, sponge stalks can serve as microhabitats for species-rich assemblages of suspension-feeder epifauna (Beaulieu, 2001), or for the attachment of octopod egg clutches during brooding (Purser et al., 2016). Co-occurrence of xenophyophores and ophiuroids has been previously documented in eastern Pacific seamounts (Levin et al., 1986; Levin & Thomas, 1988). Levin (1991) suggested that xenophyophore tests represent a substrate that can function as refuge from predators and or nursey habitat for juvenile mobile metazoans, like ophiuroids. Xenophyophore test substratum has shown to play a crucial role in the regulation of meiofauna and macrofauna communities at the CCZ (Gooday et al., 2017b), and our results suggest that these may also be important in the functional structuring of megafauna.

Heterogeneity diversity measures indicated clearly reduced diversity in the Trough relative to Flat and Ridge areas, markedly so in the case of 1/D index (Fig. 4.C). The dominance component of diversity was higher in the Trough (Fig. 5.A) unless xenophyophores were included (Fig. 5. B). The lower metazoan heterogeneity diversity of the Trough was caused by a general decrease in the density of most morphospecies, combined with a clearly higher abundance of the sponge Porifera msp-5, possibly better adapted to a presumably more disturbed environmental regime in this area. Porifera msp-5 was amongst the smallest morphospecies we detected (mean diameter: 13.1 ± 3.1 mm; without elimination of individuals >10 mm: 8.8 ± 3.4 mm) and was predominantly found (>70%) encrusting nodules. A recent study revealed a similar dominance, also exhibited by a small nodule-encrusting sponge (Plenaster craigi) in the eastern CCZ (Lim et al., 2017). Our results highlight the importance of a standardized detection of small -and usually predominant- taxa for robust assessment of heterogeneity diversity in CCZ megafauna communities.

Previous CCZ megafauna studies related the presence of nodules with increased metazoan richness (Amon et al., 2016; Tilot et al., 2018; Vanreusel et al., 2016). Although we found no direct correlation between nodule availability and sample diversity (of any order), it is possible that the overall lower nodule availability of the Trough played an important role in the reduction of evenness we observed there, since most of the
APEI6 metazoan abundance was composed by nodule-dwelling taxa. However, the survey design applied in this study was optimised for the detection of patterns at a relatively broad scale (few kilometres), compared to the tens of meters at which nodule coverage variations usually occur at the CCZ (Peukert et al., 2018). Moreover, our sampling effort evaluation highlighted that two samples did not contain a sufficiently large specimen coverage (<500 ind) to reliably assess richness patterns, and that this may also have affected the estimation of richness in previous studies. Further analysis of the APEI6 dataset at a finer spatial scale (in prep.) shall further expand and contextualize the precise relation between nodules and both the richness and evenness components of megafauna diversity.

Statistically significant differences in megafaunal density, functional composition, evenness and taxon composition were variously apparent between the landscape types studied. Previous studies showed that even modest topographic elevation (i.e. hills) has substantive effect on abyssal megafaunal compositions (Durden et al., 2015; Leitner et al., 2017; Stefanoudis et al., 2016). However, in this study the assemblages of the Flat and Ridge (in previous studies: plain and hill areas, respectively) showed a higher similarity, as compared to the Trough area, where most taxa densities were somewhat reduced and the dominant morphospecies shifted from colonial bamboo corals to a small-encrusting sponge. The higher availability of nodule and xenophyophore-test substrata in the Ridge and the Flat possibly increase the heterogeneity of these areas, enhancing the development of a more even assemblage type. Variations in heterogeneity commonly regulate niche diversification processes (Tews et al., 2004), exerting a fundamental influence on the diversity and structure of deep-sea benthic communities (Levin et al., 2001). Thus, our results suggest that by regulating nodule and xenophyophore test availability-and presumably bottom current speeds-geomorphological variations play a crucial role in the structuring of the CCZ megabenthos at the landscape scale.

### 4.4. Ecological significance of megafaunal xenophyophores

Test densities were almost four times higher in Ridge samples than in the Trough, and almost twice as dense as within the Flat area. Previous studies have also described higher relative xenophyophore densities in sites with sloping topography and enhanced water motion (Levin & Thomas, 1988; Stefanoudis et al., 2016). The feeding modes and strategies of xenophyophores remain uncertain (Gooday et al., 1993; Laureillard et al., 2004), with passive particle-trapping, suspension or deposit feeding mechanisms noted (Kamenskaya et al., 2013; Levin & Gooday, 1992). Accepting our inability to distinguish living specimens, that *A. monile* specimens alone represent over 70% of all megafauna observed in the Ridge area suggests considerable ecological significance for this taxon, and the xenophyophores as a group. Note that our identification of 23 xenophyophore morphospecies is undoubtedly an underestimate of their true species diversity, particularly in the CCZ where these are exceptionally diverse (Gooday et al., 2017b; Kamenskaya et al., 2013).

Inclusion of xenophyophores substantially affected the assessment of biological diversity, particularly in respect to heterogeneity diversity. It is conceivable that this was a ‘true body size’ mismatch effect. For example, Levin and Gooday (1992) suggest a protoplasm volume of 1 to 0.01% of test volume. This means that the mean test biomass of *A. monile* at the APEI6 was possibly <1 mg fwwt ind⁻¹ - provided its devoid of protoplasm test interior (Gooday et al., 2017a)- while the mean biomass of the smallest taxa recorded in the metazoan fraction ranged between 40-60 mg fwwt ind⁻¹. As smaller individuals are largely more abundant in the abyss (Smith et al., 2008a), it is likely that the inclusion of xenophyophores artificially reduced the heterogeneity diversity, given that ~ 1 mg fwwt sized individuals from other taxa were not possible detect
and hence not represented in analyses. Consequently, general interpretation of diversity is probably best limited to the metazoan only assessments.

5. Conclusions

This paper presents an ecological assessment of megabenthic faunal distribution in response to seafloor geomorphology at the CCZ. Differences in the megafaunal ecology between landscape types of the APEI6 manifested as changes in standing stock, functional structure, diversity, and community composition. This shows that local geomorphological variations can play an important role in the structuring of the CCZ megabenthos. Our assessment somewhat concurs with previously reported differences between abyssal hills and adjacent plains in North Atlantic megafauna (Durden et al., 2015), and in fish populations at the CCZ (Leitner et al., 2017). Yet we have added a level of abyssal landscape heterogeneity (troughs), where megafauna showed the clearest variations. Analyses of sampling effort support our results: the collected sample size enabled a stable estimation of key biological metrics, but also highlighted limitations in understanding of some parameters.

Benthic ecology has been suggested to be regionally controlled by a gradient of POC-flux to the seafloor at the CCZ (Smith et al., 2008b; Veillette et al., 2007). However, local environmental factors presumably regulated by local geomorphology, such as bottom water flows (Mewes et al., 2014), or the availability of nodule (Peukert et al., 2018) and xenophyophore test (this study) substrata may play a key role at the local level, possibly influencing habitat heterogeneity across the CCZ. This complexity needs to be reflected in both local (claim-scale) and regional (CCZ-scale) management plans (Durden et al., 2017a; Levin et al., 2016) and in the design of future monitoring strategies aimed to characterise and preserve biodiversity in the CCZ.

6. Acknowledgements

We thank the captain and crew of RRS James Cook, and the Autosub6000 technical team for their assistance during cruise JC120. We grateful to all the taxonomic experts consulted (directly or indirectly) during the generation of our megafauna catalogue: Diva Amon, Tina Molodtsova, Andrey Gebruk, Andrew Gates, Sergi Taboada, David Billelt, Henk-Jan T. Hoving, Tammy Horton, Tomoyuki Komai, Daniel Kersken, Pedro Martinez Arbizu, Christopher Mah, Michel Roux, Jeff Drazen, Rich Mooi, David Pawson, Tim O’Hara, Helena Wiklund, Mary Wicksten, Andrei Grischenko, Astrid Leitner, Craig Young, Dhugal Lindsay, and Janet Voight. We also acknowledge the inputs of Katleen Robert in geomorphological analysis, and Sabena Blackbird and James Hunt in sediment analyses.

This work forms part of the Managing Impacts of Deep-seA reSource exploitation (MIDAS) project of the European Union Seventh Framework Programme (FP7/2007-2013; grant agreement no. 603418), and was additionally funded by the UK Natural Environment Research Council. VAIH was also funded through the European Research Council Starting Grant project CODEMAP (grant no. 258482).
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Table A.1. Summary metadata for each sampling unit analysed during the present study. Coordinates (latitude, longitude; in decimal degrees) indicate the central position of each sampling unit. Images (n) are the total number of images processed per transect. Average percentage of polymetallic nodule coverage (± St Dev) was calculated as the mean of the percentage coverages values obtained for each transect image using the CoMoNoD algorithm. Visual annotations: Total abundance of fauna (total counts >10 mm), total morphospecies richness, and percentage of fauna detected on hard substratum (OHS), separated into metazoan and xenophyophore taxa. Landscape types: FL= Flat, RI= Ridge, TR=Trough

| Sampling unit | Centre latitude (°) | Centre longitude (°) | Images (n) | Seafloor area (m²) | Nodule cover (%) | Abundance | Taxa | OHS (%) | Abundance | Taxa | OHS (%) |
|---------------|---------------------|----------------------|------------|--------------------|-----------------|-----------|------|--------|-----------|------|--------|
| FL 3          | 17.262              | -123.072             | 774        | 1322               | 12.3 (± 3.2)    | 745       | 68   | 75     | 1722      | 18   | 49     |
| FL 33         | 17.233              | -123.027             | 775        | 1321               | 5.4 (± 1.2)     | 532       | 76   | 72     | 2952      | 18   | 58     |
| FL 26         | 17.225              | -123.013             | 781        | 1323               | 10.5 (± 4.0)    | 634       | 71   | 72     | 2749      | 18   | 55     |
| FL 39         | 17.217              | -123.001             | 778        | 1321               | 12.4 (± 5.6)    | 677       | 67   | 56     | 4365      | 19   | 59     |
| RI 2          | 17.282              | -122.878             | 720        | 1324               | 9.1 (± 8.0)     | 537       | 63   | 65     | 5687      | 20   | 36     |
| RI 9          | 17.297              | -122.883             | 729        | 1322               | 5.5 (± 2.1)     | 552       | 67   | 61     | 4731      | 20   | 28     |
| RI 15         | 17.310              | -122.888             | 666        | 1323               | 3.6 (± 1.1)     | 629       | 59   | 47     | 4508      | 19   | 39     |
| RI 21         | 17.323              | -122.891             | 765        | 1321               | 6.2 (± 7.5)     | 720       | 70   | 69     | 6379      | 20   | 30     |
| TR 15         | 17.264              | -122.830             | 694        | 1324               | 1.7 (± 1.4)     | 382       | 54   | 60     | 1202      | 18   | 26     |
| TR 18         | 17.248              | -122.821             | 555        | 1322               | 8.0 (± 3.4)     | 506       | 72   | 43     | 4217      | 18   | 52     |
| TR 25         | 17.223              | -122.817             | 709        | 1324               | 3.2 (± 2.3)     | 546       | 65   | 67     | 1237      | 19   | 45     |
| TR 29         | 17.220              | -122.820             | 623        | 1323               | 1.8 (± 1.9)     | 280       | 47   | 56     | 419       | 18   | 37     |
Fig. A.1. Sediment grain-size distributions plots generated for different sediment horizons sampled at the APEI6 seafloor. Lines representing mean frequency across each of the five replicate megacore samples collected per landscape type. Shadowed areas representing maximum and minimum values per replicate set. Each core was initially sliced and split into nine different sediment depths (0-5, 5-10, 10-15, 15-20, 20-30, 30-50, 50-100, 100-150, and 150-200 mm). Sediment grain-size distributions at each horizon were measured independently by laser diffraction. Horizons 0-5, 5-10, 10-15, 15-20, 20-30, 30-50 were averaged into a 0-50 mm depth.
Table A.2. Particle size statistics calculated applying a geometric method of moments for different sediment horizons sampled at the APEI6 seafloor. Values representing maximum and minimum ranges across each of the five replicate megacore samples collected per landscape type. Each core was initially sliced and split into nine different sediment depths (0-5, 5-10, 10-15, 15-20, 20-30, 30-50, 50-100, 100-150, and 150-200 mm). Sediment grain-size distributions at each horizon were measured independently by laser diffraction. Horizons 0-5, 5-10, 10-15, 15-20, 20-30, 30-50 were averaged into a 0-50 mm depth, prior to the statistical processing.

| Horizon       | Statistic | Flat          | Ridge         | Trough         |
|---------------|-----------|---------------|---------------|----------------|
|               | Mean      | 7.15 - 7.61   | 6.71 - 9.21   | 7.60 - 8.50    |
|               | St dev    | 2.82 - 3.03   | 2.54 - 4.77   | 2.99 - 4.04    |
|               | Skewness  | 0.96 - 1.50   | 0.46 - 2.02   | 0.86 - 1.86    |
|               | Kurtosis  | 4.50 - 7.35   | 3.22 - 8.29   | 3.79 - 7.50    |
|               | Mode      | 7.19          | 7.19          | 7.19           |
| 0 to 5 cm     | D50       | 6.47 - 6.70   | 6.29 - 7.40   | 6.61 - 7.03    |
|               | Mean      | 6.50 - 8.52   | 6.56 - 8.72   | 7.49 - 11.16   |
|               | St dev    | 2.73 - 3.66   | 2.71 - 2.78   | 2.95 - 3.97    |
|               | Skewness  | 0.89 - 1.56   | 0.46 - 1.15   | 0.67 - 1.07    |
|               | Kurtosis  | 4.18 - 6.60   | 3.20 - 5.82   | 2.73 - 5.20    |
|               | Mode      | 7.19          | 7.19          | 7.19           |
| 5 to 10 cm    | D50       | 5.97 - 6.89   | 5.98 - 7.95   | 6.63 - 8.17    |
|               | Mean      | 6.06 - 7.24   | 6.33 - 11.67  | 6.47 - 20.08   |
|               | St dev    | 2.10 - 3.00   | 2.43 - 6.73   | 2.48 - 4.72    |
|               | Skewness  | 0.06 - 1.08   | 0.12 - 1.64   | 0.06 - 0.90    |
|               | Kurtosis  | 2.75 - 5.02   | 2.34 - 6.26   | 1.79 - 6.14    |
|               | Mode      | 7.19          | 7.19          | 7.19           |
| 10 to 15 cm   | D50       | 6.04 - 6.48   | 5.87 - 9.50   | 6.29 - 16.45   |
|               | Mean      | 5.77 - 8.55   | 6.07 - 10.61  | 6.35 - 20.15   |
|               | St dev    | 2.19 - 4.28   | 2.50 - 2.94   | 2.56 - 5.07    |
|               | Skewness  | 0.01 - 1.93   | 0.13 - 1.18   | -0.14 - 1.85   |
|               | Kurtosis  | 2.59 - 7.50   | 2.35 - 6.17   | 1.77 - 8.79    |
|               | Mode      | 7.19          | 7.19          | 7.19 - 115.00  |
| 15 to 20 cm   | D50       | 5.69 - 6.63   | 5.70 - 10.28  | 5.93 - 31.85   |
Fig. A.2. Morphospecies rarefaction curves extrapolated to ~three times the area sampled at each landscape type for the present study. Triangles showing the total size of the sample analysed at each geomorphology. Expected richness with sample coverages of 15,000 m² show a lower richness at the Trough (~107 msp) compared to the Flat (~130 msp) and the Ridge (~134 msp) areas, but confidence intervals continued to overlap between curves. Whole geomorphological units sample size (5280 m²) covered 85-90% of the expected richness > 15,000 m².

Fig. A.3. Metazoan morphospecies surveyed for the present study. Venn diagram showing the total number of metazoan taxa shared between each combination of landscape types of the APEI6. In brackets: singleton morphospecies.
Fig. A.4. Variations of the coefficient of variation with increasing sample size calculated for the main ecological estimators used in the present study. Coefficients of variation were calculated as the standard deviation divided by the mean of each estimator at each different sampling effort (see methods), for the whole metazoan dataset collected for each landscape type of the APEI6.
Fig. A.5. Relative variations of the coefficient of variation with increasing sample size calculated for the main ecological estimators used in the present study. Coefficients of variation were calculated as the standard deviation divided by the mean of each estimator at each different sampling effort (see methods), for the whole metazoan dataset collected for each landscape type of the APEI6, and then divided by the minimum value exhibited in each along the sample size spectrum assessed.