25th
INTERNATIONAL SEABED AUTHORITY
ANNIVERSARY
1994 - 2019

Deep CCZ Biodiversity Synthesis Workshop

Friday Harbor, Washington, USA, 1-4 October 2019

WORKSHOP REPORT

Contents

INTRODUCTION

1. The International Seabed Authority (ISA) is the organization through which, in accordance with the UN Convention on the Law of the Sea ("the Convention") and 1994 Agreement relating to the implementation of Part XI of the Convention ("1994 Agreement"), the States Parties to the Convention administer the mineral resources of the Area, and control and organize current exploration, as well as future mining activities, in the Area for the benefit of mankind as a whole.

2. The Authority is also mandated to take necessary measures with respect to activities in the Area to ensure effective protection for the marine environment from harmful effects from activities in the Area and to adopt appropriate rules, regulations and procedures for, *inter alia*, the prevention, reduction and control of pollution and other hazards to the marine environment, the protection and conservation of the natural resources of the Area and the prevention of damage to the flora and fauna of the marine environment.[1]

3. Pursuant to this mandate, the Council of ISA (Council), during its seventeenth session in 2012, on the basis of the recommendation of the Legal and Technical Commission, approved, in its decision ISBA/18/C/22, an Environmental Management Plan (EMP) for the Clarion-Clipperton Zone (CCZ).[2] This included the designation of a network of nine "Areas of Particular Environmental Interest" (APEIs) as an integral part of that plan.

4. Likewise, in accordance with the above-noted decision, the Council requested the Legal and Technical Commission (Commission) to report to the Council on the implementation of the environmental management plan, highlighting that the Plan will be applied in a flexible manner so that it may be improved as more scientific, technical and environmental baseline and resource assessment data are supplied by contractors and other interested bodies. Furthermore, it requested the Commission to make recommendations, where appropriate, to the Council relating to the network of APEIs, on the basis of the results of workshops with a view to redefining, where necessary, the details of the size, location and number of required areas of particular environmental interest.

5. Subsequently, in July 2016, the Commission considered a report prepared by the secretariat [3] in which the progress made in the implementation of the plan and the steps to be taken until 2021 were recalled. In its deliberations, the Commission also noted the suggestion to create two additional APEIs, based on new work by contractors. The Commission decided to consider holding a scientific workshop to determine the suitability or need for amendment of the APEIs, indicating that such a scientific workshop should define the size, location and number of additional areas for APEIs in order to enable the Commission to make a recommendation to the Council. [4]

6. The design of the APEI network was based on recommendations formulated in a workshop in 2007 organized/chaired by Craig R. Smith, J. Tony Koslow and Malcolm R. Clark with funding from the Pew Charitable Trusts. The workshop examined elements of protected-area design and synthesized the biodiversity and ecosystem data from the CCZ available at the time. Since then, a substantial number of research and contractor surveys have been conducted within the CCZ region. As a result, seafloor biodiversity and ecosystem data have been collected in the CCZ, including from several APEIs, which

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[1] United Nations Convention on the Law of the Sea, art.145.

[2] See ISBA/17/LTC/7; ISBA/17/C/19 and ISBA/18/C/22.

[3] ISBA/22/LTC/12

[4] See ISBA/22/C/17.

provide the basis for a new scientific review and synthesis in support of a review of the CCZ APEI network.

7. Part of the review process currently underway by the LTC includes the consideration of potential additional APEIs that could be established in order to close some “gaps” in the existing network. Hence, the new data from existing APEIs, and evaluation of biodiversity patterns relative to contractor areas, are highly relevant to considering the effectiveness of the current APEI network and to the siting of new APEIs if additional protection is warranted.

8. In light of this context, the ISA and the DeepCCZ Project (University of Hawaii) convened an expert workshop titled *Deep CCZ Biodiversity Synthesis* in Friday Harbor, Washington, USA, from 1 to 4 October 2019, with financial support from the Gordon & Betty Moore Foundation, Pew Charitable Trusts, the University of Hawaii and the International Seabed Authority.

9. The workshop aimed to review the biodiversity and environmental data collected since the initial design of the CCZ APEI network at the 2007 workshop and final adoption by ISA in 2012, and to generate a broad synthesis of biodiversity information along and across the CCZ. In particular, the workshop focused on: (i) reviewing and analyzing recent seafloor ecosystem data from the CCZ; (ii) synthesizing patterns of benthic and benthic-boundary layer biodiversity, community structure, species ranges, genetic connectivity, ecosystem function, and habitat heterogeneity within the CCZ, (iii) assessing the representativity of the APEIs relative to exploration contract areas, and (iv) identifying areas where additional representative APEIs could be situated to include habitats poorly represented in the current APEI network.

10. The meeting was attended by experts from Belgium, France, Germany, Japan, Jamaica, Republic of Kiribati, the Netherlands, New Zealand, Poland, Portugal, Republic of Korea, Singapore, Sweden, Trinidad and Tobago, United Kingdom, and the United States of America. The full list of participants is included in Annex I.

ITEM 1. OPENING OF THE WORKSHOP

11. Dr. Luciana Genio delivered opening remarks on behalf of the secretariat of the International Seabed Authority. She started by thanking the Gordon & Betty Moore Foundation, the Pew Charitable Trusts and the University of Hawaii for providing financial support through the DeepCCZ project. She also expressed her appreciation to the co-chairs, Dr. Craig Smith and Dr. Malcolm Clark, for their contribution in preparing the workshop in close collaboration with the Secretariat, and to the participants and other experts for contributing scientific data/information as inputs to the workshop. She highlighted the substantial amount of research and contractor surveys undertaken by ISA contractors that have been conducted in the CCZ, including in several APEIs. Their scientific work through exploration activities has generated substantial level of new knowledge that will be crucial for the LTC to undertaking its review of the existing APEI network in the CCZ. Such review would include the consideration of potential needs for additional APEIs. She emphasized the broader context through which the ISA is mandated to develop and implement regional environmental management plans (REMPs) in all regions where exploration contracts currently exist. REMPs are a critical element and will be an integral part of measures to implement Article 145 of the Convention, which establishes the Authority’s role to ensure effective protection of the marine environment. Finally, she highlighted the Authority’s efforts to compile and share environmental data through the ISA database (*DeepData*), which provides an important platform to

engage relevant stakeholders and scientific communities, and can give key inputs to the development and implementation of the REMPs.

12. Dr. Mary Turnipseed delivered opening remarks on behalf of the Gordon & Betty Moore Foundation. Dr. Turnipseed indicated that the Gordon & Betty Moore Foundation was pleased to be a co-supporter of this critically important workshop, that the Moore Foundation prides itself on its support for groundbreaking scientific endeavors, and that this workshop is a good example. The Moore Foundation believes that the scientific synthesis and science-based recommendations on preserving the biodiversity and ecosystem function of the CCZ developed at the workshop will be essential to the development of sound policy for the management of this area. Moore also recognizes the importance of authorship of scientific reports, and was glad to see that the report will provide stakeholders and policymakers with the ability to identify and reach out to the experts responsible for each section.

13. Dr. James Palardy delivered opening remarks on behalf of the Pew Charitable Trusts in support of the scientific synthesis conducted in this workshop. He welcomed all attendees and noted that decisions are made using available rather than ideal data. Dr. Palardy encouraged participants to provide rigorous science that takes into account the needs of decision makers, including identifying best available information and policy-relevant trends, supplying weights of evidence, and identifying appropriate assumptions when insufficient data are available to draw conclusions.

14. Dr. Craig Smith, the DeepCCZ project leader and co-chair of the workshop, welcomed participants to the workshop, solicited self-introductions by all participants, and explained logistical aspects of the workshop.

ITEM 2. ADOPTION OF THE AGENDA AND ORGANIZATION OF WORK

15. After a brief introduction of the procedures by the co-chairs, Drs. Craig Smith and Malcolm Clark, the participants adopted the agenda and the proposed organization of work (see Annex II).

16. It was agreed that the workshop would be organized in plenary and breakout-group sessions as per the agenda. Breakout groups would have facilitators and rapporteurs to lead and record discussion.

ITEM 3. WORKSHOP BACKGROUND, SCOPE AND EXPECTED OUTPUTS

17. The participants were provided with several presentations as background and context to the workshop deliberations, as below:

a. Dr. Craig Smith gave a presentation on the goals, rationale, and design of the original APEI network.

b. Dr. Malcolm Clark gave a presentation on the relevance of the workshop to the LTC review of the CCZ EMP.

c. Dr. Travis Washburn introduced a report entitled "*Data to Assist the Deep CCZ Biodiversity Synthesis Workshop (1 - 4 October 2019, Friday Harbor, WA USA) in Synthesizing Patterns of Biodiversity, Community Structure, Species Ranges, Genetic Connectivity, Ecosystem Function, and Habitat Heterogeneity along and across the CCZ, and to assess the*

Representativity of the APEIs for Exploration Contract Areas”. This is a ~100-page document compiling, summarizing, and mapping environmental and biodiversity data available for synthesis in the workshop. The report was an internal document for use of workshop participants, and it is not fully available for wider circulation to allow contributing scientists to publish new, unpublished data sets contained within the report. A summary of the data sources, scientific articles or reports and original datasets, was circulated in the background document of the workshop. Dr. Washburn also discussed some of the issues associated with extracting data from the ISAdatabase *(DeepData*) for use in the workshop.

18. Summaries of the above presentations are provided in Annex III.

19. The co-chairs then briefed the meeting on the workshop objectives and expected outputs/outcomes of the workshop.

20. The following scientific questions (a-e) were proposed to guide the work of breakout groups, which would then allow participants to consider whether, given the available data, the current network of APEIs appears to capture the full range of biodiversity, species distribution, scales of connectivity, ecosystem functions, and habitat heterogeneity in the CCZ, and also to identify needs for additional APEIs and key scientific data gaps.

a) **Biodiversity:** For key biotic components (i.e., microbes, meiofauna, macrofauna and megafauna) does **species/taxon richness, evenness and community structure** vary along and across the CCZ? Do claim areas have similar levels of species/taxon richness and evenness, and similar community structure, to the proximal APEI(s)? Do morphological taxonomy, barcoding and eDNA approaches yield similar results? What taxonomic gaps hinder biodiversity (and biogeographic) syntheses for each biotic component (how well is the fauna known taxonomically)?

b) **Biogeography:** For key biotic components (microbes through megafauna), are **species ranges** (based on morphology and barcoding) generally large compared to the distances between APEIs and contractor areas? What is the degree of species (or lowest OTU) overlap/community similarity between different study locations across the CCZ? For what types of biota (small versus large, common versus rare, taxa, planktonic dispersal versus direct development) do we have information on species ranges?

c) **Genetic Connectivity:** For key biotic components (microbes through megafauna), what are **latitudinal and longitudinal scales of genetic connectivity**? Are scales of connectivity large or small compared to the distances between APEIs and contractor areas? Is there evidence of genetic connectivity (e.g., shared haplotypes) between APEIs and/or contractor areas?

d) **Ecosystem Structure, Functions and Drivers:** How do ecosystem structure and functions (e.g., **SCOC, C-cycling and C-flows, rates and depths of bioturbation, carbon burial, calcium carbonate dissolution, nutrient fluxes, biotic abundance, biomass**) and potential drivers (including seafloor POC flux, ocean depth, sediment properties [grain size, TOC, nodule abundance and properties]) vary along and across the CCZ? Do APEIs have similar levels and ranges of ecosystem functions and ecosystem drivers to contractor areas?

e) **Habitat Modeling:** Do APEIs have a similar range of habitats (i.e., **habitat diversity**) to proximal contractor areas, based on habitat modeling studies?

ITEM 4. PRESENTATIONS OF RELEVANT SCIENTIFIC DATA SETS AND PRELIMINARY DATA ANALYSES TO BE USED IN THE WORKSHOP

21. The workshop had before it the data report as presented under Item 3 by Dr. Travis Washburn. This document provided a basis for further discussion and analyses within Breakout Groups.

22. Despite efforts by some workshop participants and the ISA Secretariat, the use of data contained in the ISA database (*DeepData*) for the workshop deliberation was rather constrained by issues relating to data quality, data validation and completeness of information submitted in digital data templates (e.g. metadata) to the ISA. These issues, and the inputs provided by workshop participants, will need to be addressed by the ISA, and plans are to consider this as an item when the LTC meets in February-March 2020.

23. Workshop participants, including DeepCCZ project leaders and other scientific experts, who had submitted relevant scientific information prior to the workshop, were invited to present their reviews of the different data themes. These presentations occurred during the latter half of Day 1 and first part of Day 2, and covered data sources, data compilations, analyses and preliminary results relevant to the main questions posed for the workshop in Item 3.

24. Summaries of these presentations, written by the presenters and members of the Breakout Groups, are provided in Annex IV.

25. For the effective analysis and synthesis of available scientific information to describe biodiversity and ecosystem patterns along and across the CCZ, the workshop participants were initially split into 11 Breakout Groups organized by topic, each with a lead facilitator and rapporteur, as follows:

i. ***Microbes:*** Matthew Church, lead; Emma Wear, rapporteur
ii. ***Metazoan Meiofauna:*** Daniela Zeppilli, lead; Ann Vanreusel, rap.
iii. ***Foraminifera*****:** Andrew Gooday, lead; Swee Cheng Lim, rap.
iv. ***Macrofauna*****:** Craig Smith, lead; Travis Washburn/Lenaick Menot, rap.
v. ***Invertebrate megafauna:*** Daniel Jones, lead; Erik Simon-Lledo, rap.
vi. ***Fishes and Scavengers:*** Jeffrey Drazen, lead; Astrid Leitner, rap.
vii. ***Genetic Connectivity****:* Thomas Dahlgren, lead; Guadalupe Bribiesca Contreras, rap.
viii. ***eDNA***: Erica Goetze, lead; Franck Lejzerowicz, rap.
ix. ***Ecosystem functions:*** Andrew Sweetman, lead; Marta Cecchetto, rap.
x. ***Fossilized Fauna:*** Diva Amon and rapporteur
xi. ***Habitat Mapping and Environmental Data***: Kerry Howell, lead; Kirsty McQuaid, rap.

26. The Breakout Groups were flexible, and participants moved among them as analyses and related discussions progressed. This flexibility was important to ensure that the most appropriate combination of expertise was available when considering the best way to analyze or interpret the many data sources and results, and allow participants to contribute their expertise on multiple topics.

27. The Breakout Groups were requested to review and synthesize the information available, focusing on answering the scientific questions in Item 3, and to consider, among other things:

a. Any additional information made available at the workshop or appropriate analyses;
b. Sampling efficiency and method adequacy, including geographic limitations;
c. Occurrence of rare-species;
d. Taxonomic certainty (e.g. higher or lower taxonomic level at different spatial scales);
e. Habitat stratification (seamounts, abyssal plains, sediment, nodules, benthic boundary layer, water column);
f. Data sources (additional datasets to follow up post-workshop);
g. Degree of confidence (statistical robustness, quantitative or qualitative);

ITEM 5. REVIEW, ANALYSIS, AND SYNTHESIS OF RELEVANT SCIENTIFIC DATA ON BIODIVERSITY AND ECOSYSTEM PATTERNS ACROSS THE CLARION CLIPPERTON ZONE AND THE EFFECTIVENESS OF THE EXISTING NETWORK OF AREAS OF PARTICULAR ENVIRONMENTAL INTEREST (APEIs)

28. Each working group reported at plenary sessions on the progress of their considerations on key biotic and abiotic patterns of CCZ ecosystems, within and across the different taxa, the representativity of APEIs, and identification of data gaps to improve scientific understanding or interpretation of results.

29. Each working group prepared a final account of their data, analyses, and results. These accounts are presented for each taxon/breakout group in Annex V.

ITEM 6. OVERARCHING SYNTHESIS OF PATTERNS OF BIODIVERSITY AND ECOSYSTEM FUNCTION ACROSS THE CCZ

30. Comments addressing the key workshop questions synthesizing patterns of benthic and benthic-boundary layer biodiversity, community structure, species ranges, genetic connectivity, ecosystem function, and habitat heterogeneity along and across the CCZ are presented here.

a. What are the overall levels of biodiversity in the CCZ and how well are they sampled?

Incomplete sampling, and a focus on the eastern CCZ, limits a clear understanding of the levels of biodiversity across the CCZ. However, species richness in this region is substantial, with at least 500 species collected/observed in many of the major faunal groupings, including metazoan meiofauna (~500 species), macrofauna (>500 species), invertebrate megafauna (~630 species), and foraminifera (>1000 species). Within the prokaryotes, i.e., bacteria and archaea, greater than 10,000 molecular operational taxonomic units (equivalent to species) have been sampled from sediments and nodules at a single site in the eastern CCZ. Species-richness estimators suggest 25 - 75% of the total species found at any sampled site remain to be collected, indicating that many thousands of faunal species occur across the CCZ.

Despite sampling limitations, it is clear the CCZ hosts unusually diverse assemblages of megafaunal xenophyophores (22% of the global species richness of these giant foraminiferans), as well as relatively diverse mobile-scavenger assemblages. Population genetic studies indicate that genetic diversity in eight common CCZ macrofaunal and megafaunal species is higher than expected based on studies of marine invertebrates from other regions, such as continental shelves and ephemeral habitats in the deep sea. A high diversity (and abundance) of seafloor fossils from extinct and extant cetaceans and sharks has also been found across the CCZ.

As for many abyssal regions, rare species dominate the diversity for nearly all faunal size classes/groups (except for mobile scavengers and fish) for all sites, substrates (sediment versus nodules), and habitats (abyssal plains versus seamounts) thus far sampled. In general, species numbers still rapidly increase with additional sampling effort for most groups. The majority of diversity in microbes, metazoan meiofauna, foraminifera, macrofauna, and invertebrate megafauna falls within undescribed species (i.e., species that are new to science).

Most sampling of biodiversity has been concentrated in the eastern CCZ in contractor areas. Little or no biodiversity data were available for our synthesis from the west-central region of the CCZ (constituting about 50% of the management area). The core areas of most APEIs remain unsampled, and biodiversity data from the few APEIs that have been sampled is very limited.

b. How do biodiversity, community structure and ecosystem functions vary along and across the CCZ and what are the key environmental drivers (e.g., POC flux, nodule abundance, water depth, and/or seafloor topography)?

Microbes: Based on very limited data, there are no significant within-habitat variations in bacterial and archaeal taxonomic diversity across the CCZ (east to west). However, sediments and nodules harbored greater bacterial and archaeal diversity and greater variability in community composition, than the overlying seawater.

Metazoan meiofauna: Based on quantitative morphological data primarily from the eastern CCZ, metazoan meiofaunal abundance, diversity, and community structure vary at local and regional scales.
POC flux exhibits a strong positive correlation with meiofaunal abundance, as well as with diversity, on local and regional scales. Meiofaunal diversity, based on eDNA studies, appears to vary across APEIs 1, 4 and 7 with POC flux. Within APEIs 4 and 7, meiofaunal diversity is higher in seamount than in abyssal-plain sediments. However, meiofaunal biodiversity data are limited to the eastern CCZ for morphology, and the western CCZ for eDNA, restricting our ability to synthesize patterns across the entire CCZ.

Foraminifera: There are gradual shifts in the species composition of foraminiferal assemblages in relation to environmental gradients (e.g., POC flux) across the CCZ. Available morphological and molecular data suggest that meiofaunal foraminiferal diversity is lower in the western than the eastern CCZ. Many of the megafaunal xenophyophores dwell on nodules, indicating that nodule occurrence is a key driver of xenophyophore diversity, and that xenophyophore diversity will be relatively low in areas with little/zero nodule abundance.

Sediment macrofauna: Macrofaunal abundance, diversity, and community structure vary substantially across the CCZ, and the equatorial North Pacific in general, with variations in POC flux, nodule abundance, and water depth implicated as potential drivers. Very limited APEI data suggest there might be lower abundance, diversity, and a different community structure in APEI 3 compared to contractor contract areas 600-900 km away. No other direct comparisons can be made between APEIs and contractor areas.

Invertebrate megafauna: Megafaunal abundance, community composition, and the distribution of common morphotypes show high variability at relatively small spatial scales. There is a trend of increasing megafaunal abundance from west to east across the CCZ, and possibly higher density in the mid areas of the CCZ compared with peripheral APEIs. Nodule cover is a key environmental correlate with invertebrate megafaunal abundance and community composition. Numerous individual morphotypes have been found exclusively on nodules, suggesting a high diversity of nodule-dependent megafauna. This implies that areas with low nodule abundance will have lower megafaunal diversity.

Fishes and scavengers: The community structure and diversity of mobile scavengers (megafaunal invertebrates and fishes attracted to bait) and fishes (counted in imaging surveys away from bait) vary significantly across the CCZ, and across the Pacific, suggesting that even for these highly mobile species, not all regions of the CCZ are equivalent. Seamounts have significantly different scavenger communities than neighboring abyssal plains, thus contribute to regional diversity.

Ecosystem functions: Sediment-community oxygen consumption (SCOC) varies across the broader central equatorial Pacific region and is positively correlated with POC flux. Nodule abundance is not correlated with these broad regional variations in SCOC. Within the CCZ area, ecosystem function data are limited, and although SCOC does vary between sites, and with POC flux, SCOC trends across the CCZ region are not statistically significant (possibly due to small sample size and biased data from the eastern end). Seafloor dissolved phosphate and silicate fluxes vary significantly across the broader equatorial Pacific region and are correlated with POC flux. There are too few data from within the CCZ to determine trends.

Metazoan eDNA: ASV/OTU richness (i.e., richness at the species or higher taxonomic level) in APEIs 4 and 7, which receive moderate POC flux, was higher than in low-POC-flux APEI 1, consistent with POC flux acting as a key environmental driver of community diversity in the CCZ. Limited comparisons between seamount and abyssal-plain sediments indicate higher diversity in seamount sediments, suggestive of bathymetric/topographic influences on sediment-community diversity.

Fossilized fauna: This work is in progress and further analyses are needed, but preliminary results show abundance and diversity of fossilized fauna varies across the CCZ, with highest abundances found in the eastern CCZ.

Climate-change impacts: Changes in key environmental parameters, including POC flux, resulting from climate change are projected to vary along and across the CCZ by 2100, including among APEIs. The current set of APEIs is broadly representative of potential climate change hazards within the CCZ. APEIs 4 and 6 may be climate change refugia, while APEIs 1 and 9 may experience the largest climate change impacts.

Habitat modeling and classification: Twenty-four habitat types were identified using environmental variables implicated in the workshop as important drivers of biodiversity, community composition and ecosystem functions. The variables used were POC flux, nodule abundance, slope, and Benthic Position Index. There is substantial habitat variability across the CCZ, with most classes being found only in eastern, central, or western, and/or northern or southern regions-and not throughout the CCZ.

Summary: Biodiversity, community structure, ecosystem function, and potential climate-change impacts vary substantially across the CCZ for most biotic groups and ecosystem functions, with POC flux, nodule abundance, water depth and topographic variability indicated as key ecological drivers. Community structure, and often biodiversity, varied between sediment and nodule substrates, and between seamount and abyssal-plain habitats, for microbes, metazoan meiofauna, foraminifera, macrofauna, invertebrate megafauna and to some degree, mobile scavengers. Habitat modeling and classification, using the key ecological drivers mentioned above, yielded 24 habitat types within the CCZ, with substantial habitat variability across the region. These results generally support the division of the CCZ into the nine ecological subregions originally used for siting the APEIs, as outlined in ISA (2008) and Wedding et al. (2013).

c. How large are faunal species ranges (based on morphology and molecular approaches) in the CCZ?

Metazoan Meiofauna: Most (>60%) nematode and harpacticoid species (both morphological and molecular data) have been recorded from only one site in the CCZ. While some species are shared across two or more sites separated by ~200 to >1000 km, only a few species appear to have very broad ranges throughout the region as well as other ocean basins. However, because most of the meiofaunal species are locally rare, we cannot determine whether species have been collected only at single sites because they have small ranges (e.g., <200 km) or are simply under sampled (i.e., they are "pseudo endemics"). Without more sampling, we must assume that many meiofaunal species may have ranges of <200 km.

Foraminifera: Some foraminiferan morphospecies present in CCZ samples (mainly multi-chambered globothalameids) are known from literature records to be widely distributed in the deep ocean. Two species are confirmed to range across multiple ocean basins. Approximately 6% of meiofaunal foraminiferal morphospecies sampled within the CCZ, representing 44% of all individuals, occur broadly (at 9-11 sites) across the CCZ, indicating that some abundant foraminifera have large ranges within the region. However, most foraminiferal morphospecies and molecular species are rare and have been collected only at 1-2 sites; under-sampling combined with the rarity of many species make it impossible to distinguish small species ranges ("endemism") from inadequate sampling "(pseudo-endemism"). For two species of megafaunal xenophyphores, large ranges spanning 3,800 km within the CCZ have been confirmed using genetic techniques, but for most xenophyophores, data are too limited to determine ranges.

Sediment Macrofauna: Some abundant macrofaunal species range over 400-900 km, and two occur in both the western and eastern CCZ, spanning a range of ~3000 km. However, the vast majority (~80%) of species appear to be rare and have been collected only at single sites. Thus, as for the metazoan meiofauna, we cannot determine whether most species have small ranges (<200 km) or are under sampled (i.e., pseudo endemics). Because rarity is often correlated with small species ranges for macrofaunal sized animals, we cannot assume that rare species in the CCZ are widely distributed, and simply under sampled. Thus, until more intensive sampling proves otherwise, we must assume that many macrofaunal species within the CCZ may have ranges of less than 200 km.

Invertebrate megafauna: There were mixed results for species ranges. Over 50 morphotypes were shared across distant sites (e.g., APEI 6 and Kiribati, 5000 km separation), and most sites which had reasonable sampling shared at least 20 morphotypes, suggesting large ranges across the CCZ for some invertebrates. However, many morphotypes (102) were only found at one site, indicating potentially relatively narrow species ranges or pseudo-endemism due to under-sampling.

Fishes and scavengers: Many of the fishes and scavengers sampled in the CCZ have very large, even global ranges in abyssal habitats, but there are a few species that may be restricted within the CCZ. Regional variations in community composition are largely the result of differing abundances rather than species presence/absence.

Metazoan eDNA: In a comparison of APEIs 1, 4, and 7, a small proportion of the community (<16% of OTUs) occurred in more than one APEI, while most (≥ 84%) were sampled in only a single APEI. This could be a result of limited sampling, or reflect distributional ranges of <700 km (the distance between sites sampled in APEIs,1 4, and 7).

Summary: A very small proportion of the >2000 faunal (metazoan) species identified within the CCZ show relatively wide species ranges spanning the CCZ or, in some cases, occurring in multiple ocean basins. Most species of metazoan meiofauna, foraminifera, and sediment macrofauna have been found only at single sites and at low abundances (often as singletons). We cannot differentiate the hypotheses that most benthic species in the CCZ have small ranges (<200 km), or that many species have broader ranges but are simply under sampled. A precautionary approach, until proven otherwise, is to consider that these species may have ranges of <200 km (which is less than the scales of APEI core regions (200 x 200 km) or individual exploration contract areas).

ITEM 7. ASSESSMENT OF THE REPRESENTATIVITY OF THE APEIs RELATIVE TO EXPLORATION CONTRACT AREAS, AND IDENTIFICATION OF AREAS WHERE ADDITIONAL REPRESENTATIVE APEIs COULD BE SITED

26. In general, there were insufficient data to evaluate the overall representativity of APEIs. Few APEIs have been sampled, and often there were no comparable data from adjacent contract areas in the CCZ for many of the size classes/groups. However, some brief results and comments, where applicable, for the questions posed as part of the CCZ Biodiversity Assessment are given below:

a. Do APEIs capture/protect the full range of biodiversity and community structure observed along and across the CCZ, and especially within contractor and reserve areas (i.e., are they fully representative)?

The data are insufficient to fully address this question. However, the biodiversity and community structure of all the size class/groups, except the microbes, varied within the CCZ. Key environmental drivers of biodiversity patterns are thought to include POC flux, nodule abundance, depth, locations on seamounts, and topographic variability (captured by BPI). Thus, to be representative, APEI locations should capture the full range of these habitat variables across the CCZ (see habitat modeling and classification discussed below).

b. Are similar levels of ecosystem structure and function (e.g., POC flux, abundance, biomass, sediment community oxygen consumption (SCOC), etc.) demonstrated or expected in contractor/reserve areas and APEIs?

Substantially more ecosystem function data are required to make such an evaluation, but there was evidence that SCOC and nutrient fluxes changed with POC flux, highlighting the need for APEIs to capture the full range of POC fluxes in order to protect different levels of ecosystem functions.

c. Do APEIs capture (and replicate) a similar range of habitats as contractor and reserve areas, based on habitat classification and mapping?

Habitat classification and mapping identified 24 habitat types across the CCZ, 16 of which have >10% of their total area within contractor and reserved areas combined (i.e., within “mining areas”). Ten of these 16 habitat types are relatively well represented within APEIs, with APEIs 4 and 6 having a high representation of CCZ habitats. However, the six habitats characterized by high nodule abundance (>11 kg m^{-2}) are common in contractor and reserve areas, and very poorly represented (0 to 5% of total habitat area) within the current APEI network. These nodule-rich habitats are concentrated along the east-to-west central axis of the CCZ (Fig. 1).

The habitat classification indicates that significant areas of nodule-rich habitats that currently occur in "mining areas" and not in APEIs, can be found at the east end of the CCZ, to the northeast of the CCZ, within the central CCZ, and in the northwestern CCZ.

Two other habitats classes (15 and 16) with moderate nodule abundance are also poorly represented in the APEIs, occurring in no, or only one, APEI.

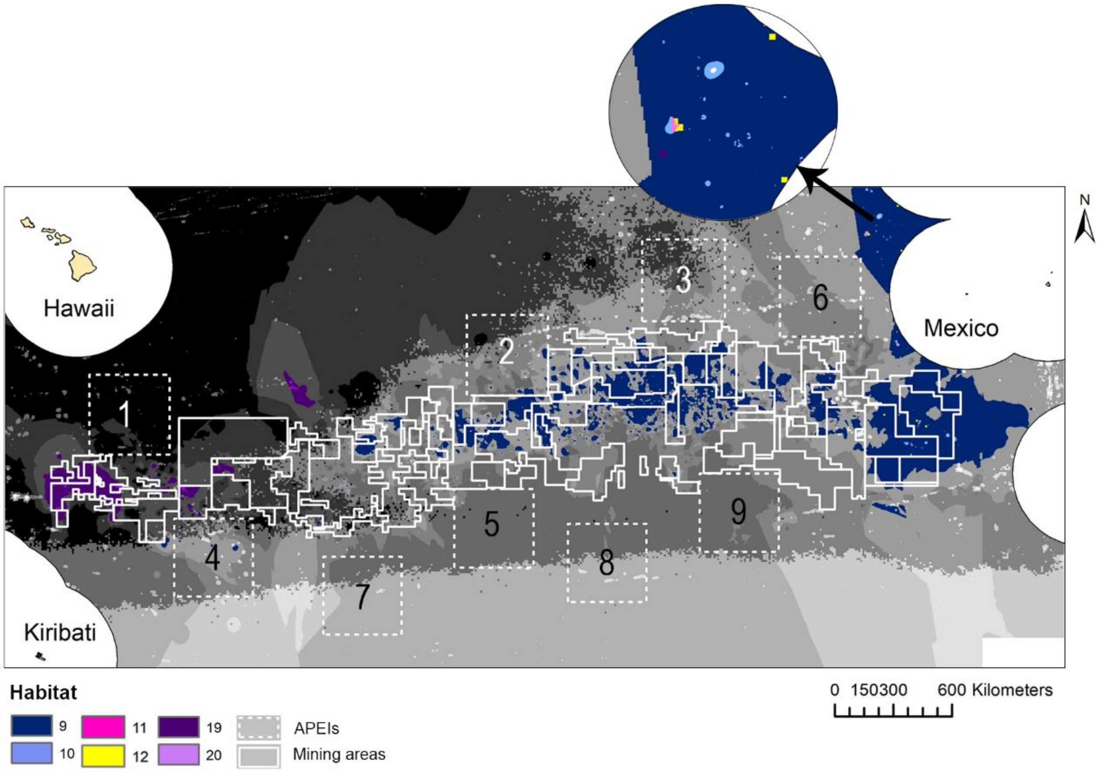

Figure 1. *Distribution of the six nodule-rich habitat classes (classes 9, 10, 11, 12, 19 and 20) across the CCZ, relative to contractor areas, reserve areas and APEIs.*

d. Do species ranges and genetic connectivity appear to bridge APEIs and contractor areas?

A small percentage of metazoan meiofauna, foraminifera, macrofauna and invertebrate megafauna have species ranges and genetic connectivity indicated to span the distances (200 – 800 km) between APEIs and contractor areas. However, most CCZ species are locally rare and have been collected only at one site, and we cannot distinguish possible small ranges from under-sampling.

ITEM 8. KEY DATA GAPS LIMITING BIODIVERSITY SYNTHESIS ACROSS THE CCZ

27. An important consideration during the workshop was discussion and determination of key data gaps that restricted the ability to address patterns and trends, and to fully answer the questions posed for the workshop. Each breakout group was asked to list the main data, methods and analytical gaps or issues that should be a priority for future surveys and sampling. These compilations of key gaps are not meant to be comprehensive, exhaustive, or even consistent across groups, but rather to focus on the major limitations identified in compiling the data and synthesizing biodiversity patterns across the CCZ.

28. ISA secretariat provide an update of the ongoing work of uploading environmental data to the ISA database (*DeepData*), highlighting that additional data may become available in the future from the contract areas through the *DeepData*. At the time of the workshop, several issues were identified with the ISA database constraining its use for analysis at the time of the workshop. It was suggested that this should be the subject of future discussions within the ISA Secretariat and the Legal and Technical Commission.

29. Below is a listing of key data gaps, by Breakout Group topics.

Microbes:

- Available data provide very poor spatial and temporal resolution in the CCZ; however, this may improve through inclusion of forthcoming datasets and through inclusion of samples from contractor claim areas.
- Available sequence data are too limited to assess representability of APEIs (due to poor sampling of mining-claim areas adjacent to APEIs).
- Methodological differences among the existing microbial studies make comparisons across studies difficult.
- Only taxonomic classification of microorganisms is possible given the available sequence data; microbial molecular taxonomy cannot be directly related to ecosystem function in these poorly studied deep-sea taxa. Thus, standardized approaches to connect environmental microbial taxonomy to function (e.g., metagenomics, isotopic tracers) are needed.
- Data are insufficient to assess how removal and/or disturbance of specific abyssal habitats will impact ecosystem services currently provided by microorganisms. Additional observations, including measures of microbial biomass and biogeochemical function, are needed.

Metazoan meiofauna:

- Metazoan meiofauna are under-sampled, as demonstrated by the fact that most rarefaction curves do not reach an asymptote.
- APEIs are very poorly sampled: There is only one dataset for meiofauna from one eastern APEI and another from three western APEIs.
- There is a major lack of taxonomic work (e.g., species descriptions, barcoding, integration of working species across programs) that would allow comparison of species distributions over wider geographical scales.

Sediment Macrofauna:

- There has been no quantitative macrofaunal sampling in core areas of eight of the nine APEIs, and extremely limited sampling in the ninth (APEI 3).
- There has been no quantitative macrofaunal sampling in nearly all of the central and western CCZ, i.e., over an area covering >50% of the CCZ region.
- Full macrofaunal diversity has not been sampled at any site (species are still accumulating rapidly with additional sampling efforts) so overall diversity levels and species ranges are poorly constrained.
- Most species are undescribed.

Invertebrate megafauna:

- Temporal variability has not been addressed in the CCZ, but seasonal and interannual variations in megafaunal abundance have been documented in other abyssal plain sites (albeit at higher latitudes).
- The ability to compare between APEIs and adjacent contract areas is limited by lack of sampling. More standardized survey designs are needed.
- Evaluation of the relationships between POC flux and megafaunal community parameters is hindered by lack of direct measures of POC flux or sediment community oxygen consumption for most sites (forcing use of coarse resolution (in space and time) modelled data).

Scavengers and fish:

- Baited camera data exist for eastern CCZ contract areas and western CCZ APEIs only, with major gaps in the central CCZ. Methods need to be standardized, and applied throughout the CCZ, to allow regional comparisons.
- Standard methods (e.g., viewing area, duration, and amount/type of bait) are critical for comparisons of baited-camera data among different areas.

Foraminifera:

- Inconsistencies in sample sizes and analytical methods (notably sieve mesh sizes) limit the comparison of data across wide areas of the CCZ.
- Under-sampling is a recurrent problem at all sites studied (pseudo-endemism cannot be distinguished from true endemism) and increased sampling efforts are needed at multiple spatial scales.
- Foraminiferal data, both morphological and molecular, are very limited from the western CCZ.
- There are some eDNA data from a small region of the eastern CCZ, and no quantitative (e.g., abundance) data or eDNA data yet available for foraminifera from APEIs.
- There are currently insufficient molecular data to analyze the population genetics of any foraminiferal species from the CCZ.

Ecosystem functions:

- *In situ* POC flux measurements (from sediment traps and/or SCOC) are needed from each APEI and adjacent contract areas to compare particle flux and SCOC in space and time.
- Temporal variability of SCOC and other ecosystem functions are needed; most studies encompass measurements made over hours to days.
- There are little or no data available for most benthic ecosystem functions (e.g., bioturbation, calcite dissolution) across the CCZ.

Population connectivity:

- Population connectivity has been studied for only eight abundant macrofaunal/megafaunal species. Nothing is known about connectivity for the vast majority of meiofaunal, macrofaunal and megafaunal benthic species in the CCZ, most of which are rare.
- The absence of taxonomic data on almost all CCZ fauna has hindered studies of species ranges and connectivity. In most cases, almost none of the species-level data collected by different contractors or researcher groups is comparable. High-quality taxonomic projects should be a priority in future research efforts in the CCZ.

Metazoan eDNA:

- eDNA metazoan diversity is under-sampled at all spatial scales in the CCZ.
- Spatial coverage of sampling for individual programs is limited and insufficient at present to assess representativity of APEIs in comparison to contractor or reserve areas.
- Taxonomic classification is problematic for mtCOI reads, the most commonly used marker for putative species-level assessment of metazoans. Alpha-taxonomy combined with DNA barcoding of meiofaunal taxa should be a high priority to enhance classification of reads to fill this gap.

Fossilized fauna:

- Despite an extensive amount of data from the eastern CCZ, there is no baseline fossil information or imagery available for the western contract areas. Data are also lacking for APEIs 2, 5, 8, and 9. More standardized survey designs are needed.
- The fossil-imagery data that do exist have surveyed only relatively small areas of seafloor in five contract areas, five APEIs and two areas outside of the CCZ.
- Image data varies widely in quality, limiting the ability to resolve smaller fossils, such as shark teeth, or features needed for species identification.

ITEM 9. SCIENTIFIC CONCLUSIONS FROM THE WORKSHOP OF RELEVANCE TO THE LTC REVIEW OF THE CCZ EMP

30. Scientific conclusions from the workshop that may be useful for consideration in the review of the CCZ EMP by the Legal and Technical Commission are as follows:

a. Total species richness of all biotic size classes appears relatively high, but is poorly sampled and described taxonomically at all CCZ sites, and for nearly all biotic groups, studied. Biodiversity data available for this synthesis has been collected primarily in the eastern CCZ in contractor areas, with limited sampling of most groups in the rest of the CCZ, including core areas of APEIs.

b. Nonetheless, the available data show that biodiversity and community structure for most biotic size classes vary substantially within the CCZ, with key environmental drivers including POC flux, nodule abundance, depth, and topography. Ecosystem functions also appear to vary with these drivers. These environmental proxies, as used in the original EMP formulation, as well as habitat classification and mapping, are useful to assess the representativity of current and future APEIs across the CCZ.

c. Workshop results and discussion confirmed that APEIs should be broadly distributed along and across the full CCZ to capture the range of measured variability in biodiversity, community structure, ecosystem functions, and key ecological drivers. Climate change sensitivity should also be considered. In addition, the possibility that many faunal species may have small ranges (<200 km) suggests that APEIs should be broadly distributed to protect species with narrow distributions.

d. The size of the core regions of APEIs (200 x 200 km) remains appropriate given a 2016 review of mean dispersal scales of deep-sea benthos[5] that supports the 100-km scale used in the original APEI design.

e. One of the goals of the APEI network is to protect 30% of the managed area within APEIs, and this should include areas representative of the full range of habitats, biodiversity and ecosystem structure and function. Habitat mapping suggests the current APEI network captures a good representation of many of the 24 habitat types found within the CCZ, covering a range of POC fluxes, depths, and topographic variability. However, the 6 habitat types characterized by high nodule abundance are poorly represented within the APEI network and could be better protected by placing additional APEIs in the easternmost, central and western CCZ.

f. The presence of high densities of fossils in the eastern CCZ suggests that additional consideration is warranted regarding conservation and management measures for fossil protection in the Environmental Management Plan.

ITEM 10. CLOSURE OF THE WORKSHOP

31. The workshop was closed at 18.30 p.m. on Friday, 4 October 2019.

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[5] Baco, Amy R., et al. “A Synthesis of Genetic Connectivity in Deep-sea Fauna and Implications for Marine Reserve Design.” *Molecular Ecology*, vol. 25, no. 14, 2016, pp. 3276–3298.

Annex I

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Annex II

WORKSHOP AGENDA

30 Sept - Monday

PM – Participants arrive at Friday Harbors Labs and check-in after 3 pm. Dinner in town in self-organized groups.

1 Oct - Tuesday - Chair Malcolm Clark

830 - 845 **Plenary in the Commons -** Welcomes from ISA Secretariat, Moore

Foundation, Pew Charitable Trusts and DeepCCZ Project

845 - 900 **Plenary -** Participant Introductions and FHL Logistics

900 - 945 **Plenary -** Workshop rationale: Original design of the APEI network (C. Smith),

the CCZ-EMP and the LTC discussion on CCZ EMP review (M. Clark).

945 – 1045 **Plenary -** Workshop goals (see Workshop Goals at end of Agenda), deliverables and organization. Present and discuss initial **Data Report** and template for **Final Report**

1045 - 1115 Coffee break in Dining Hall/rowboat orientations

Plenary - Data presentations led by topic leaders

1115 – 1145 **Bacteria and Archaea -** Emma Wear, Matt Church et al.

1200 – 1245 Lunch in Dining Hall

Plenary - Data presentations led by topic leaders – cont.

1245 – 1315 **Macrofauna** – Travis Washburn, Craig Smith, et al.

1315 – 1345 **Invertebrate megafauna** – Daniel Jones, et al.

1345 – 1415 **Fishes and Scavengers** – Jeff Drazen and Astrid Leitner

1415 – 1445 **Foraminifera** – Andrew Gooday

1445 –1500 Coffee break in Dining Hall

Plenary - Data presentations by led by topic leaders – cont.

1500 – 1530 **Genetic Connectivity –** Thomas Dahlgren, Adrian Glover, Guadalupe Bribiesca-Contreras et al.

1530 – 1545 **Metazoan eDNA** – Erica Goetze

1545 – 1615 **Ecosystem Functions** –Andrew Sweetman, Marta Cecchetto, et al.

1615 – 1645 **Habitat Mapping and Environmental Data** – Kerry Howell and Kirsty McQuaid

1645 – 1700 **Fossilized Fauna** – Diva Amon et al.

1700 – 1730 **Climate Change -** Chih-Lin Wei via Craig Smith

1730 - 1800 **Plenary -** Review of progress with data summaries

2 Oct – Wednesday – Chair Craig Smith

Plenary - Data presentations led by topic leaders – completed

830 – 900 **Metazoan Meiofauna** — Daniella Zeppilli et al.

900 – 930 **Plenary -** Identification of breakout group topics, leaders, rapporteurs and marching orders

930 - 1030 **Breakout groups** begin consideration of key questions for their topic, data availability and syntheses – rooms TBA

1030 – 1045 Coffee break in Dining Hall

1045 – 1200 **Breakout groups** continue data evaluation, analyses and syntheses

1200-1245 Lunch in Dining Hall

1245 – 1300 **Plenary –** Brief discussion function/needs of breakout groups

1300 - 1630 **Breakout groups** continue data evaluation, analyses and syntheses (coffee breaks by group)

1630 – 1800 **Plenary -** Breakout groups report progress (10 minutes per group)

3 Oct – Thursday – Chair Malcolm Clark

830 – 930 **Plenary -** Discussion of general breakout group progress, gaps (do we need additional breakout groups?), relocation of participants, how are syntheses and analyses going?

930 – 1200 **Breakout groups** continue syntheses and *each develops an outline for write-up of synthesis for their topic (key question addressed, data available and used, results, major gaps, conclusions concerning representativity of APEIs, any recommendations for modifications/additions to APEI network)*. (coffee breaks by group)

1200 – 1245 Lunch in Dining Hall

1245 – 1430 **Plenary -** Breakout groups present outline of syntheses in bullet form, i.e., answers to key workshop questions, gaps, conclusions, recommendations, etc. (8 – 10 min per group).

1430 – 1530 **Plenary – Initial discussion and formulation of overarching results, conclusions, and recommendations**, with assignment of writing tasks for overarching summary. Formulation of **Overarching Synthesis Breakout group.**

1530 – 1545 Coffee Break

1545 – 1700 **Original breakout groups** begin write-up of syntheses of topics and conclusions and using outlines previously generated. **Overarching Synthesis Breakout Group** begins synthesis.

4 Oct – Friday – Chair Craig Smith

830 – 915 **Plenary -** Breakout groups report on writing progress (4 min each).

915 – 1045 **Plenary – Final formulation of overarching results, conclusions, recommendations**, assignment of writing tasks for overarching summary. Formulation of additional breakout group(s) as needed.

1045 – 1200 **Breakout groups** continue write-up of syntheses of topics and overarching results and recommendations (coffee breaks by group).

1200 – 1245 Lunch in Dining Hall

1245 – 1500 **Breakout groups** continue write-up of syntheses of topics and overarching results and recommendations.

1500 – 1515 Coffee break in Dining Hall

1515 – 1745 **Plenary –** breakout groups report on progress with topic write ups, tasks remaining, and people responsible for completing these task (15 min per group)

1745 – 1815 **Plenary –** Workshop conclusion – review progress made, synthesis/writing tasks remaining, and formulate plans and timelines for completing these tasks

5 Oct – Saturday

830 – 1100 Most participants check out of FHL

1100+ Some key participants remain to compile workshop report

Annex III

SUMMARY OF INTRODUCTORY PRESENTATIONS

Presentations delivered under Item 3 above.

a. Goals, rationales and design of the original CCZ APEI network

by Craig R. Smith, University of Hawaii

This presentation summarized (a) the goals and outcome of the original Pew funded workshop conducted in 2007 to design a network of representative marine protected areas (now called APEIs) to safeguard biodiversity and ecosystem function on the CCZ during manganese nodule mining, (b) the key design elements recommended by the workshop for APEIs in the CCZ, and (c) lessons learned from the APEI design process of general relevance to the development of Regional Environmental Management plans. The design of the APEI network in the CCZ was based on recommendations from a workshop, sponsored by the Pew Charitable Trusts, and organized/co-chaired by Craig Smith, J. Tony Koslow, and Malcolm Clark in October, 2007 at the University of Hawaii. The workshop convened 22 experts, including scientists, international lawyers, mineral geologists, and representatives from the ISA. The original Pew workshop considered threats to the deep seafloor habitats in the CCZ from mining and other impacts, and reviewed data on patterns of abundance, biomass, biodiversity, species ranges, and gene flow across the region, and their relationships to environmental (i.e., habitat) variables in the CCZ. The workshop developed general goals for APEIs in the CCZ and used MPA design principles to develop specific APEI recommendations for this seafloor region. APEI goals included protection of 30-50% of management area (CCZ), capturing the full range of habitat variability in the CCZ, maintaining sustainable populations within the benthic fauna, replicating across the region to capture E-W and N-S turnover of biota, and making the APEIs large enough that their core regions are buffered from impacts of mining sediment plumes.

General APEI elements included the following:

(1) The APEIs should be managed across the CCZ region as a whole (i.e., in a REMP).
(2) The CCZ region can be divided into three east-west and three north-south habitat strata (or subregions) because of strong E-W and N-S productivity-driven gradients in ecosystem structure, yielding nine distinct subregions within the CCZ, each requiring an APEI.
(3) The core area of each MPA should be at least 200 km in length and width, i.e., large enough to sustain populations for species potentially restricted to a subregion of the CCZ.
(4) Each APEI should contain the full range of benthic habitat types found within its subregion (e.g., dense nodule fields, abyssal plains, abyssal hills, seamounts and fracture zones).
(5) Each APEI core area should surrounded by a buffer zone 100-km wide to ensure that the APEI core is not affected by mining plumes.

In summary, nine 400 x 400 km APEIs were recommended, one in each of the 9 CCZ subregions defined by productivity gradients and faunal turnover. The APEIs were situated to avoid or minimize overlap with existing mining exploration and reserved claim areas and to protect as many seamounts as possible within a subregion. Within and between spacing of APEIs were of roughly similar scales (400-800 km), allowing the APEIs to potentially function as a connected network.

The APEI recommendations for the CCZ were presented to the ISA Legal and Technical Commission in March - May, 2008 (Smith et al., 2008; Wedding et al., 2013), strongly endorsed by the ISA Legal and

Technical Commission (LTC) in concept, and provisionally adopted in 2012, with repositioning by the LTC of two APEIs from the core to the periphery of the CCZ.

Some of the lessons learned from the APEI design and setup process in the CCZ include the following:

(1) APEI networks should be erected within a REMP before many exploration claims are granted (otherwise the ability to design viable networks may be compromised).
(2) Deep-sea species/community distributions and connectivity patterns (i.e., for thousands of species, many of them rare) are not knowable within a region on time scales necessary to develop APEI networks (and REMPs) for deep-sea mining **-** *We must use a representative APEI approach to fully protect biodiversity and ecosystem functions in the deep sea***.**
(3) There can be pushback from stakeholders to reduce/relocate APEIs, especially from areas containing prime-grade mineral deposits.
(4) However, because mineral grade (geochemistry) and biota are often linked, the precautionary approach requires protecting high-grade areas in absence of extensive knowledge of regional patterns of biodiversity and connectivity.

References:

Smith, CR et al. 2008. Rationale and recommendations for the establishment of preservation reference areas for nodule mining in the Clarion-Clipperton Zone. Fourteenth session. Kingston, Jamaica, 26 May–6 June 2008. http://www.isa.org.jm/en/ sessions/2008/documents: Legal and Technical Commission, International Seabed Authority. Technical document no. ISBA/14/LTC/2.

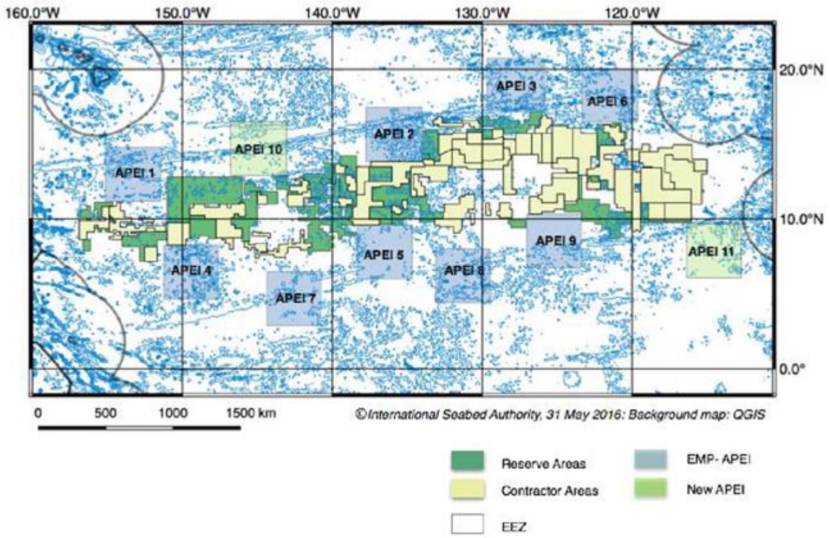
Wedding LM, Friedlander AM, Kittinger JN, Watling L, Gaines SD, Bennett M, Hardy SM, Smith CR. 2013 Fromto practice: a spatial approach to systematic conservation planning in the deep sea. Proc R Soc B 280: 20131684. http://dx.doi.org/10.1098/rspb.2013.1684

b. Review of the CCZ Environmental Management Plan: relevance of the workshop to the LTC

By Malcolm Clark, NIWA, New Zealand

An environmental management plan (EMP) for the Clarion Clipperton Zone (CCZ) was approved by the ISA Council and Assembly in 2012. This had several strategic aims to ensure environmentally responsible seabed mining, to enable effective protection of the marine environment, and to sustainably manage the CCZ as a whole. The EMP involved a collective and collaborative approach (contractors, scientists, managers) to data collection and environmental management, as well as consideration of multiple operations and cumulative impacts. A key concept of the EMP was the designation of Areas of Particular Environmental Interest (APEIs) to protect regional-scale biodiversity. One of the conditions of the EMP was that of regular review, including evaluating the efficacy of the APEI network.

The LTC in 2016 responded to new data suggesting that dispersal capabilities of several benthic taxa were more limited than the current spacing of some of the APEIs where distances between them exceeded 1000 km. APEI numbers 10 and 11 were proposed to fill gaps to the NW and SE of the CCZ (see figure below).



This proposal was considered by a new LTC in 2017. An environmental subgroup of the LTC discussed this recommendation, and also corresponded with, and sought information from, several experts outside the LTC. There was agreement that the proposed APEI 10 was appropriate, as there is an obvious gap in existing coverage in the northwestern area of the CCZ, and its placement in between APEIs 1 and 2 would satisfy the <1000 km dispersal concern. However, the location of APEI 11 was not as straight-forward, due to a number of potential issues with proximity to EEZs, size, spacing from neighboring APEIs, and location relative to the central CCZ resource band. It was generally agreed that locating an APEI is not just a matter of filling in gaps on the map, but should link back to the underlying purpose and objective of APEIs:

-to maintain sustainable populations and to capture the full range of habitats and communities by being large enough to maintain minimum viable population sizes for species potentially restricted to a subregion of the Clarion-Clipperton Zone without being affected by mining plumes from any activities immediately adjacent to an area

There were several key conclusions reached by the working group:

1) The original nine sub-areas which are proxies for the north-south and east-west productivity gradient need to be considered as a guide to representation of APEIs as a network.
2) Distance separation is a difficult “rule” as it varies between taxa, reproductive mode, and oceanographic conditions. However, a maximum of 1000 km should be observed.
3) Coverage in the northwestern sector of the CCZ is light, and a new APEI #10 was thought a good proposal to reduce the large distance between APEI # 1 and 2.
4) The central band of the CCZ should be included where possible in APEI location. Hence, there was a preference to locate APEI 11 to the north in between the EEZs and to maximize its size so it includes the “core” 200 km square, and as much of the 400 km buffer zone as possible. Consider options between contract areas (even if size smaller?)
5) If APEI #11 is placed east, this would leave a large distance gap in the southeast, and a third APEI could be needed.

6) Topography is also a consideration, which requires including abyssal hills and seamounts in APEIs as a different habitat to that of nodules on the abyssal plain. However, it was felt that this criterion should not push APEIs further away from the central axis than necessary.

At this stage, the working group felt it was not appropriate to sign off the location of the proposed APEIs, but plan on a more detailed discussion of the role, design, criteria, and effectiveness of existing APEIs, and utilize additional data collected during recent major research voyages which included sampling inside APEIs. This was delayed from 2018 due to a focus on Draft Exploitation Regulations, but picked up again in collaboration with the DeepCCZ Project and this workshop in 2019.

c. An Introduction to the Data Report for the DeepCCZ Data Synthesis Workshop

by Travis Washburn, University of Hawaii

A data report was created by Travis Washburn and Craig Smith, with the assistance of the DeepCCZ Synthesis working group leaders, for use by all participants during the workshop. Variables were first identified that could possibly influence deep-sea biological communities and physical processes. These variables included: physiographic data, data on nodule resources, sediment and water-column data, flux data, biogeographic data, and climate change data as well as brief summaries of data sets to be used in the workshop by each working group and some preliminary analyses. Data for environmental variables were obtained from online resources (e.g., the World Ocean Atlas, GeoMapApp, etc.), from the published literature, and from direct solicitations to scientists. Maps of all datasets were then created for each variable (or where data were unavailable, previously created maps were used) and included in the data report. After initial presentations by Craig Smith and Malcolm Clark, the contents of the data report were reviewed to assist all working groups with their analyses. Data for various layers (e.g., nodule abundance, POC flux, depth, etc.) were made available with the assistance of Kerry Howell and Kirsty McQuaid. Use of data outside the report was contingent on permission by workshop co-chairs as well as the data contributors.

During the discussion of the data report, the recently-released ISA database *DeepData* was also discussed. Problems with the database, including difficulties in data extraction and a lack of necessary metadata, prevented the inclusion of these data for the workshop synthesis.

Annex IV

SUMMARY OF DATA THEME PRESENTATIONS

Presentations delivered under Item 4 above in the order presented.

a. Microbes – Emma Wear, Matthew Church, Beth Orcutt, and Rob Young

The introductory talk on microbial data included overviews of the methodology of sampling Bacteria and Archaea in marine habitats, the extant microbial datasets, and preliminary conclusions regarding habitat-type and spatial variability. We discussed the necessity of using genetic techniques to assess bacterial and archaeal community composition, as cellular morphology is not useful for assigning taxonomy for this group of organisms. Our preliminary meta-analysis incorporated samples primarily from the northeastern CCZ, including those from ABYSSLINE01 and ABYSSLINE02 covering the UK-1 and OMS claim areas and APEI-6, and the Malaspina survey project, with a smaller number of samples from the western CCZ, in particular the COMRA claim area. Caution is called for in comparing bacterial and archaeal community data generated using different analytical techniques, in particular those sequenced using different PCR (polymerase chain reaction) primers, as primer choice has been shown to impact both relative abundances of specific taxonomic groups and richness and diversity parameters. Given these caveats, we presented broad, preliminary trends in microbial communities. The clear primary control on microbial alpha and beta diversity was habitat type: water column (upper or deep ocean), sediments, or nodules, with notably higher richness in nodules and sediments. In contrast, we observed no or minimal spatial differences in overall community composition, richness, or diversity within those habitat types between the UK-1 strata, OMS, and APEI-6. On a very qualitative level, taxa present within specific habitats were generally consistent within samples from the eastern and western CCZ, although uneven sample sizes prohibited a formal analysis. We concluded with a brief discussion of the need for the deep-sea microbial community to move towards a more intercomparable sampling approach, even though the rapid rate of change in DNA sequencing technology remains a challenge.

b. Macrofauna – Travis Washburn and Craig Smith

Macrofaunal datasets comprised of box core samples collected in UKSRL1, IFREMER, GSR, BGR, IOM, OMS, Republic of Korea, and Russia exploration claims as well as in or near APEIs 3, 6, and 9 were acquired from Dr. Craig Smith from the University of Hawaii, Dr. Lenaick Menot from IFREMER and Dr. Magda Blazewicz from the University of Lodz through the JPIO project, Dr. Se-Jong Ju from KIOST, Dr. Ellen Pape from Ghent University, Dr. Koh Siang Tan from the National University of Singapore, and Slava Melnik from Yuzhmorgeologiya. Historical datasets were used from the western and southern CCZ as well. Different datasets included different components of the macrofaunal community (from a single class to all taxa) as well as different levels of taxonomic distinction (from species to order or higher). These differences across datasets required analyses to be performed on polychaete, tanaid, and isopod communities separately and at both the species and family level.

Macrofaunal studies were focused in the eastern and central CCZ, with only historical datasets representing the western CCZ. Polychaete and tanaid abundances showed a trend of increasing numbers from west to east across the CCZ. Species diversity was highly variable across sites which was likely partially the result of differences in taxonomists among studies, highlighting the inability to combine datasets at the species level and the need to standardize taxonomy across the CCZ and sampling programs. Family diversity appeared to be a better measure for comparisons among studies because family-level identifications are fairly standardized. For the majority of studies, the number of species recorded as singletons (or represented as only one individual in the entire study) was greater than 50% of all species recorded, showing the need for additional sampling to capture the full species richness diversity of macrofaunal communities throughout the CCZ.

Macrofaunal data from the ABYSSLINE Project was presented to illustrate some of the key biodiversity patterns evident some far in the eastern CCZ. Within two 30x30 km sampling areas (called strata) with the UK-1 exploration area, a total of 24 box core samples were collected to sample macrofauna. From these 24 box cores (a total area of 6 m^2 913 polychaete individuals were sampled, belonging to 42 families and 154 species (>70% of which potentially are new to science. Based on several species richness estimators, the total polychaete species richness across these two strata is estimated to substantially exceed 250 species. Most of the polychaete species in these samples were rare, with >50% of individuals and >90% of species richness occurring in species which each constituted <2% of total polychaete abundance; i.e., as group, rare species common in these abyssal macrofaunal communities. The UK-1 polychaete community appeared to be very rich in families and species compared to similarly sampled (sediment cores) and identified (morpho-taxonomy by the Natural History Museum) collections from the CCZ, and from the deep-sea globally.

c. **Invertebrate Megafauna – Daniel Jones and Erik Simon-Lledó**

The patterns in invertebrate megafauna (animals >10 mm in maximum size) in the Clarion-Clipperton Zone (CCZ) were assessed using information from image-based assessments, ground-truthed, where possible using megafaunal specimens. The focus on assessments from imagery means that there are some limitations, particularly in the level of taxonomic resolution of the faunal identifications. We used the term morphotypes as our unit of identification as species-level identifications are rarely possible and the taxonomic level of identifications varies. To try and improve consistency between surveys the megafaunal group have made considerable efforts to create a standardized morphotype catalogue and use this to re-annotate images. Such standardization is facilitated by modern annotation platforms, such as Biigle. The morphotype catalogue has synthesized all available data from the CCZ and contains a total of 632 morphotypes, dominated by echinoderms, cnidarians, sponges and arthropods. We were able to identify datasets from nearly 20 sites and nearly 60 transects, including information from 6 APEIs. Data analysis was split into a “meta-analysis”, which did not recheck or align the species but made the higher groups included consistent, and a “standardized analysis”, which was fully re-annotated following the morphotype catalogue. The datasets showed some clear patterns. A west to east increase in faunal density generally occurred across all available datasets. The west to east pattern also corresponded to a decrease in depth. Patterns in morphotype diversity were not as clear but will be explored further. The megafauna appear to respond to changes in the environment at a wide range of scales, from local to regional. These patterns will be further explored at the workshop and new data incorporated into the synthesis.

d. **Fishes and Scavengers – Jeff Drazen and Astrid Leitner**

Fishes and mobile scavenger communities were evaluated across the CCZ and Pacific using baited cameras and video or photo transecting methods. Data for scavenger diversity, relative abundance and community composition were compiled from baited camera deployments throughout the Pacific at depths below 3000 m, both unpublished and published studies (n=12 studies, 157 deployments, 43 in the CCZ). Data in the eastern CCZ were from 3 contract areas (UK1, OMS, BGR) and the data in the western CCZ were from 3 APEIs (1,4, 7). Methodological variations existed between studies that made comparisons challenging and reduced the dataset substantially for comparison. Scavenger identifications were standardized across the studies but in several cases this was not possible. Diversity varied between locations and community composition was significantly different between the SW Pacific, western CCZ, eastern CCZ, Hawaii and western CCZ seamount sampling locations. Though many of the taxa were widespread their relative abundances change across the CCZ region. Data for fish diversity, density and community composition was also compiled from video or photo transecting methods (ROV, AUV, and towed cameras) in the CCZ and neighboring regions. A standardized fish identification guide was created and the authors identified all of the fish in transects to ensure taxonomic standardization. Data from 5 contract areas (UK-1 TOML areas B, C, and D, Kiribati EEZ) and 4 APEIs (1, 4, 6 and 7) were available. It was clear that many studies did not sample large enough areas to adequately assess fish diversity and/or density because fishes are often sparsely distributed taxa. Fish community composition varied across the CCZ with similarity declining with increasing distance from east to west between sites. By combining both baited camera and transect data sets for fishes we evaluated species ranges and, not surprisingly, found that many species are broadly distributed; however, a few only appeared on deep (>3000 m) seamounts.

e. **Foraminifera – Andrew Gooday, Franck Lejzerowicz, Swee-Cheng Lim, and Brygida Wawrzyniak-Wydrowska**

Foraminifera constitute an important part of the meiofauna and macrofauna and nodule-encrusting fauna across the CCZ, while giant species (xenophyophores, many sessile on nodules) often dominate the megafauna. Faunal and genetic data are concentrated in contract areas located in the eastern half of the Zone but with few data available from any APEIs. Diversity is very high; for example, single megacore samples typically yield well over 100 meiofauna-sized morphospecies, with previously unseen species being added with each new sample. The numbers of molecular species (OTUs) are even higher. Morphological and environmental DNA (eDNA) data reveal that assemblages in all size categories are dominated by poorly-known, single-chambered monothalamids, the vast majority of them undescribed, while the better-known multichambered agglutinated and calcareous foraminifera are less common. Literature records based on test morphology suggest that many of the multichambered species known from the CCZ have wide geographical distributions at abyssal depths. On the other hand, the distributions of undescribed monothalamids are poorly known, and the majority are rare (often singletons or doubletons), making it impossible to conclude anything about their ranges. For two xenophyophore species, however, we have genetic confirmation for ranges extending at least 3,800 km from the western to the eastern CCZ. Across smaller distances of several 100s of kilometers (the scale of an individual contract area), foraminiferal assemblages seem to be relatively uniform, but with some indication of gradual change in composition with increasing spatial separation. Many data gaps remain, reflecting the scarcity of samples from the western CCZ and APEIs.

f. **Genetic Connectivity – Thomas G. Dahlgren, Guadalupe Bribiesca-Contreras, Helena Wiklund, and Adrian G. Glover**

Genetic data (DNA sequences) were available for analysis at the workshop from two main sources: published data on international databases linked to peer-reviewed publications, and unpublished data brought to the workshop by participants. Including both these sources of data, information from 4 Areas of Particular Environmental Interest (APEIs) and 8 contracted exploration zones was available. These data can be used to assess three important questions critical to the development of a Regional Environmental Management Plan (REMP): How many species are there as determined by genetics? How widely distributed are these species? How connected are their populations?

Sequence data are available from a range of faunal components that include several functional groups (e.g both nodule-dwelling animals and animals living associated with the sediment) but in general, the majority of data are from sediment-dwelling macrofauna and mobile scavengers collected in baited traps (typically macrofaunal-sized amphipods). In terms of biodiversity, genetic data typically recovers more species in analyses compared to morphological data. In global comparisons, CCZ biodiversity does seem relatively high compared to other regions, e.g. the North Sea. In terms of biogeography, sites within the CCZ (e.g contractor regions or APEIs) are characterized by a long list (e.g. 100-200 species) that are found only in those sites. However there are also many species shared, e.g. between APEI-6 and the UK-1/OMS sites there are 14 species shared across a distance of >1000 km. Population connectivity data show a universal pattern of high genetic diversity (with the exception of some nodule-dwelling taxa) and genetic connectivity across large distances e.g. with evidence of geneflow within species between the

CCZ and DISCOL experimental disturbance sites. However, this may be subject to sampling bias as the taxa sampled for connectivity analyses are necessarily the ones with high abundances and broad distributions.

g. Metazoan eDNA - Erica Goetze

Environmental DNA methods can be informative in addressing workshop questions related to biodiversity, biogeography and genetic connectivity (of the 5 primary topics). The DeepCCZ eDNA project goals were to: (1) conduct baseline surveys of invertebrate and vertebrate metazoans using eDNA methods, (2) assess seawater, sediments and nodules as source material for eDNA surveys, and (3) to evaluate whether seamounts are biodiversity hotspots and potential refugia that could serve as larval sources for populations on the abyssal plains that are impacted by mining. Multi-gene eDNA metabarcoding was conducted on seawater, sediments, and polymetallic nodules sampled across seamounts and abyssal plains in APEIs 1, 4, and 7 in the western Clarion Clipperton Zone, with metazoan animals as organismal targets. eDNA metabarcoding was effective at capturing the distinct biotas known to occur in association with different substrate types (e.g. nodule-specific fauna), with distinct community composition and very few ASVs and OTUs shared among sample types. We find evidence that seamounts in the western CCZ are ASV/OTU richness hotspots and reservoirs of unique metazoan diversity in comparison to the surrounding abyssal plains. Seamounts were characterized by significantly higher proportions of the community that were unique to a specific seamount or were cosmopolitan across plain and seamount habitats, with a higher proportion of plain fauna widespread in abyssal plain habitats but absent from deep seamounts. Across a gradient of low to moderate POC flux, we find lowest taxon richness and evenness at lowest POC flux, with community composition on nodules also influenced by nodule size. Key limitations for this dataset and type are (1) lack of broad-scale sampling, in particular across both claim areas and APEIs, (2) lack of reference sequences for the highly diverse meiofaunal sediment community (alpha taxonomy with COI-barcoded specimens), and (3) unknown timescales of eDNA persistence in the deep ocean (sediments).

h. Ecosystem Functions – Andrew Sweetman, Marta Cecchetto, Frank Wenzhofer, and Tanja Stratmann

We have collected ecosystem function (EF) data from numerous studies from across the central Pacific, CCZ, as well as gathered unpublished data. The data-sets spanned a wide geographical area (15°S to 23°N; 103°W to 158°W) and range of depths (4300-5400m depth). The data sets comprised data on sediment community oxygen consumption (SCOC) rates, nutrient fluxes across the sediment-water interface (N, P, Si), bioturbation rates (cm^2 yr^{-1}), sediment organic C and carbonate content (%), and POC flux information from traps and modelling exercises. We decided to focus our efforts on SCOC data and nutrient flux data as more of this data existed for the CCZ and central Pacific. Our main findings were that SCOC was positively and significantly related to POC flux across the region, while multiple regression analysis showed that nodule abundance did not seem to exert much of an effect on SCOC. Nevertheless, only 20% of the variance in SCOC was explained by modeled POC flux, suggesting other factors (e.g., organic matter quality) also need to be measured in baseline studies. We found that the significant relationship observed between SCOC and POC flux did not hold when only data-sets from the CCZ were used. Although a positive relation was still observed, it highlights the need for more data on benthic ecosystem function in the region. We found that the available in situ SCOC rates from the western APEIs were within the range of flux measurements from the central equatorial Pacific and CCZ but are at the low end of the scale. In terms of nutrient fluxes, we found a significant and positive effect of POC flux on silica and phosphate fluxes across the central equatorial Pacific, but not nitrate. These significant relationships were not seen when data from the CCZ were plotted against POC flux and nodule abundance, which highlights the need for more benthic ecosystem functioning data in the region. In terms of the silicate, phosphate and nitrate fluxes, flux estimates from APEIs 1,3, and 7 appeared to be outliers (confirmed from Cook's Distance analysis) when compared to the available flux data from other areas.

However, due to the limited number of APEI data-sets available, it was not possible to robustly conclude whether benthic function (nutrient fluxes) within these and other APEIs were unique/ similar with respect to the greater CCZ/ nearby contract areas.

i. Habitat Mapping and Environmental Data – Kerry Howell and Kirsty McQuaid

Habitat classifications are a versatile tool that can be used by managers to support spatial planning in large, data-poor areas. Classifications identify and delineate different types of habitats, which are used to represent biological communities. During this introductory session we presented a top-down, broad-scale habitat classification of the CCZ, which used environmental surrogates to represent variation in species distributions. The environmental variables used in the classification have been shown to influence species distribution in the CCZ, and included topography, POC and nodule abundance. The classification was carried out using a clustering algorithm, which grouped areas into habitat classes with different environmental properties. The classification identified 46 habitats across the CCZ, and these were hypothesized to support different fauna.

We then presented an assessment of the habitat representativity of the current APEI network, using the classification to identify gaps. This highlighted that several unique habitats with high nodule abundance in the central CCZ are not currently protected and showed that many of these habitats overlap with exploration and/or reserved areas. We suggested that additional APEIs should be established in peripheral areas and conservation-focused PRZs in mining areas, to protect habitats receiving less than 10% protection. The classification will be further refined during the workshop and a comparison of the distribution of values of key environmental variables contained within the APEI network versus mining exploration and reserved areas will also be carried out.

j. Fossilized Fauna – Diva Amon, Erik Simon-Lledó, Daphne Cuvelier, Thomas Dahlgren, Jennifer Durden, Adrian Glover, Kerry Howell, Daniel Jones, Kirsty McQuaid, and Craig Smith

The CCZ is thought to host unique, abundant and novel biodiversity in all size classes, so it is widely acknowledged that caution should be exercised with regards to deep-sea mining. As additional evidence of the potential sensitivity of this area to mining disturbances, we report the presence of fossils on the abyssal seafloor of the CCZ. This is based on observations from the following CCZ areas: UKSRL 1, OMS, GSR, BGR, OMCO, APEI 1, APEI 3, APEI 4, APEI 6, APEI 7. Additionally, image datasets from the DISCOL site in the southeast Pacific and the Kiribati EEZ will be used. Observed fossils were mostly cetacean bones, as well as shark teeth encrusted in polymetallic ore. While organic falls are known from this area, there has been little published on fossil falls, although there are records stretching back to the Challenger Expedition. In the near future, we plan to elucidate and publish a scientific paper on the types of fossils, their densities and potential ages, although given the known encrustation rate of the polymetallic ore, they are likely millions of years old. Additionally, many of these fossils host fauna either attached to the fossil itself or sheltering underneath (poriferans, cnidarians, polychaetes, ophiuroids, asteroids, etc.), showing that these fossils act as an additional source of hard substratum and may impact the benthic community. The presence of easily accessible and potentially unique fossils, as well as the potential damage resulting from mineral exploitation, has led us to conclude that special attention and perhaps additional conservation and management measures should be considered in the Regional Environmental Management Plan for the CCZ.

k. Seabed Climate Projections: Models, Thresholds and Outcomes – Chih-Lin Wei, William W. L. Cheung, and Lisa Levin (via Craig Smith and Daniel Jones)

The effects of climate change on the deep ocean include warming (Levitus *et al.*, 2000), oxygen loss (Breitburg *et al.*, 2018), acidification (Gehlen *et al.*, 2014), changes in ocean circulation (Toggweiler and Russell, 2008) and changes to surface productivity (Steinacher *et al.*, 2010) that reduce food supplies to the seafloor (Jones *et al.*, 2014). Projections of these changes in environmental conditions already exceed their historical variability (or annual standard deviation between 1951 and 2000) in many parts of the world deep ocean and will continue to expand over the next 80 years and beyond (Sweetman *et al.,* 2017). Current projections suggest that climate-related impacts will occur in all areas targeted for deep-sea mining (Sweetman *et al.,* 2017). The impacts of climate change will lead to effects on deep-sea life including direct impacts to key biological processes including metabolic rates, growth, and reproduction leading to increases in mortality and range shifts. Circulation variations will alter connectivity among populations and limit their ability to recover. This will lead to direct effects on ecosystems and the functions and services they provide. It is likely that cumulative effects from climate drivers and mining disturbance will interact.

Present projections suggest that the abyssal Pacific (>3000 m depth), which includes the Clarion-Clipperton Zone, is expected to experience dissolved oxygen changes of –0.37 to 0.03% and changes in the flux of particulate organic carbon (food supply) to the seafloor of –31.8 to 9.8% between present conditions and 2100 (Sweetman *et al.,* 2017). Temperature is expected to increase in the abyssal Pacific by 0.02 to 0.47 °C by 2100. These changes, particularly those in seafloor POC flux, are expected to lead to ecosystem-scale changes in the deep sea, such as reductions in seafloor biomass (Jones *et al.*, 2014).

In the coming decades, climate-induced changes will affect all areas of nodule-mining interest and are expected to be spatially heterogeneous across CCZ. Overall, the current network of APEIs broadly represent the climate hazards across the entire region (including exploration, reserved and background areas), i.e., they contain area expected to be climate refuges (little change) or have more resilience (greater baseline variability). In particular, APEIs 4 and 6 may be the climate-change refugia (undergoing relatively little change by 2021), and APEIs 1 and 9 may experience the largest impacts (i.e., be pushed farthest beyond their baseline variability in climate driven parameters such as POC flux. Thus, it is important to consider climate changes in conjunction with mining impacts to improve the effectiveness of environmental management. For example, it may be important to try to manage and differentiate impacts, such as species loss, directly caused by local mining from those generated at distance or by climate change (Levin *et al.*, 2019). Incorporating climate change into the management process for mining is an important consideration for cumulative impact assessment.

This research will be reported by a forthcoming paper:

Lisa Levin, Chih-Lin Wei, Daniel C. Dunn, Diva J. Amon, Oliver S. Ashford, William Cheung, Ana Colaço, Elva Escobar, Harriet Harden-Davies, Jeffrey C. Drazen, Khaira Ismail, Daniel O. B. Jones, David Johnson, Jennifer T. Le, Franck Lejzerowicz, Satoshi Mitarai, Telmo Morato, Sandor Mulsow, Paul Snelgrove, Andrew Sweetman, Moriaki Yasuhara (in preparation) Climate Change Considerations are Fundamental to Management of the Deep Sea.

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l. Metazoan Meiofauna — Daniela Zeppilli, Ann Vanreusel, Pedro Martinez, Ellen Pape, Tania Nara Bezerra, Freija Hauquier Katja Uhlenkott, Annemiek Vink, and Chisato Murakami

Meiofauna is defined as all benthic metazoans retained on a 32 µm sieve. Meiofauna has a key position in the food web and a significant role in ecological processes and ecosystem functioning. Outside chemosynthetic ecosystems, meiofauna tends to dominate the benthic compartment with increasing water depth. Meiofauna represents the most diversified group in the marine realm, encompassing 24 of the 35 animal phyla. However, considering metazoan meiofauna, only two groups are dominant: nematodes the most abundant taxon (50-90%), followed by copepods.

In nodule ecosystems, meiofauna is abundant in the first centimeters of sediment and the general trend is confirmed with the dominance of nematodes (80%) followed by copepods (10%). Nodule meiofauna is characterized by a high diversity at local scale. When compared to nodule-free abyssal plains nodule nematodes show lower abundance, but a similar genus composition and even higher biodiversity (EG51). Fifty percent of described species in CCZ are also present in the Peru Basin, showing that these species can range over 5000 km.

Methodological standardization is fundamental to compare the results from different studies (e.g. in abundances, SR, Evenness). For this workshop, data from 173 MUC samples from 7 claims (IFREMER, GSR, BGR, IOM, UKSRL, OMS, DORD) and 1 APEI (APEI3) were collected in addition to published data from Singh et al. 2016 and various Radziejewska et al. papers.

The first evidence analyzing data is that CCZ meiofauna is characterized by spatial and temporal variability. Spatial variability is demonstrated by the analysis of samples from the So239 cruise in 2015 visiting 4 contract areas and 1 APEI (3) where a clear gradient in densities and diversity was shown which followed a gradient in POC flux. Molecular data largely confirmed the morphological results demonstrating the high number of taxa restricted to one area, and the relatively low number of shared taxa between sites.

Temporal variability is also an important factor shaping CCZ meiofauna. In the BGR claim, meiofauna abundance in two sites was investigated along a time series of 5 years (2013, 2014, 2015, 2016, 2018). Mean values are relatively constant between years, but with an important increase in abundance and variance in 2015.

In the BGR claim, the meiofauna abundance of 88 MUCs coming from different areas and cruises (years) showed important differences between sites, between years but also high variance within a single MUC. The high variability inside claims is confirmed when different spatial and temporal datasets are analyzed. Finally, the meiofauna group identified potential strategies and datasets to answer the main workshop questions:

- Does species/taxon diversity vary along and across the CCZ?
  Meiofauna group strategy:
  Inclusion of the new data with literature
  Testing the POC flux and local topography (and the combination of both) as driver structuring meiofauna communities
- Are species ranges generally large compared to the distances between APEIs and contractor areas?
  Meiofauna group strategy:
  Combination of morphological and genetic data
- What is the degree of species overlap/community similarity between different study locations across the CCZ?
  Meiofauna group strategy:
  Working in progress this week but limited to the Eastern part
- Do claim areas have similar levels of species/taxon diversity to the proximal APEI(s)?
  Meiofauna group strategy:
  Limited meiofauna data in APEIs (only from one) but we can try to predict

Annex V

BREAKOUT GROUP REPORTS SYNTHESIZING BIODIVERSITY, ECOSYSTEM FUNCTION AND HABITAT PATTERNS ACROSS THE CLARION CLIPPERTON ZONE, AND THE EFFECTIVENESS OF THE EXISTING NETWORK OF APEIs

a. MICROBES: BACTERIA AND ARCHAEA –

Taxonomic diversity of Bacteria and Archaea in the Clarion-Clipperton Zone of the North Pacific Ocean

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Introduction

The functioning and health of marine ecosystems are tightly connected to extant biodiversity. The diversity of life has direct influence on numerous ecosystem functions, including energy capture, nutrient cycling, and organic matter production and consumption. In many regions of the ocean, the activities of microorganisms, defined here as unicellular organisms <2 μm in diameter, dominate many of these critical ecosystem services. Marine microorganisms are genetically and metabolically diverse, and include all three domains of life (Eukarya, Archaea, and Bacteria). Through their collective metabolic activities, these tiny organisms play globally significant roles in catalyzing the cycles of numerous elements. Moreover, marine microorganisms are a major fraction of ocean biomass, particularly in deep sea ecosystems that comprise some of Earth's largest biomes (Whitman et al. 1998; Orcutt et al. 2011; Kallmeyer et al. 2012). Despite their importance to habitability in these ecosystems, to date, our knowledge of microbial diversity and function in deep sea ecosystems remains poor. These gaps in our existing knowledge partly reflect chronic under-sampling of these remote habitats; however, such gaps also reflect difficulties inherent to studying organisms too small to observe without high-powered microscopes, and generally poor success in culturing naturally-occurring microbes. As a result, contemporary studies of marine microorganism diversity frequently rely on cultivation-independent methodologies, most notably including DNA sequencing of individual genes, suites of genes, or entire genomes (Giovannoni and Stingl 2005).

The lack of distinguishing morphological structure often observed among microorganisms hinders traditional taxonomic approaches to classify these organisms. As a result, comparative assessment of gene sequences remains the "gold standard" for assessments of microbial biodiversity. Such approaches have highlighted the enormous genetic reservoir of diversity found among marine microorganisms, and provide novel insights into specific ecosystem functions mediated by these organisms (DeLong 2009). The most common of these approaches relies on polymerase chain reaction (PCR) amplification and sequencing of ribosomal RNA (rRNA) genes. While other approaches, including those based on non-PCR-dependent methodologies (*e.g.,* shotgun metagenomics), provide relevant information for assessing microbial biodiversity, analyses of microbial biodiversity in ocean ecosystems remain rooted in rRNA gene phylogenies (Pace 2009).

As part of a larger International Seabed Authority (ISA) effort to synthesize ecosystem-scale patterns in extant biodiversity throughout the Clarion-Clipperton Zone of the North Pacific, this report summarizes data relevant to assessment of microorganism diversity. Large areas of the seafloor in the CCZ are rich in polymetallic nodules, making the region a likely target for future mining of ocean minerals. The overall goal of this report is to review available data from this region to evaluate patterns in microbial diversity. Where possible, the report synthesizes information on microorganism diversity in various sampled habitats (*e.g.*, water column, sediments, and nodules) of the CCZ, inclusive of mining claim areas and Areas of Particular Environmental Importance (APEIs). To assess the novelty of the CCZ region microbial biodiversity to other deep-sea habitats, we also include comparisons to select published datasets. For comparability among studies, this report focuses exclusively on diversity of Bacteria and Archaea, based on published and unpublished, PCR-amplified, 16S ribosomal RNA (rRNA) gene sequence data. The 16S rRNA gene is currently the most widely used genetic indicator for assessing bacterial and archaeal taxonomy, facilitating use of this gene marker to assess microorganism diversity in the CCZ.

16S rRNA sequence data utilized for synthesis

There are relatively few studies characterizing microbial biodiversity in the CCZ. For this report, 16S rRNA gene sequence data specific to the CCZ region were obtained from public DNA sequence repositories (e.g., the National Center for Biotechnology Information's GenBank and the European Bioinformatics Institute's European Nucleotide Archive), which included published works and several unpublished datasets. Together, the resulting rRNA gene sequences derived from studies spanned nearly a 20-year period (2000-2018), and included samples collected from contract areas contractd to the United Kingdom, Singapore, China, and Germany, as well as several APEIs (Figure 1, Table 1). The resulting collection of sequence data included key microbial habitats in this region, including polymetallic nodules, abyssal sediments, and water column (spanning from the sunlit near-surface waters to the near-bottom benthic boundary layer). For purposes of comparability, only 16S rRNA gene sequences derived from PCR-dependent approaches were included for this report.

Sequence data utilized for this synthesis were analyzed using several bioinformatic pipelines (e.g., DADA2, QIIME, mothur; however, all alpha diversity measures reported are Amplicon Sequence Variants from DADA2, Callahan et al. 2016), with taxonomic assignments based on the SILVA v132 reference database (Quast et al. 2013). Habitat-specific and geographic patterns in bacterial and archaeal taxonomic diversity were evaluated based on estimates of species richness (Chao1 index) and diversity (Shannon diversity, H'). The selection of PCR primers used to amplify rRNA genes varied among studies (Table 1; see discussion of "Limitations of CCZ microbial sequences analysis", below). In practice, this limited the scope of direct comparisons of community composition between several studies included in the meta-analysis. Further, primer choice has been shown to influence measures of richness and diversity in microbial communities (Klindworth et al. 2013; Wear et al. 2018) and therefore the comparisons herein should be interpreted cautiously. There are existing and forthcoming pending datasets for this region relying on non-PCR-based methodologies (*e.g.*, shotgun metagenomic sequencing) that will likely be useful for refining assessments of microbial diversity and function in selected regions of the CCZ (see Supplemental Tables).

Table 1. Microbial sample sets used for report analyses. PCR primers indicates the name of the primers used for PCR amplification of rRNA genes. Sequencing technology indicates the approach used to sequence the DNA libraries. Category totals do not include resequenced samples or analytical replicates

Dataset	**Location**	**Number of samples**	**Number of sites**	**PCR primers**	**Sequencing technology**	**References**
Nodules		**Total: 129**	**Total: 40**			
Abyssline01	Eastern CCZ: UK-1 Stratum A	36	8	515F, 806R	Illumina	Shulse et al. (2017)

MIDAS	Eastern CCZ: APEI 6 and UK-1	14	2	515F, 806R	Illumina	Jones, Hollingsworth, Young et al. in prep.
Abyssline01, resequenced	Eastern CCZ: UK-1 Stratum A	13	8	341F, 805R	Illumina	Lindh et al. (2017)
Abyssline02	Eastern CCZ: UK-1 Stratum B, OMS Stratum A, APEI 6	76	27	341F, 805R	Illumina	Lindh et al. (2017, 2018)
Blöthe 2015	German claim area	1	1	8F, 1492R & 109F, 912R	Clone libraries	Blöthe et al. (2015)
Wu 2013	Western CCZ, probably in COMRA claim area	2	2	8F, 1492R & 571F, U1204R	Clone libraries	Wu et al. (2013)
Sediment		**Total: 409**	**Total: 64**			
Abyssline01	Eastern CCZ: UK-1 Stratum A	80	9	515F, 806R	Illumina	Shulse et al. (2017)
MIDAS	Eastern CCZ: APEI 6 and UK-1	58	19	515F, 806R	Illumina	Jones, Hollingsworth, Young et al. in prep.
DeepCCZ landers	Western CCZ: APEIs 4 and 7	2	2	515F, 806R	Illumina	Sweetman, Cecchetto in prep.
Abyssline01, resequenced	Eastern CCZ: UK-1 Stratum A	37	9	341F, 805R	Illumina	Lindh et al. (2017); Shulse et al. (2017)
Abyssline02	Eastern CCZ: UK-1 Stratum B, OMS Stratum A, APEI 6	262	27	341F, 805R	Illumina	Lindh et al. (2017, 2018)
Wang 2010	Mid-CCZ: east and west regions of COMRA contract area, one site near the edge of the	4	4	27F, 1492R	Clone libraries	Wang et al. (2010)

	InterOceanMetal claim area					
Wu 2013	Western CCZ, probably in COMRA claim area	2	2	8F, 1492R & 571F, U1204R	Clone libraries	Wu et al. (2013)
Xu 2005	Western CCZ, COMRA claim area	1	1	Eubac27F, Eubac1492R & Arch21F, Arch958R	Clone libraries	Xu et al. (2004)
Water column		**Total: 110**	**Total: 19**			
Abyssline01	Eastern CCZ: UK-1 Stratum A	24	3	515F, 806R	Illumina	Shulse et al. (2017)
Abyssline01, resequenced	Eastern CCZ: UK-1 Stratum A	6	3	341F, 805R	Illumina	Lindh et al. (2017); Shulse et al. (2017)
Abyssline02	Eastern CCZ: UK-1 Stratum B, OMS Stratum A, APEI 6	80	10	341F, 805R	Illumina	Lindh et al. (2017, 2018)
Malaspina	Transect, NE corner of CCZ	6	6	515F-Y, 926R	Illumina	Not published (PIs: Sarmento and Gasol)

Biodiversity and biogeographic patterns in Bacteria and Archaea

Analyses of the available 16S rRNA gene sequences from both mining claim areas and APEIs highlighted the overall paucity of data currently available to evaluate microbial biogeography across the CCZ (Figure 1). For example, an overall lack of data from adjacent mining claim areas and APEIs make the available data of limited use for assessing the representativity of the APEIs. Moreover, the vast majority of data currently available from this region derive from the northeastern portion of the CCZ, largely restricted to sampling conducted in 2015 (including UK1, Ocean Mineral Singapore (OMS), and APEI 6 sites). There are currently very sparse sequence data available from the central and western CCZ (Table 1; Figure 1). However, we are aware of research cruises conducted over the past 2 years (e.g., DeepCCZ in 2018, Mining Impacts in 2019) that will likely improve spatial coverage of this region, including APEIs in the underrepresented western CCZ (APEIs 1, 4, and 7; see Supplemental Table). Such data will likely provide important additional information for assessing geographic variability in microbial taxonomic diversity in the CCZ. Notably, while these forthcoming datasets will extend the longitudinal coverage across the

CCZ, there remains a paucity of data across much of the central CCZ and lack of data from mining claim areas adjacent to APEIs.

Estimates of Chao1 (metric of species richness) and Shannon diversity indices suggest regional similarity in taxonomic diversity for those areas sampled in the CCZ. Both of these microbial diversity indices appear similar in claim areas and APEIs (Figure 2). However, for both metrics, there is clear variability by habitat type and between cruises (which were analyzed with different primers, different sequencing depths, etc.). In contrast to the apparent spatial stability in microbial diversity observed among the different CCZ sampling sites, the available data suggest distinct habitats sustain differences in overall diversity. This conclusion was also apparent based on rarefaction analyses of amplified sequence variants (ASVs) derived from the different studies (Figure 3). For example, estimates of Shannon indices from microbial communities inhabiting polymetallic nodules and sediments were greater than those of corresponding microbial communities inhabiting the overlying water column (Figure 2). Diversity and richness associated with polymetallic nodules was typically comparable to the surrounding sediments (Figures 2 and 3). For these analyses we did not differentiate microbial communities based on nodule age, size, or chemical composition; although such metadata have not typically been reported in studies examining microbial communities associated with nodules, such metadata could provide additional information on factors influencing microbial communities.

These differences in both Chao1 and Shannon diversity were also associated with unique overall community composition between habitats, as shown by a non-metric multi-dimensional scaling analysis of Bray-Curtis distance estimations of microbial communities sampled from the UK1 and OMS claim mining areas and APEI 6 (Figure 4A). In particular, sediment and nodule habitats clustered separately from seawater samples (Figure 4A), with no clear pattern dependent on where samples were collected. Notably, nodules encompassed almost as much between-sample diversity as the water column samples, despite coming from a much more limited range of source locations. This analysis also revealed that the CCZ nodule microbial communities appear distinct from microbial communities sampled from ancient (23 million years old) ferromanganese rinds on seafloor basalts elsewhere in the eastern Pacific Ocean (Figure 4B, basalt-area data from Lee et al., 2015).

These habitat-specific differences in microbial diversity were further evident in examining relative abundances of key microbial taxa within the various CCZ regions and habitats sampled (Figure 5). Although qualitative, several patterns emerge from these analyses: 1) Vertical patterns in the relative abundances of key microbial taxa were consistent with known depth-dependent distributions. For example, several major groups of microorganisms known to be abundant in the upper ocean, including the photosynthetic cyanobacteria and abundant chemoheterotrophs (consumers of organic matter for energy and nutrition, *e.g.*, clades of SAR11), were dominant above 300 m. Similarly, other taxa, including presumed chemoheterotrophs and chemoautotrophs (organisms that fix carbon dioxide using energy from oxidation of reduced chemicals, *e.g.*, Thaumarchaeota, Marinimicrobia, and members of the Deltaproteobacteria), demonstrated elevated relative abundances deeper in the water column consistent with known distributions of these microorganisms. 2) Sediment-associated taxa tended to be distinct from those found in the water column; similarly, several microbial taxa appeared preferentially associated with nodules, including several often considered copiotrophic (those growing optimally under conditions of elevated nutrients and organic matter, such as members of the Gammaproteobacteria family Alteromonadales). 3) Nodules appeared highly enriched in microbial taxa known to catalyze key nitrogen redox transformations (specifically nitrification, *e.g.*, members of the Thaumarchaeota and members of the Planctomycetes belonging to the family Pirellulaceae).

Limitations of CCZ microbial sequences analyses

Synthesis of these existing 16S rRNA gene sequence data from the CCZ revealed several important limitations that complicated comparative analyses across studies. The selection of PCR primers utilized to amplify rRNA genes varied between different studies, largely as an effect of the primers in favor at the time when the study was conducted (Table 1). In some cases, the different primers amplified different regions of the rRNA gene, hindering direct comparisons of the resulting sequences between studies. In addition, as with any PCR-based approach, amplification biases specific to the PCR primers have a direct influence on the representability of available rRNA gene sequences. In particular, some of the sequences utilized as part of this synthesis relied on 16S rRNA gene primers with known amplification biases to specific taxa of Bacteria and Archaea, including taxa known to be abundant in marine ecosystems (*e.g.*, Apprill et al. 2015; Walters et al. 2015; Parada et al. 2016). The combination of biases and different target regions have additionally been shown to influence measures of diversity (Klindworth et al. 2013; Wear et al. 2018), making comparative analyses between studies difficult. Among the data utilized for this report, those deriving from amplification and lllumina sequencing of the V4 region of the 16S rRNA gene formed the largest dataset for preliminary assessment of geographic and biodiversity comparisons.

In addition to these primer-dependent limitations, the 16S rRNA gene sequence database collected for this report derived from studies utilizing Sanger-based sequencing of cloned PCR amplified genes, and from studies that utilized high-throughput (Illumina) sequencing of barcoded PCR amplicons. These two methodological approaches yield vastly different numbers of sequences per sample (Figure 6) and sequence read lengths. For example, the number of sequences per sample reported from studies relying on cloning were often <100 sequence reads per sample, while studies reporting sequences based on barcoded amplicons typically reported >30,000 sequence reads per sample (Figure 6). As a result, we opted not to include measures of richness and diversity from the clone library samples, as these metrics would certainly be underestimates; however, this does eliminate our ability to comment on the alpha diversity of claim areas in the western CCZ at this time, as those areas have exclusively been sequenced using clone libraries.

Summary of Gaps and Limitations

- The currently available data provide very poor temporal and spatial resolution in the CCZ; however, this may improve through inclusion of forthcoming datasets (*e.g.*, DeepCCZ in the western APEIs, Mining Impacts in the eastern CCZ) and through inclusion of contractor claim area samples.
- The available sequence data limit our ability to assess representability of APEIs; there is currently poor spatial sampling resolution of mining claim areas adjacent to APEIs.
- Methodological differences (*e.g.*, PCR primers, sequencing approaches) among the existing data make comparing across studies difficult.
- The existing data currently allow taxonomic classification of microorganisms; however, a limitation of such taxonomic data is the inability to connect taxonomy to ecosystem function in uncultured and minimally studied deep-sea taxa. There are approaches to connect environmental microbial taxonomy to function (e.g., metagenomics, isotopic tracers), but these approaches are rapidly evolving and currently require specialized training and significant resources. Moreover, many of these approaches are not currently standardized for baseline monitoring.

Conclusions

- For those sites where we have large sequence datasets (claim areas UK1 and OMS, and APEI 6, and sediments from APEIs 4 and 7), we have sufficient data to examine bacterial and archaeal taxonomic diversity.
- Based on the limited data currently available, we do not see significant regional (East to West) or claim area to APEI-specific differences in bacterial and archaeal taxonomic diversity (species richness, Shannon diversity, or broad trends in community composition).

- rRNA gene amplicon sequencing and analyses are useful for insight into microbial taxonomic diversity in the CCZ abyssal seafloor habitats.
- Bacterial and archaeal biodiversity varies significantly among deep-sea habitats (i.e., sediments, nodules, and seawater), with both greater Shannon diversity and greater variability in community composition observed among sediments and nodules.
- The emergent data suggest habitat-specific (sediment, nodules, seawater) taxonomic distributions, and that these differences in microbial communities may result in different ecosystem services (i.e., nitrogen and carbon cycling).
- We currently do not have sufficient information to assess whether removal or disturbance of specific abyssal habitats will impact ecosystem services currently provided by microorganisms. Information needed to inform understanding of how disturbance may alter ecosystem services will require additional observations, including measures of microbial biomass and biogeochemical function.

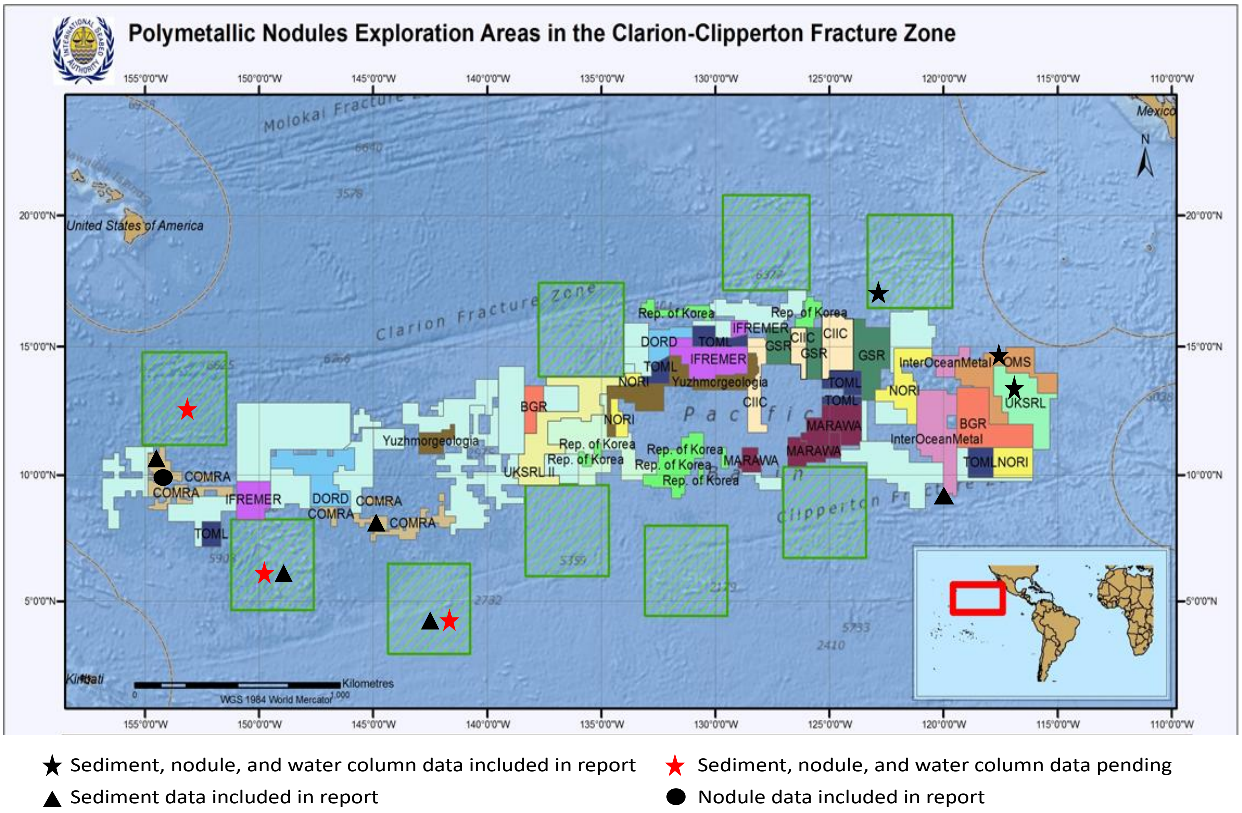

Figure 1. Map of CCZ mining claim areas and APEIs. Symbols depict study regions where samples were collected for assessment of microbial taxonomic diversity.

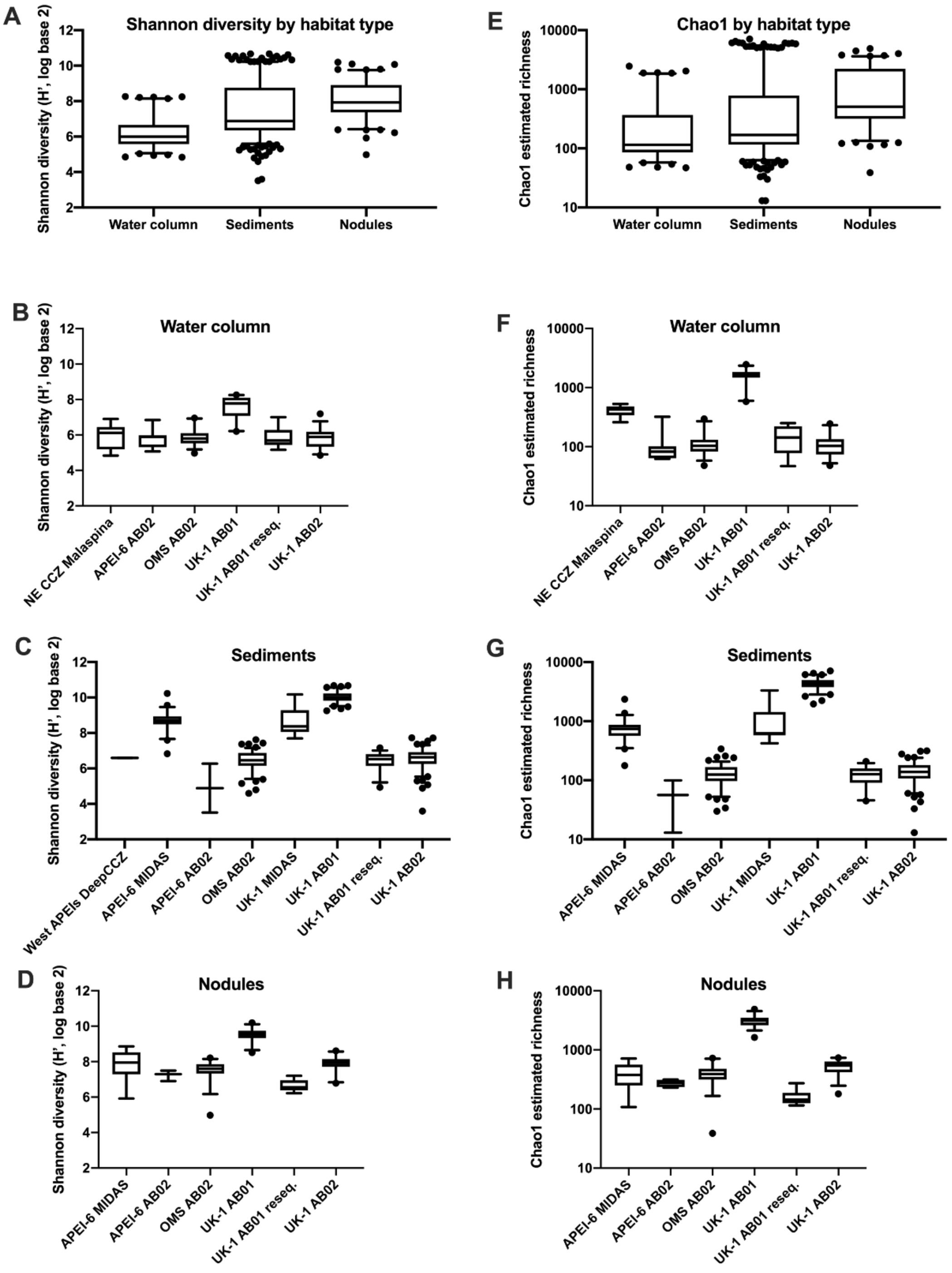

Figure 2. Shannon diversity (log base 2) and Chao1 estimated richness, based on amplicon sequence variant relative abundances from all datasets sequenced using Illumina. Panels A and E: Data from all sites are grouped together, by habitat type. Panels B-D and F-H: Samples were subdivided by habitat type then by sampling region and cruise (AB01 and AB02 here are the Abyssline cruises). Sampling sites are arranged roughly from west to east along the X-axis. In all panels, the boxes represent the median, 25th and 75th percentiles of the data, error bars the 5th and 95th percentiles, and filled circles outliers.

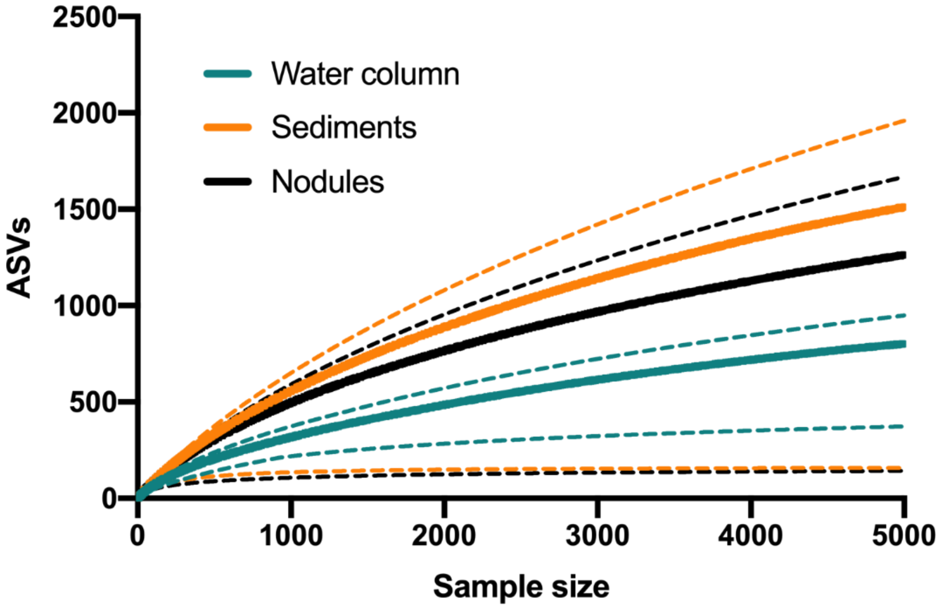

Figure 3. Rarefaction curves depicting richness of amplified sequence variants in different CCZ habitats. Only data from Abyssline01 and MIDAS studies are included in these analyses because these studies utilized the same PCR primers. The solid line is the median rarefaction curve of all samples from a habitat, and the dashed lines are the maximum and minimum curves. Samples were rarefied to a standard size of 5000 sequences, which was smaller than the observed sequence yield for samples in these datasets.

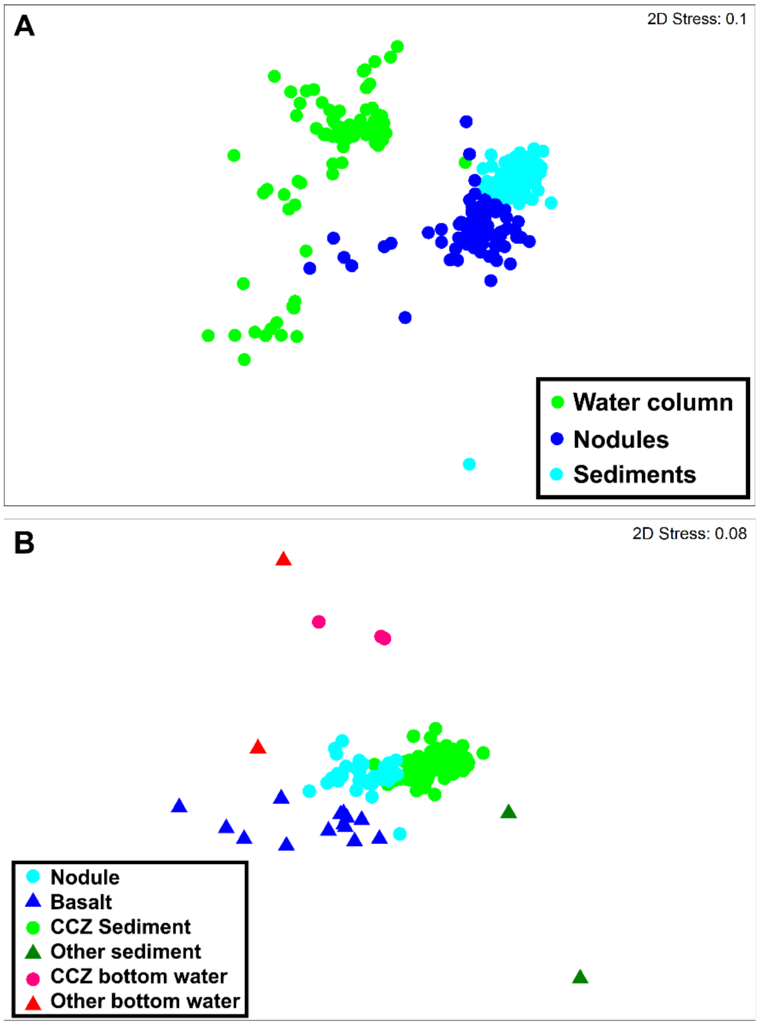

Figure 4. Beta diversity of CCZ microbial communities. A. Non-metric multidimensional ordination of bacterial community composition data from the UK1 and OMS mining claim areas and APEI 6 (based on sequence data from Abyssline02 and Abyssline01, with individual points coded by habitat type. Each point represents a full community and proximity of points indicates overall community similarity. Note the clear distinction between water column samples and benthic substrates (sediment and nodules). B. Benthic CCZ communities appear distinct from those in parts of the deep seafloor where the crust is dominated by basalt, even when communities are compared at the relatively coarse phylum level, as was the case here (CCZ samples re-analyzed from Shulse et al, 2017; basalt-area samples from Orcutt et al. in review).

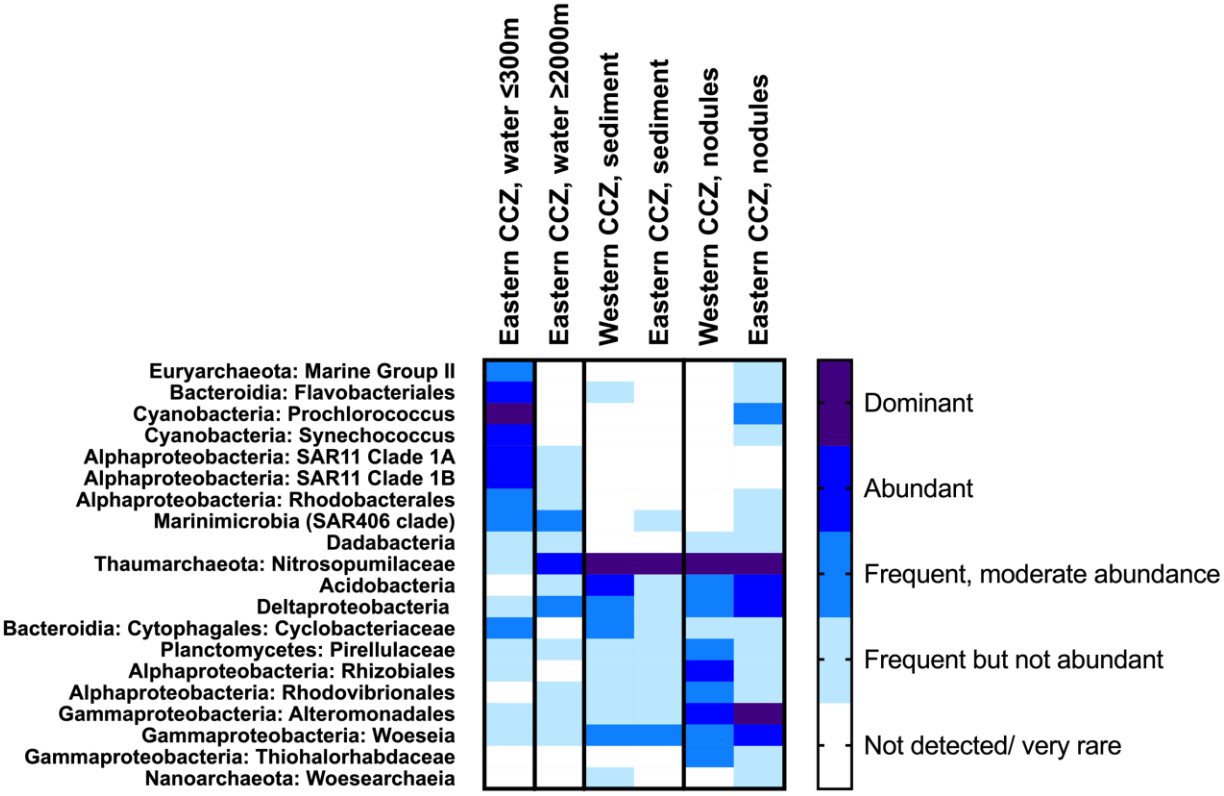

Figure 5. Qualitative frequency data of bacterial and archaeal groups at various taxonomic levels of interest, separated by habitat type and broad CCZ location. Dark blue squares and white squares are assessed at a high confidence; the intermediate colors are abundances about which we are less confident, given our current sample availability.

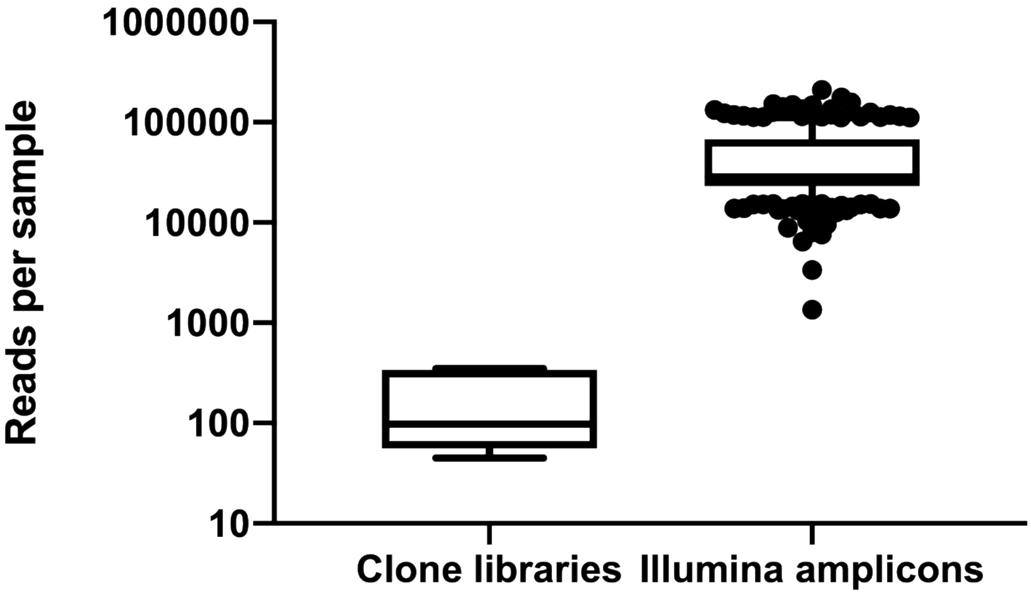

Figure 6. Reads per sample in the clone libraries and a representative subset of the Illumina amplicon studies included in this study. The box plots represent the median, 25th and 75th percentiles, the error bars depict 5th and 95th percentiles, and individual dots represent outliers.

Supplemental Tables. Additional microbial datasets currently available from the CCZ not incorporated in this meta-analysis. Amplicon datasets were not included because the raw sequence data were not publicly available at the time the meta-analysis was conducted. Metagenomic samples were not included because the sequence density is not comparable to the amplicon datasets that currently constitute the bulk of the extant data.

Dataset	**Location**	**Number of samples**	**Number of sites**	**PCR primers**	**Sequencing technology**	**References**
Nodules		**Total: >3**	**Total: 2**			
Cho 2018	Central CCZ: KODOS	3	1	27F, 1522R and 21F, 958R	Clone libraries	Cho et al. (2018)
Ye 2010	Western CCZ: COMRA	Unknown	1	Unknown	Clone libraries	Ye et al. (2010)
Sediment		**18**	**12**			
Cho 2018	Central CCZ: KODOS	3	1	27F, 1522R and 21F, 958R	Clone libraries	Cho et al. (2018)
Dong 2016	Western CCZ	9	9	Unknown	Illumina	Dong et al. (2016)
Jing 2016	Western CCZ: COMRA	6	2	Unknown	Clone libraries	Jing et al. (2016)
Metagenomes		**Total: 7**	**Total: 5**			
Abyssline01: nodules	Eastern CCZ, UK-1 Stratum A	2	2	N/A	Illumina HiSeq	Evans, Shulse et al. in prep
Malaspina: Pernice 2016	Northeastern CCZ	1	1	N/A	Illumina HiSeq	Pernice et al. (2016)
Tara Oceans: water column	Eastern CCZ	3	1	N/A	Illumina HiSeq	Pesant et al. (2015)
Xu 2007	Western CCZ: in or near COMRA	1	1	N/A	Cosmid library	Xu et al. (2007)

Microbial datasets from the CCZ anticipated in the near future. Microbial sequence datasets in progress at the time of the workshop.

Dataset	**Location**	**Sample type(s)**	**Contacts**
DeepCCZ	Western CCZ: APEIs 1, 4, and 7	Nodules: ~10 samples over 2 sites Sediments: ~100 over 5 sites Water column: ~144 over 12 sites And select metagenomes	M. Church, E. Wear, University of Montana
JPI Oceans: MiningImpact 2 project	Eastern CCZ: Belgian and German contract areas	Sediments Water column, especially benthic boundary layer	Max Planck Institute and Alfred Wagner Institute Project coordinator: M. Haeckel, GEOMAR
GEOTRACES	Western CCZ: meridional	Water column	BioGeoTraces group: D.

GP15	transect		Chappell, S. Clayton, Old Dominion U.; P. Berube, MIT

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b. **METAZOAN MEIOFAUNA** —

Metazoan meiofauna

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Introduction

In this chapter the main patterns in biodiversity and biogeography of metazoan meiofauna over the CCZ and adjacent areas taxa will be discussed. Meiofauna is the group of sediment-dwelling, small-sized organisms that are retained on a 32-µm sieve (see LTC guidelines). Meiofauna represents among the most diversified communities of the marine realm (Figure 1). They mainly consist of nematodes representing up to 90 % of the total community followed by copepods representing about 10 % or less. Several other taxa are occasionally present in various numbers. They include taxa such as Tardigrada, Kinorhyncha, Ostracoda and Loricifera which are considered as permanent meiofauna while also some taxa are represented by juveniles such as Polychaeta, Amphipoda and Isopoda. The latter are considered temporary meiofauna. Their high abundance and diversity, their general distribution, their rapid generation time and fast metabolic rates make meiofauna important in ecosystem functions such as nutrient cycling and energy transfer to higher trophic levels (Woodward 2010). Furthermore, meiofauna can be used as a proxy for responses of benthic communities to environmental changes and anthropogenic impacts (Zeppilli et al. 2015). The benthic size class of the meiofauna is the most numerous and may be the most diverse metazoan taxa in nodule areas and adjacent abyssal plains (Radziejewska 2014).

By demonstrating patterns in biodiversity and distribution of taxa in the Clarion Clipperton Fracture Zone we will answer a set of specific questions which are related to the main objectives of this workshop.

- Does meiofauna diversity vary along and across the CCZ?
- Do claim areas have similar levels of biodiversity to the proximal APEI(s)?
- What is the degree of community similarity between different locations across the CCZ?
- Are species ranges generally large compared to the distances between APEIs and contractor areas?

Figure 1. Illustration of dominant meiofauna groups with on the left side a tardigrade, a kinorhynch and harpacticoid copepod and in the middle and right different nematode specimens © Gilles Martin/Ifremer.

Methods

Datasets

Meiofauna datasets available for the DeepCCZ workshop came from 7 contractor areas (GSR, IFREMER, IOM, BGR, UKSRL, OMS, DORD) and 4 APEIs (APEI1, APEI3, APEI4 and APEI7) for a total of 201 samples (Table 1 and Figure 2).

Table 1. Meiofaunal datasets used for the synthesis. C= contractor, RP= Research Project, x = morphologic/taxonomic/genetic data, $ = eDNA data. N MUC Dep = number of multicore deployments.

Contributor	N MUC Dep	Owner	Claim/ APEI	Meiofauna abundance	Meiofauna composition	Nematode diversity	Copepoda diversity
IFREMER	17	IFREMER (C)	IFREMER	x	x	x	x
UGent	38	GSR (C) JPIO2 (RP)	GSR, APEI3, IFREMER IOM	x	x	x	
SNG	88 10	BGR (C) UKSRL (C)	BGR, UKSRL, OMS,GSR, IFREMER, APEI3	x	x	x	x
DORD	20	DORD (C)	DORD			x	
DEEPCCZ	18 (ROV Push cores) + 10 (Nodules)		APEI1, APEI4, APEI7		$	$	$
TOTAL	201		7 claims, 4 APEI				

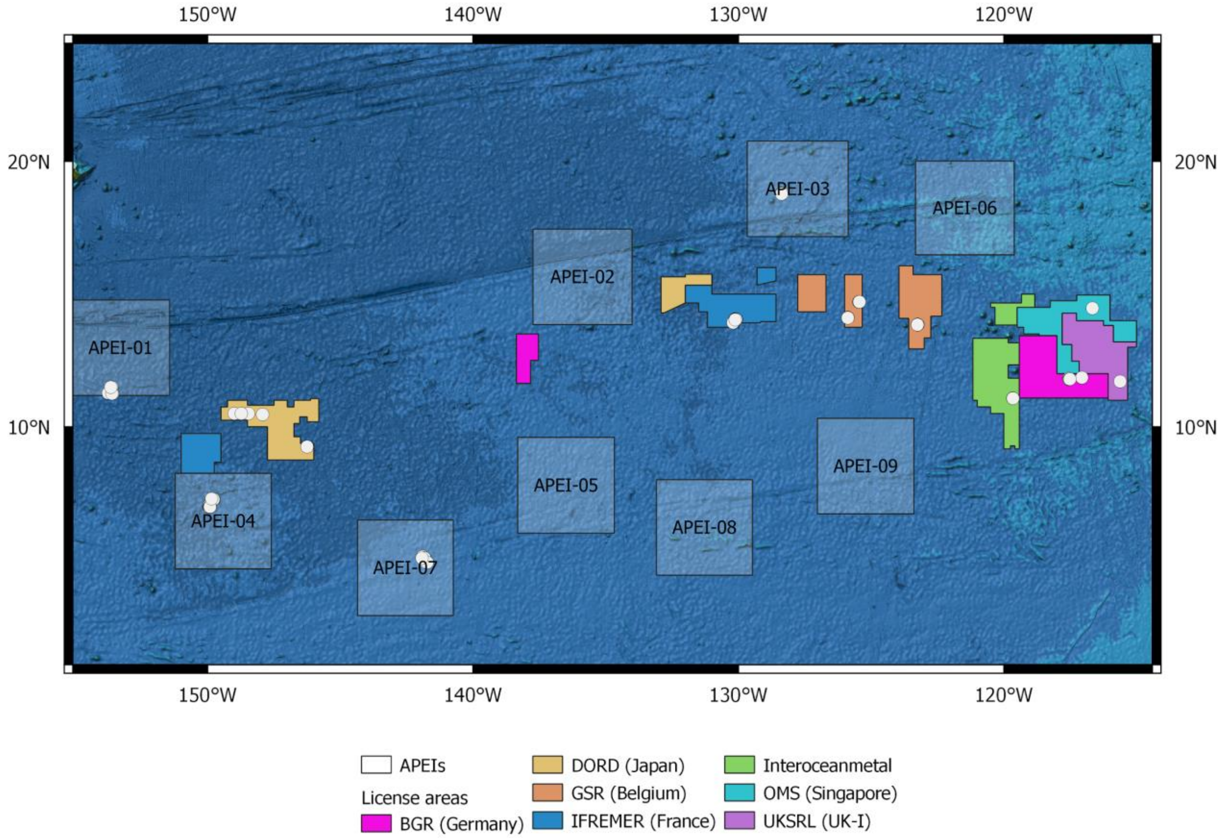

Figure 2: CCZ locations for which meiofauna data were made available for the DeepCCZ workshop.

Morphological datasets

Sampling was conducted in the CCZ during the EcoResponse cruise SO239 with RV Sonne (Martínez Arbizu and Haeckel, 2015) in March–April 2015. Six different sites situated in four contract areas and APEI3, as established by the ISA, were visited to study the meiofauna, among other things. Samples for morphological as well as molecular analysis were collected but here only the morphological data are used. In addition, samples were collected in the GSR area in the framework of contractor environmental baseline studies in 2015 and 2017. Details on sampling and processing of samples are provided respectively in Hauquier et al (2019) (Ecoresponse Cruise) and Pape et al (2017) (GSR baseline studies). Baseline studies carried out by BGR contributed 88 Multicorer deployments between 2010 and 2018 but data are not included here. Samples were collected in the east zone of the French mining claim area of the Pacific Nodule Province during the Nodinaut campaign on board of L'Atalante, May–June 2004. Ten stations were sampled from two sites category, nodules area and outside nodules area, using multicore (9.5 cm in diameter) down to 5 cm depth. During ABYSSLINE I and II cruises, samples were taken in the UKSRL area and in the OMS area with RV Melville and RV Thompson.

Molecular datasets

Deep CCZ: Environmental DNA (eDNA) biotic surveys were conducted in the western Clarion Clipperton Zone as part of the DeepCCZ field program on RV Kilo Moana cruise 18-8. Sediment and polymetallic nodules samples were collected in APEIs 1, 4 and 7. Sediments and nodules were collected on abyssal plains by ROV push cores (7 cm diameter), with 2 dives in APEI 7, 2 dives in APEI 4, and 2 dives in APEI 1, with 2-5 cores collected for eDNA on each ROV dive. Sediment samples were subsampled for eDNA at 0-2 cm and 3-5 cm sediment horizons. Polymetallic nodules were either collected using push cores or by the manipulator arm of the ROV. Eukaryotic communities were characterized by amplicon sequencing using two genetic markers, the V4 region of the 18S rRNA gene (approximately 450 base pairs [bp]) and a fragment (ca. 350 bp) of the mitochondrial COI gene. For 18S rRNA, the eukaryotic forward Uni18SF: 5′-AGG GCA AKY CTG GTG CCA GC-3′ and reverse primers Uni18SR: 5′-GRC GGT ATC TRA TCG YCT T-3′ primers (Zhan et al., 2013) were used; For COI, the universal metazoan primers mlCOIintF: 5′-GGW ACW GGW TGA ACW GTW TAY CCY CC-3′ and jgHCO2198: 5′-TAI ACY TCI GGR TGI CCR AAR AAY CA-3′ (Leray et al., 2013). Samples were sequenced on two MiSeq Illumina^{TM}lanes using V3 chemistry and paired-end sequencing (2×300 bp). Data were processed through a Qiime2/DADA2 bioinformatic pipeline, including quality filtering, denoising, merging and chimera removal. Amplicon sequence variants (ASVs), or unique genetic variants comparable to high resolution operational taxonomic units (OTU's) (Callahan et al. 2017), for nematodes, harpacticoids, and other meiofaunal organisms were analyzed in reference to environmental variables (Table 2).

Table 2: Edna ASV richness of major meiofaunal groups

Taxa	**ASV Richness**
Nematoda	775
Harpacticoida	135
Gastrotricha	26
Loricifera	24
Kinorhyncha	5
Tardigrada	0
Rotifera	1

Graphical representation

Most graphs were made in R using the package ggplot2 (Wickham, 2016) and grouped with cowplot (Wilke, 2019). The R package iNEXT was used to construct rarefaction and extrapolation curves based on nematode species (abundance data) and nematode and copepod ASV richness (incidence data) (Hsieh et al. 2016, 2019). Richness was estimated using Chao2 through the R package iNEXT (Chao and Jost 2012). Shaded, colored areas indicate the 95 % confidence intervals obtained from a bootstrap method based on 200 replicates. Horizontal dotted grey lines (top two panels) indicate maximum interpolation values for each sample type. Sampling coverage, defined as the inverse probability of adding a new species with each sample, is included to compare richness between samples of differing sampling efficiencies (Chao & Jost, 2012). Vertical dotted grey lines (bottom panel) indicate the value at base coverage, defined as the highest coverage value between minimum extrapolated values and maximum interpolated values. We performed both sample size (number of individuals for species, number of reads for ASVs) and sample coverage-based rarefaction and extrapolation, as the former may misrepresent the degree of differences in taxon richness between communities (see Chao and Jost, 2012). To visualize the number of shared and unique species and ASVs between different sites sampled, UpSet plots were created by means of the package UpSetR (Gehlenborg, 2019).

Results and Discussion

Biodiversity analysis

For the biodiversity analysis we applied a dual approach. In a first set of analyses we identified the relationship between diversity and abundances and/or seafloor POC flux. The rationale behind this approach is that areas with the highest POC flux are in general characterized by the highest abundances (Hauquier et al., 2019). Furthermore, our data show that these areas are also the most taxon rich areas. Therefore, it is important that APEIs are sufficiently representing the most food-rich parts of the CCZ with the highest regional POC flux. A strong correlation was found for Copepoda morphospecies density and abundances within core samples in the UKSRL contractor areas illustrating that an increase in numbers of specimens brings in a similar proportion of new species per sample (Figure 3). This pattern was also observed in the IFREMER contractor area in samples collected during the Nodinaut Cruise (Ramirez et al., 2010).

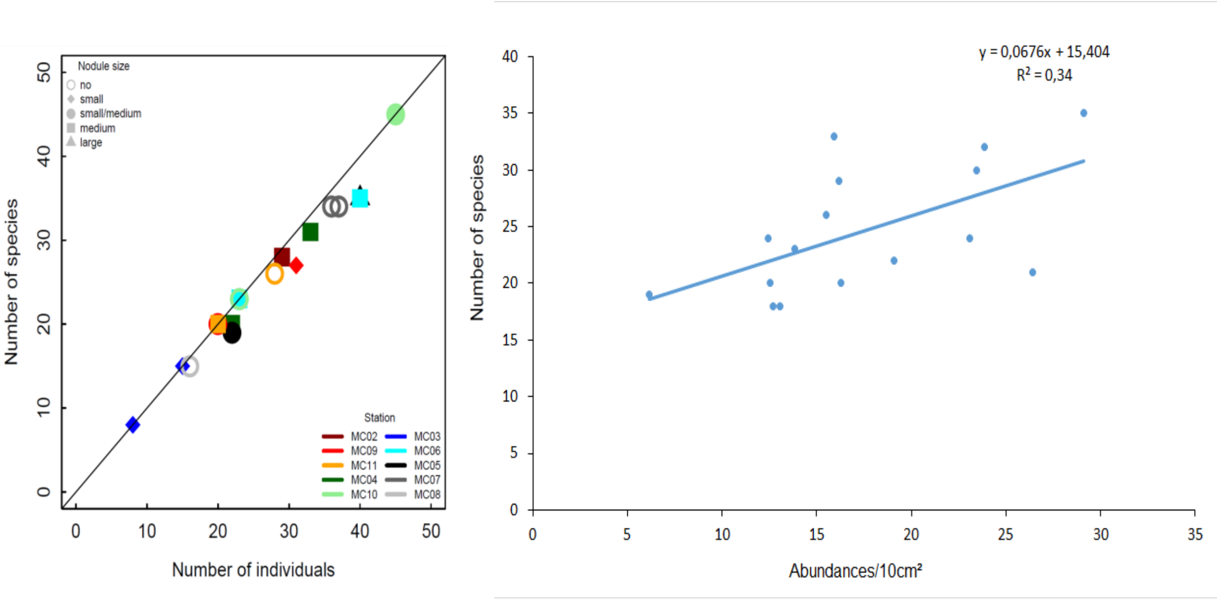

Figure 3. XY scatterplot for benthic copepod species density versus number of adult individuals per core (on the left) and versus all copepods, including juveniles (on the right). Data are provided by Menzel and Martinez (unpublished) from UK seabed resources (on the left) and by Mahatma and Martinez, unpublished from Nodinaut cruise from the eastern French Claim (on the right).

For nematode genera and species based on a dataset of 4 contract areas and APEI 3, all situated in the North eastern part of the CCZ but representing a pronounced POC gradient, a similar positive trend was found between taxa richness and total nematode densities (Figure 4). The increasing trend at morphospecies level is very similar as for the genera, though with values nearly twice as high.

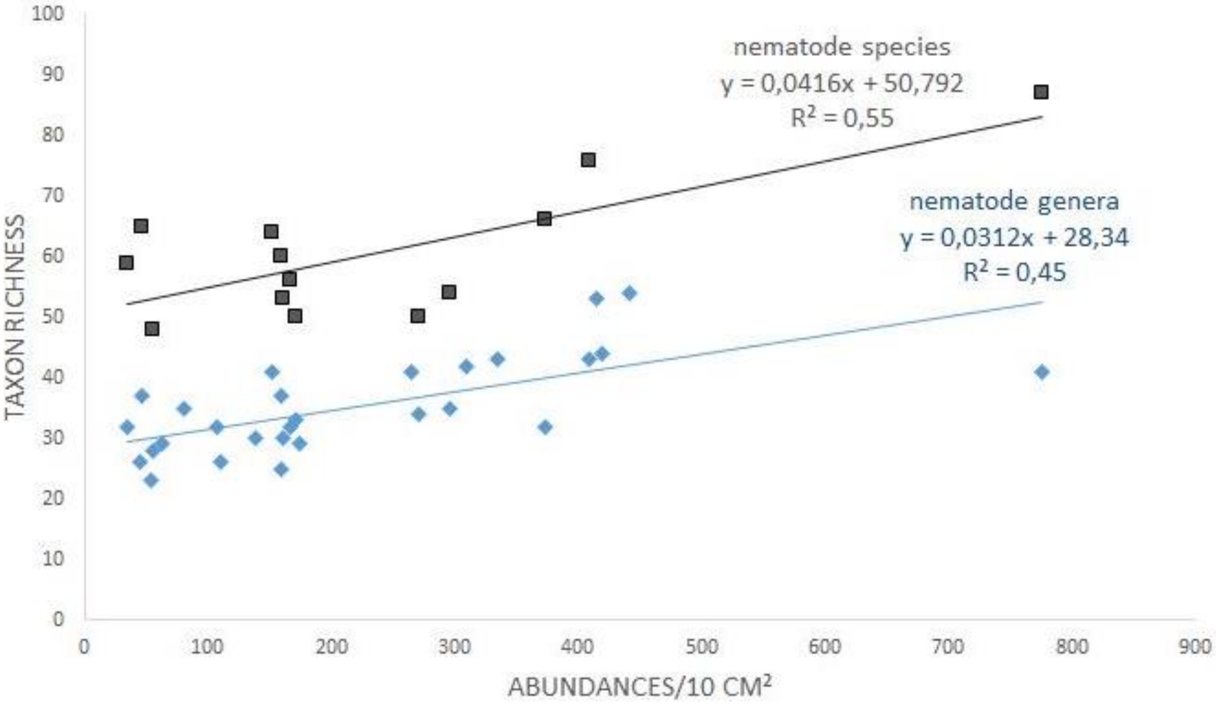

Figure 4. XY scatterplot of nematode genus (blue symbols) and species (black symbols) richness versus abundances per core. Data are from Hauquier et al (2019) and (Pape et al, 2016 and unpublished data from GSR)

Figure 5 demonstrates the distinct POC flux regimes for 3 Western APEIs (1, 4 and 7). Meiofaunal ASV richness increases with increasing POC flux ($R^2 = 0.30$). When separated by dominant meiofaunal components, this trend is strongly determined by nematode diversity. Nematodes make up over 80.3% of the ASV richness for meiofauna, while harpacticoids only make up 14%. Representation by other groups (Gastrotrichs, Kinorhynchs, Rotifers etc.) was <1%. Nematode ASV richness increased with increasing values of seafloor POC flux. This trend was however not observed for copepod ASV richness (Figure 5).

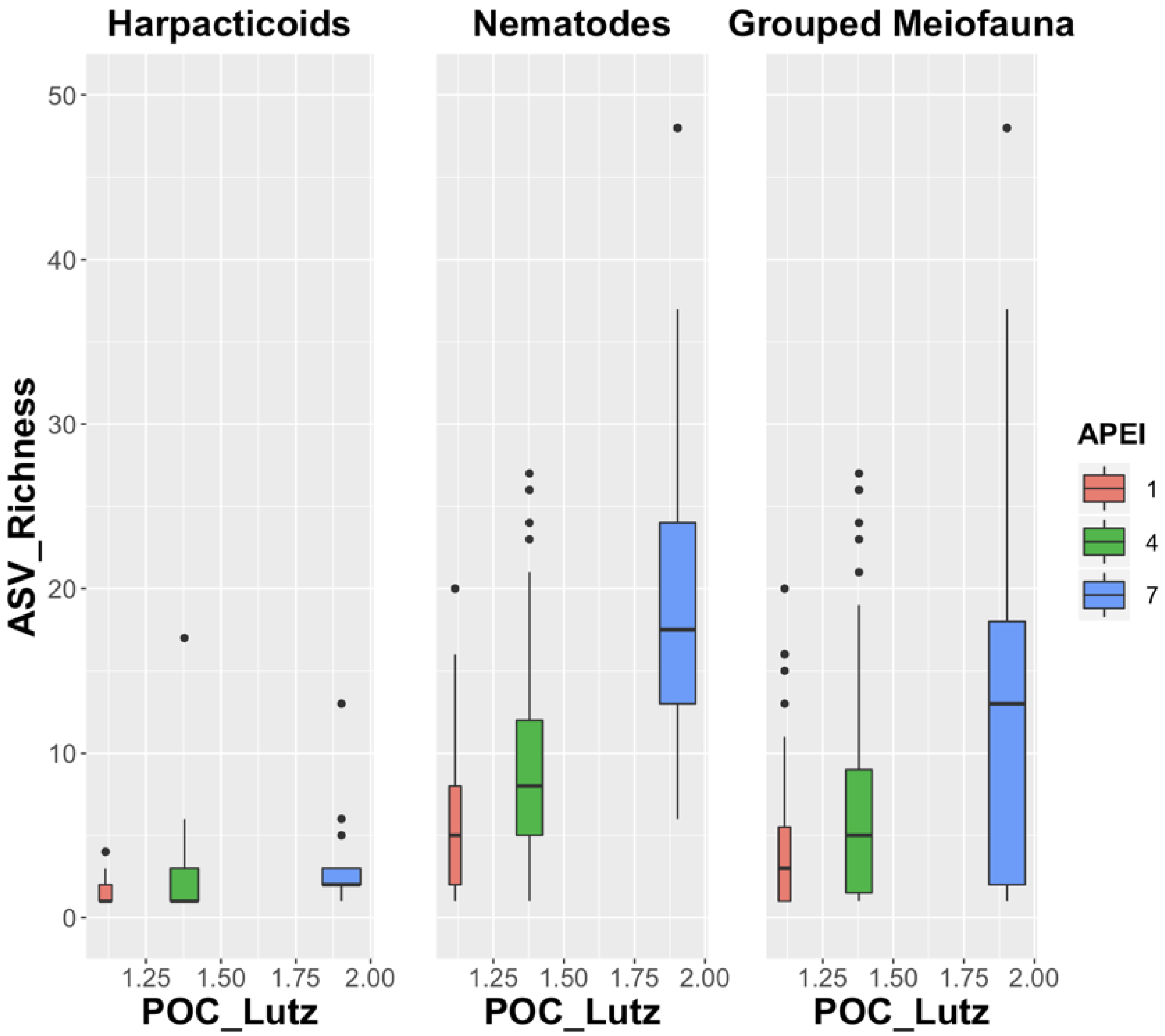

Figure 5: Meiofauna ASV richness with POC flux for Western CCZ APEIs. Data is pooled per sample from 18S eDNA and only includes samples from plains from both nodules and sediment. POC flux was extracted from the Lutz model estimated POC flux for each sampling location.

In a second approach, we identified the rarefaction curves for different datasets (Meiofauna ASV's in the Western CCZ and nematode morphospecies from the Eastern CCZ) to demonstrate the relation between regional biodiversity and POC flux.

Amplicon sequence variant (ASV) sampling coverage and richness for each APEI is reported in Figure 6. Fig 6A shows that APEI 1, 4, 7 had similar sampling efficiencies. Fig 6B shows massive under-sampling. Fig 6C is rarefaction based on sample coverage and shows under-sampling. Both the sample size (B) and sample coverage (C) based rarefaction and extrapolation curves showed the lowest species richness in APEI 1, (lowest seafloor POC flux), and highest richness in APEI 7 (highest seafloor POC flux).

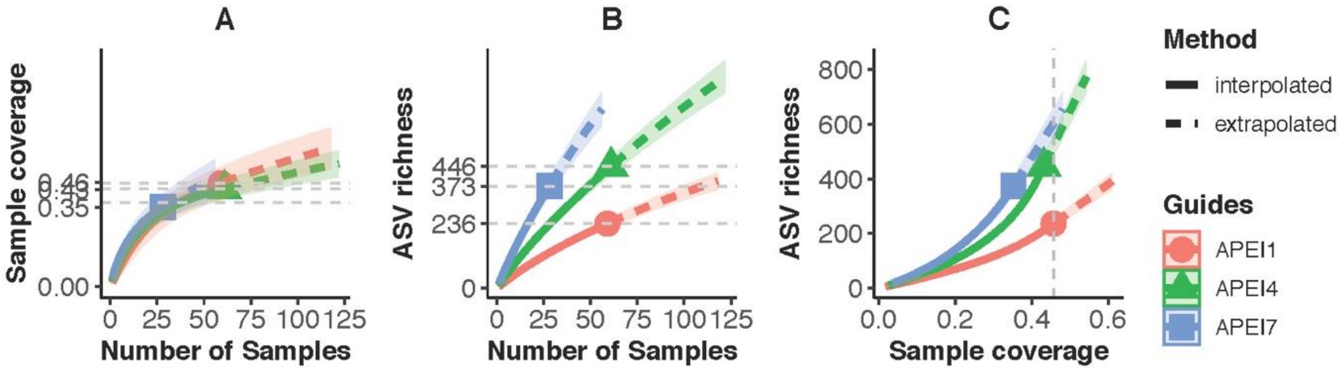

Figure 6. Amplicon sequence variant (ASV, 18S) sampling coverage and richness within APEIs 1, 4 and 7 (western CCZ). All meiofaunal groups listed in Table 2 are included.

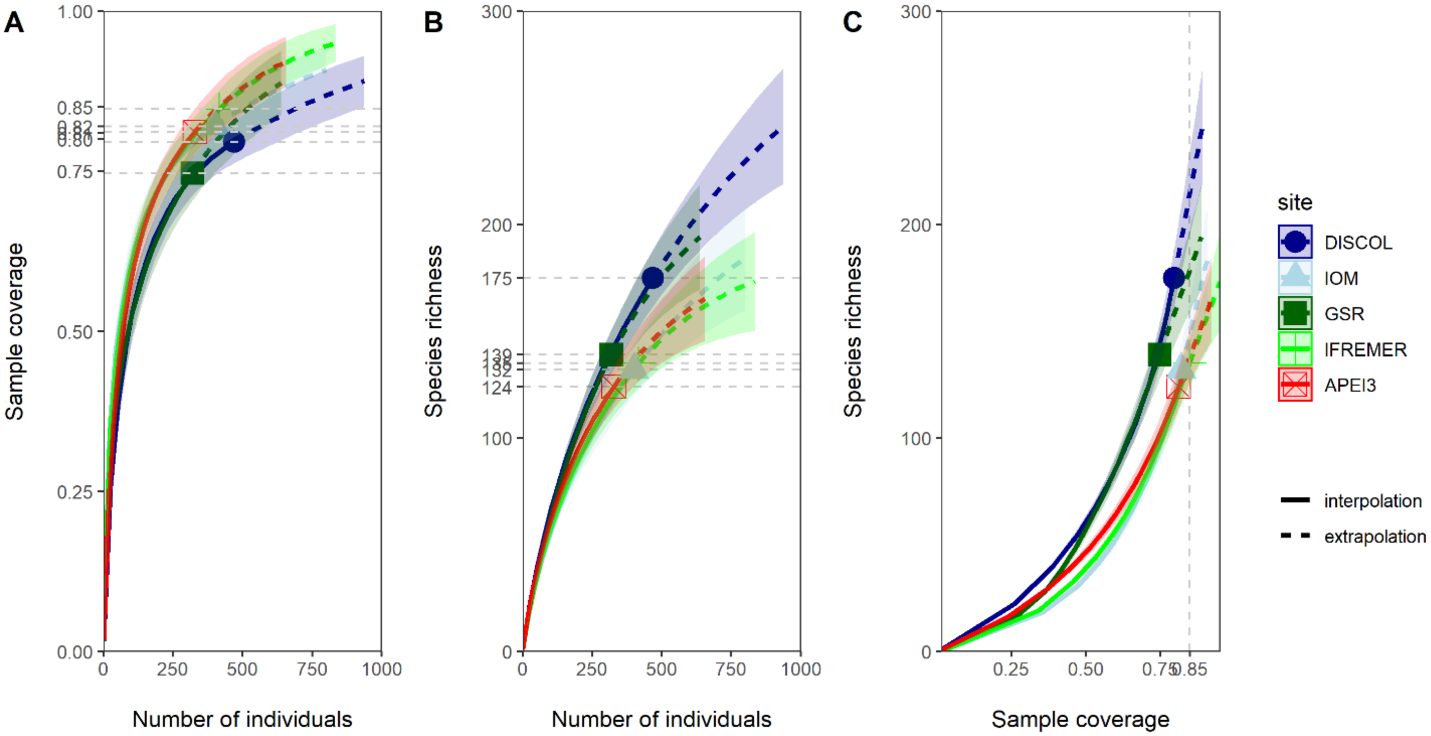

Figure 7. Nematode morphospecies sampling coverage and richness for the eastern CCZ and DISCOL area (Peru Basin).

Also for the nematode morphospecies, richness was not fully characterized by the samples taken in the 5 areas (sample coverage ranged between 75 and 85%; Figure 7A) which was also evidenced by the lack of asymptotes in the sample size-based rarefaction curves (Figure 7B). Both the sample size (B) and sample coverage (C) based rarefaction and extrapolation curves showed the lowest species richness in APEI 3, IOM and IFREMER (lowest seafloor POC flux), and highest richness in the DISCOL and GSR area (highest seafloor POC flux).

Both approaches for different datasets and areas confirm that local and regional diversity are highest in areas with the largest input of organic material.

Community composition

By using UpSet diagrams we show for different datasets (ASV's and morphospecies, Figure 8 and 9) that specific areas in the CCZ are characterized by a high number of unshared taxa (expressed as %), while the number of shared taxa between 2 or more areas is low (%) and decreasing with the number of areas involved.

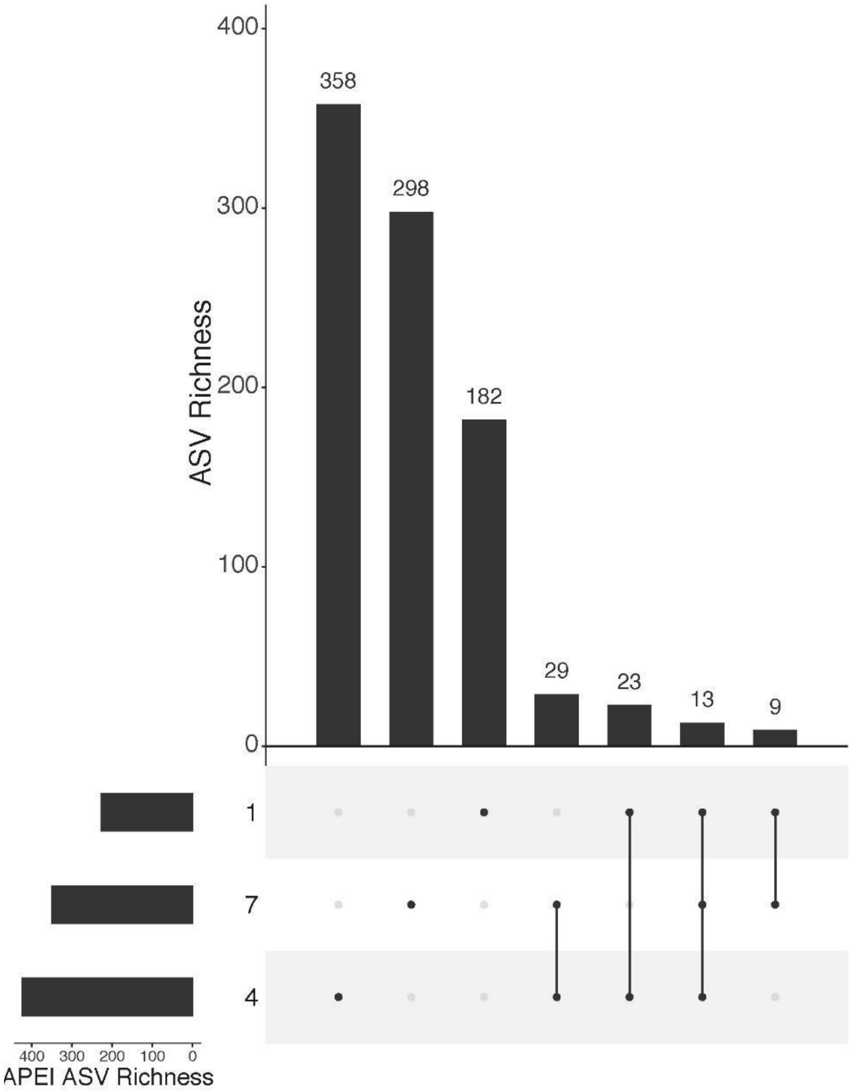

Figure 8. UpSet plot of taxon sharing between APEIs 1, 4, and 7 for nematodes and harpacticoids, from eDNA amplicon sequencing of 18S rRNA V4 of sediment samples.

Also, the UpSet plot based on nematode morphospecies demonstrates that most nematode species have been collected only in single areas, i.e. 3 CCZ contract areas, APEI 3 and the DISCOL site in the Peru Basin (see Figure 9). Only a limited number of species are shared between multiple areas. 17 out of the 424 morphospecies (4%) in total are shared between all 5 areas. DISCOL shares nearly 50% of its morphospecies with the CCZ (86 out of 176 species).

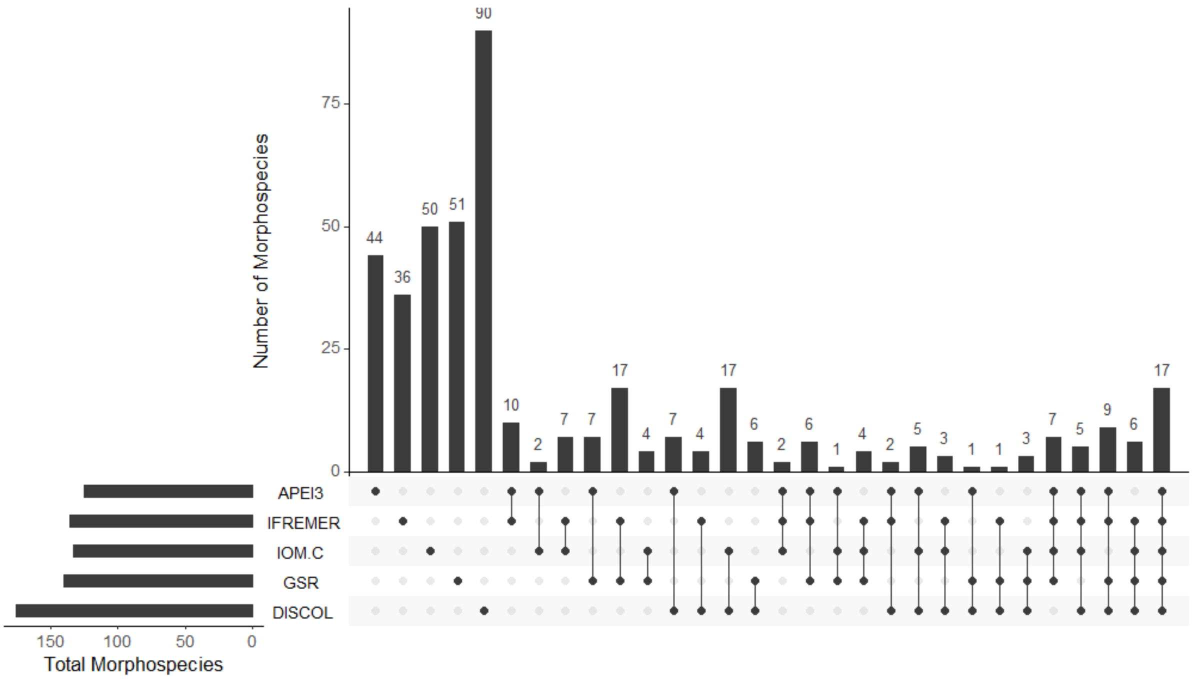

Figure 9. Upset plot of nematode (morpho)species richness in the CCZ and DISCOL (Peru Basin). In the CCZ, different contract areas and APEI 3 were sampled.

Species ranges

Despite the high number of rare taxa that are unique for one specific area, there are also several taxa that are shared not only between different areas in the CCZ but also with the Peru basin or even with areas outside the Pacific. For nematode morphospecies identifications based on the dataset of Hauquier et al (2019), 15 morphospecies present in the samples of the Eastern CCZ are also known from the Peru Basin, while 14 morphospecies had a wide distribution including Atlantic, Arctic, Mediterranean and Southern Oceans.

For harpacticoid copepods, Menzel et al. (2011) studied distribution ranges of species belonging to the abyssal genus *Mesocletodes*. From 102 species studied, only one species was exclusively from the CCZ (Nodinaut area) and 20 additional species present in the CCZ were shared with the Atlantic Ocean.

Conclusions

Since meiofaunal local diversity increases with abundances, at least for the dominant taxa such as nematodes and copepods, we can expect the highest biodiversity in the areas with highest surface productivity and resulting POC flux. Furthermore, our data effectively show that both local and regional diversity increases with POC flux. Therefore, it is important that APEIs sufficiently represent the most food rich parts of the CCZ with the highest regional POC flux.

In addition, it is important to consider the observation that there are no dominant species. Most of the species are locally rare. Only a small percentage of the species are shared between two or more claim areas. Therefore, it is equally important that APEIs are sufficiently spread across the present environmental gradients to make sure that the high number of locally rare species are protected.

Furthermore, the high number of rare species requires an increase in research effort on local biodiversity both in APEIs and claim areas. Remarkably, several species also show evidence of a wide geographical distribution. However, we assume everything is not everywhere since the environment is a determining factor in their distribution, as indicated by different meiofauna communities present in different environments (vents, seeps, abyss). There is a lack of integrated taxonomic work combining morphological descriptions with molecular and functional characterization that hinders comparison of species distributions over wider geographical scales as illustrated by the large amount of new species and the low representation of deep-sea species in GeneBank.

Conclusions

Since meiofaunal diversity increases with densities for the dominant taxa, we can expect the highest biodiversity in the areas with highest surface productivity and resulting POC flux.

- There are no dominant species. Most of the species are locally rare. Only a small percentage of the species are shared between two or more claim areas.
- Local and regional meiofaunal diversity is high and under-sampled, as demonstrated by the fact that most rarefaction curves do not reach an asymptote.
- Several meiofaunal species show evidence of a wide geographical distribution.
- However, everything is not everywhere since environment is a determining factor in their distribution.

Therefore

- It is important that APEIs sufficiently represent the most food-rich (food-poor) parts of the CCZ with the highest (and lowest) regional POC flux
- It is equally important that APEIs are sufficiently spread covering the present environmental gradients to make sure that the high number of locally rare species are protected
- It is important to increase the research effort in APEIs in comparison with claim areas because APEIs are very poorly sampled
- The high number of rare species requires an increase in research effort on local and regional biodiversity

Gaps

- APEIs are very poorly sampled: There is only 1 small dataset for meiofauna that compares an APEI with some claim areas; and another that compares meiofauna among western APEIs

- There is a lack of taxonomic work that allows comparison of species distributions over wider geographical scales
- There is a lack of reference data comparing barcoded and vouchered meiofauna specimens (i.e., comparing identifications based on molecular versus morphological approaches)
- Biodiversity data are based on limited sample sets from all areas of the CCZ, with some large areas (including most APEIs) entirely unsampled

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c. FORAMINIFERA –

Workshop Report: Foraminifera

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Introduction

The foraminifera are shell-bearing protists (protozoans) that constitute an important element of deep-sea benthic communities. Meiofauna-sized species with relatively robust multichambered shells ('tests'), often composed of secreted calcium carbonate, are well known from the geological literature, and many species have been described. However, in the abyssal deep sea these 'familiar' foraminifera are typically outnumbered by delicate single-chambered forms (monothalamids) with agglutinated or organic-walled tests. These are poorly-known and, in contrast to the multichambered taxa, are largely undescribed. They are common in meiofaunal samples but also often dominate the macrofauna collected in epibenthic sledge and box core samples. A group of even larger agglutinated foraminifera, the xenophyophores, are visible in seafloor photographs and constitute the dominant megafaunal organisms in the nodule fields of the CCZ. Finally, sessile foraminifera are very abundant on the polymetallic nodules themselves. Thus, many of the foraminifera encountered in samples from the CCZ will be undescribed monothalamids, although the more 'familiar' multichambered types are also present.

Because the foraminiferal cell is enclosed within a test, it is necessary to distinguish specimens that were alive when collected from those that were dead, most commonly by staining with Rose Bengal. It may also be necessary, particularly where monothalamids are abundant, to distinguish complete tests from fragments.

Distribution and Nature of Data

Quantitative data (abundance, species richness etc.) for meiofaunal foraminifera are available mainly from the eastern CCZ: UK-1 and OMS areas (Goineau and Gooday, 2017, 2019; Gooday and Goineau, 2019), the IOM area (Z. Stachowska et al., unpublished), and the Kaplan East (KE; Nozawa 2005 M.Sc thesis; Nozawa et al., 2006) and Kaplan Central (KC; Ohkawara 2011 PhD thesis) sites (Table 1). Two unpublished undergraduate theses based on samples from the JET site provide the only quantitative data from the western CCZ (Okamoto, 1998; Nozawa, 2003). Qualitative data (i.e., species occurrences) are derived mainly from epibenthic sledge and box core samples obtained in the UK-1, OMS, IOM, German, Belgium, French and Russian areas and APEI-3, all located in the eastern half of the CCZ (Kamenskaya et al., 2012, 2013; Gooday unpublished, Kamenskaya unpublished; Wawrzyniak-Wydrowska unpublished). Most of these data are for macrofaunal-sized species. Additional species occurrences for mainly meiofaunal-sized taxa can be derived from the quantitative datasets and from published sources (Radziejewska et al., 2006; Ohkawara et al., 2009). Genetic data (SSU rRNA gene sequences) are available for some meiofaunal species from the OMS and UK-1 areas.

Megafaunal-sized xenophyophores were collected in the Russian, UK-1 and OMS areas and APEI 6, all located in the eastern CCZ (Kamenskaya 2005; Kamenskaya et al., 2015, 2017; Gooday et al., 2017a,b,c, 2018a,b) (Table 1). A small collection of specimens was obtained in APEIs 1, 4 and 7 in the western CCZ (Gooday, Durden et al., in revision). Genetic data were derived from 23 xenophyophore species from OMS, UK-1 and APEIs 1 and 4. Qualitative data on sessile foraminifera encrusting nodules are available from the French (IFREMER), OMS, UK-1 areas, and 15°N,125°W in the eastern CCZ (Mullineaux 1987, 1988; Veillette et al., 2007). Finally, samples for environmental DNA and RNA (eDNA/eRNA) analyses were collected in the UK-1 and OMS areas during ABYSSLINE cruise, with additional samples being obtained from three parts of the BGR area (MANGAN cruise) and French area (BIONOD cruise).

Table 1. Distribution of samples from the CCZ used for foraminiferal studies. MuC = megacorer; EBS = epibenthic sled; Qt = quantitative data; Ql = qualitative data. The right-hand column shows the numbers of sediment samples analyzed for eDNA and eRNA from different areas.

Sampling site		**MuC**			**EBS**	**Xenophyophores**	**Nodule fauna**	**eDNA/ RNA**
Sieve mesh (µm)		**>32**	**>150**	**>250**				
Eastern CCZ							Ql	65/65
UK-1			Qt			Taxonomy	Ql	66/66
OMS			Qt			Taxonomy		
BGR					Ql			43/28
GSR					Ql			
IOM				Qt	Ql			
IFREMER (east)					Ql		Ql	3/3
Russian						Taxonomy		
APEI-3					Ql			
APEI-6						Taxonomy		
Kaplan East		Qt						
Kaplan Central		Qt						
Western CCZ								
French (west)							Ql	
JET		Qt						
APEI-1						Taxonomy		
APEI-4						Taxonomy		
APEI-7						Taxonomy		

Biodiversity

Morphological data

CCZ foraminifera are very diverse. Eleven megacorer samples (0-1 cm layer, >150-µm fraction) from the UK-1 Strata A and B and the OMS Stratum yielded a grand total of 580 morphospecies (represented by live and dead tests and by live and dead fragments) (Goineau and Gooday, 2019) (Fig. 1). The combined datasets included a few relatively common morphospecies (represented by >100 specimens) but the majority were uncommon and 29% were represented by singletons, a pattern typical of deep-sea foraminiferal assemblages generally. A subset of 5 samples sieved on a finer 63-µm mesh yielded 462 morphospecies, of which 170 were not present in the coarser (>150-µm) fractions (Gooday and Goineau, 2019). Three samples from the IOM area have also yielded diverse foraminiferal assemblages, comprising a total of 187 species, despite being sieved on a coarser (250-µm) mesh (Stachowska, unpublished).

In the UK-1 and OMS areas, species were still being added after 11 samples (Fig. 2), and rarefaction curves did not reach an asymptote (Fig. 3), suggesting that the total number of species (>150 µm) was higher. Estimates for total species numbers ranged from 690 (abundance-based estimator ACE) to 877 (incidence-based estimator Jacknife 2) (Goineau and Gooday, 2019).

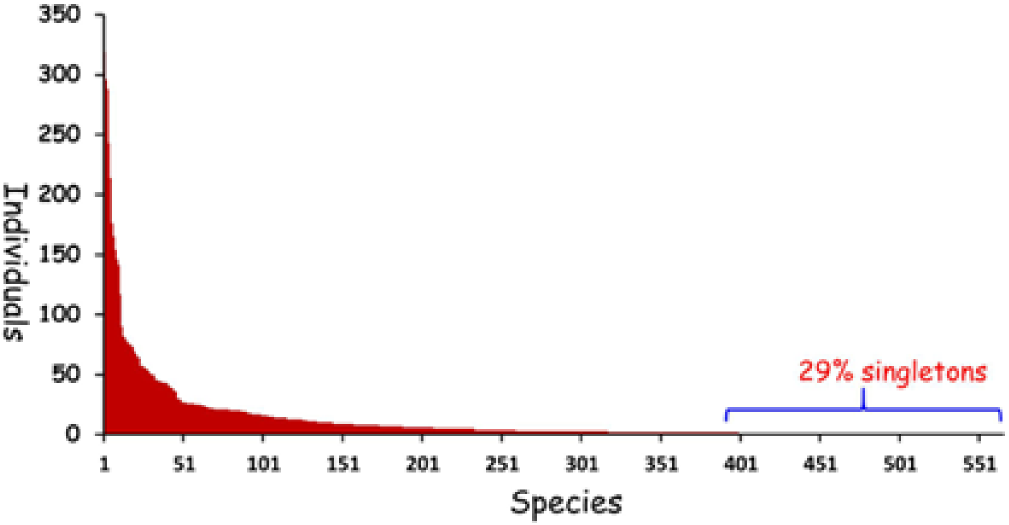

Fig. 1. Species from 11 megacorer samples (UK-1 and OMS sites; >150 µm fraction), ranked by abundance. Data from Goineau and Gooday (2019).

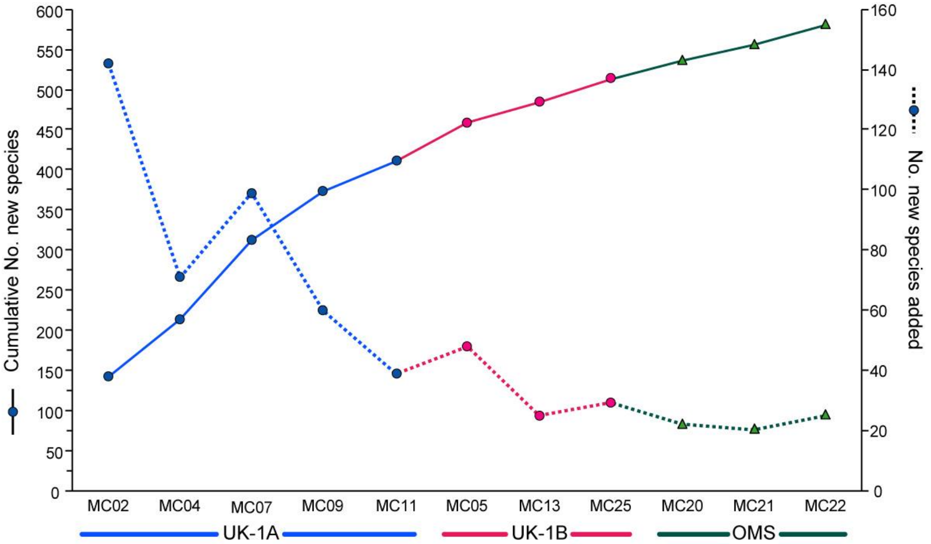

Fig. 2. Solid line: cumulative number of species in 11 megacorer samples from UK-1 Strata A and B and OMS Stratum (eastern CCZ). Dotted line: number of species added with each new sample. From Goineau and Gooday (2019).

There was no obvious difference in species richness between UK-1 and OMS strata separated by distances of up to 224 km, as indicated by the intermingling of rarefaction curves (Fig. 3).

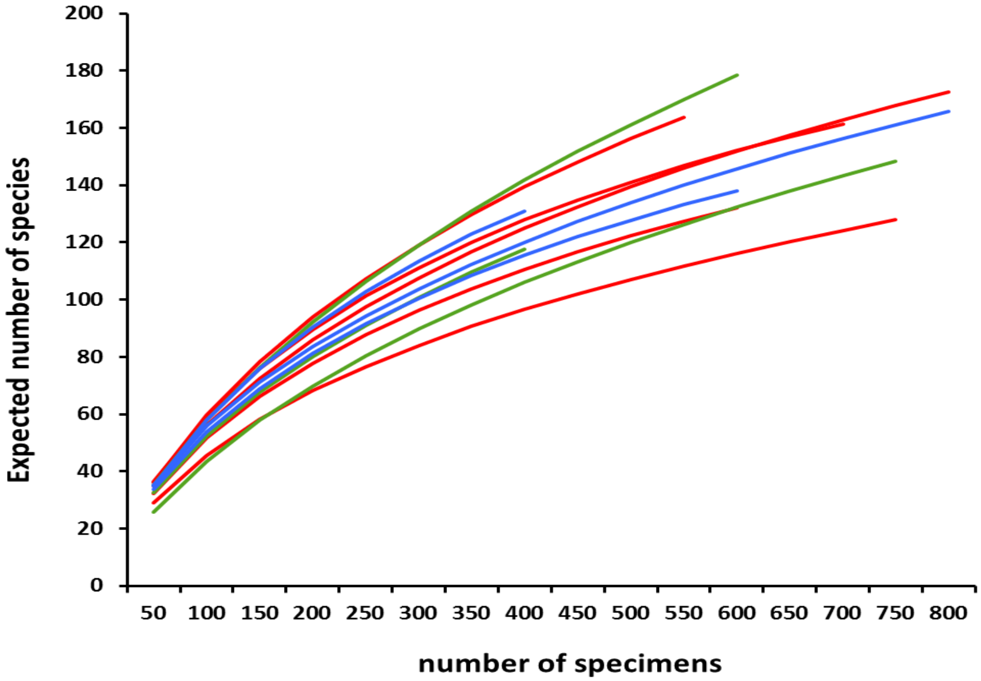

Fig. 3. Rarefaction curves for 11 megacorer samples (0-1 cm layer, >150-µm fraction) from UK-1 Strata A (red) and B (green) and OMS Stratum (blue). Data from Goineau and Gooday (2019).

Inconsistencies in sample sizes and the methods used to analyze the samples (notably sieve mesh sizes) limit the comparison of data across wider areas of the CCZ (Table 1). The only comparable quantitative samples are those obtained at the KE (eastern CCZ) and JET (western CCZ) sites, which were analyzed by the same person using the same methods (Nozawa 2003, 2006). Slightly more species represented by complete specimens were recognized in samples from the JET site (171 species among 1702 specimens; mean 31.6±15.3 per sample) compared to the those from the KE site (168 species among 983 specimens; mean 27.2±8.1 per sample). Although the difference in the mean number of species per sample was not significant (p = 0.5356), rarefied species richness is slightly higher at KE than at JET (Fig. 4).

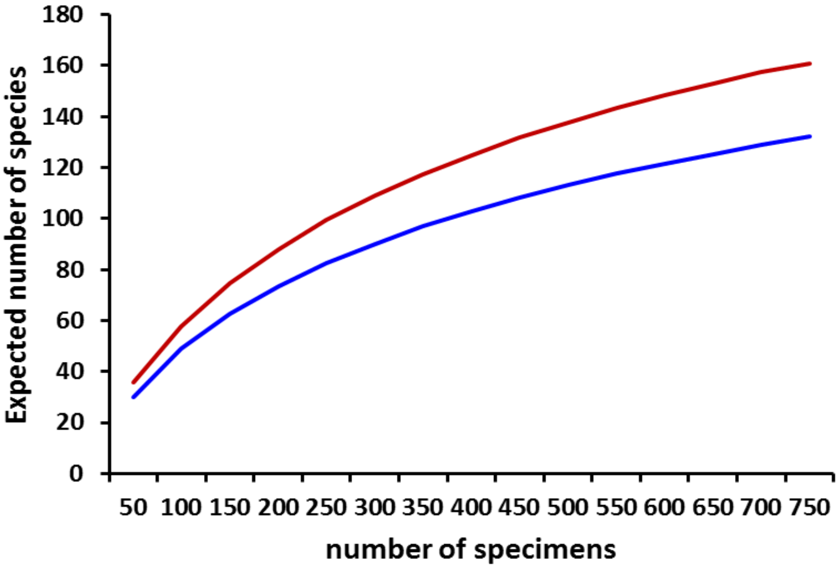

Fig. 4. Rarefaction curves for the Kaplan East (red) and JET (blue) sites. Data based on the >63 µm fraction of the 0-1 cm layer of megacorer subcores (Nozawa 2003, 2005).

Meiofaunal foraminifera in quantitative samples are dominated by monothalamids. Among complete individuals they represent around 75% of the 580 species (>150 µm) from the UK-1 and OMS sites (Goineau and Gooday, 2019), a similar proportion of 187 species (>250 µm) at the IOM site (Stachowska unpublished) and 83.7% of 168 species (>63 µm) at Kaplan East (Nozawa, 2005; Nozawa et al., 2006), all located in the eastern CCD (11-15°N, 116-120°W; 4089-4440 m). At Kaplan Central, 82.2% of species were monothalamids (Ohkawara, 2011), while the proportion of monothalamids at the JET site in the western CCZ, was, surprisingly, somewhat lower (74.2% of 171 species >63 µm) (Nozawa, 2003). Most of the remaining complete foraminiferal tests in these samples belonged to multichambered agglutinated taxa; the proportion of calcareous taxa was small: 7.9% at UK-1 + OMS, 5.9% at KC site, but less among the finer fractions at KE (<5%) and the JET (≤3.0%) site.

Qualitative epibenthic sledge samples (>300 µm fraction) typically yield abundant macrofaunal-sized monothalamids. Samples from five sites in the eastern CCZ (German, IOM, Belgium, French and APEI 3) sorted by the same people using consistent methods yielded 159 foraminiferal species, all of them monothalamids (Wawrzyniak-Wydrowska and Gooday, unpublished). A study of photographs provided by Olga Kamenskaya (Shirshov Institute, Moscow) of macrofaunal foraminifera from the French, German and Russian areas have added some 30 morphospecies to the list from the CCZ.

Eastern CCZ samples have yielded 53 xenophyophore species (megafauna-sized foraminifera), 38 in the UK-1 and OMS areas, 12 in the Russian area, and 9 in APEI 3 (Kamenskaya 2005; Kamenskaya et al., 2015, 2017; Gooday et al., 2017a,b,c, 2018a,b). In the western CCZ, xenophyophores collected by an ROV in APEIs 1, 4 and 7 have revealed a further 10 morphospecies, bringing the total for the CCZ as a whole to 63. Seventeen of these species have been formally described (the majority in the last few years). These represent 22% of the global total of described xenophyophore species and emphasize the status of the CCZ as an area of unusually high megafaunal xenophyophore diversity (Gooday et al., 2017a).

Finally, foraminifera and foraminifera-like protists, are common on polymetallic nodules. In the eastern and western French areas, Veillette et al. (2007) distinguished 68 morphospecies attached to nodules, with another 5 loosely associated species (Table 2).

Table 2. Number of foraminiferal morphospecies and nodules studied at 3 eastern and 1 western site (French claim areas). Data from Veillette et al. (2007).

Site	East site A	East site B	East site C	West site
Position	14°N, 130°W			9°N, 150°W
Number of nodules	15	50	39	131
No. morphospecies	47	63	66	63

A total of 86 sessile foraminifera attached to selected nodules from the UK-1 area were recognized by Gooday et al. (2015). A detailed study of nodule-encrusting foraminifera from the OMS area, as well as UK-1 area, will certainly increase this number further. These assemblages can be compared with those from more westerly CCZ sites (the two French areas), illustrated in the Supplementary Material to Veillette et al. (2007), as well as those illustrated by Mullineaux (1987, 1988) from the eastern CCZ (15°N, 125°W) and the central North Pacific (30°N, 157°W).

Based on these morphological analyses, we estimate that the total number of foraminiferal morphospecies across all size fractions (meiofaunal, macrofaunal, megafaunal), sessile as well as free-living, must be well in excess of 1000, just in the limited amount of material from the relatively few sites from which we have data.

Environmental DNA and RNA data

eDNA studies based on samples from the UK-1, OMS and eastern French areas, support the conclusion that foraminifera are highly diverse and include a substantial proportion of monothalamid OTUs. These include most of the existing monothalamid clades, some of which are known only from environmental samples (Lejzerowicz, unpublished data). Environmental DNA (eDNA) and RNA (eRNA) were extracted from 171 sediment samples sampled according to a nested design representing four national claims (Table 3). Only 3 samples were processed for the French site, which is situated in the more central part of the CCZ. Hence the scope of the eDNA analyses are currently limited to faunal patterns in an homogeneous region in term of nodule abundance and POC flux.

Table 3. Number of biological samples per area and per type of molecule extracted from bulk environment sediment material (Sediments), and number of technical duplicates successfully sequenced from these samples for the foraminifera-specific 37F hypervariable region of the 18S ribosomal RNA gene.

	BGR		**IFREMER**		**OMS1**		**UK1**	
	DNA	RNA	DNA	RNA	DNA	RNA	DNA	RNA
Sediments	43	28	3	3	66	66	65	65
Sequenced	66	30	6	6	128	127	130	129

We obtained 6,348 OTUs, including 27.1% and 52.2% OTUs assigned to the Globothalamea and monothalamids, respectively. The rRNA/rDNA sequences representing these OTUs were found in both technical duplicates of each sample and hence can be assumed to be free of PCR and sequencing errors (Esling et al. 2015). After further filtering that reduced to 2061 the number of OTUs detected in both the DNA and RNA version of each sample, we found similar proportion of OTUs per taxon, with 28.3% and 56.4% on average assigned to Globothalamea and Monothalamea, respectively, across claim areas and samples (Fig. 5). It is important to note that the method choice for the assignment of short environmental foraminiferal sequences remains a major challenge that affects the proportion of unassigned taxa. One standard method used for microbiome data assigns a majority of OTUs but the assignment confidence needs to be evaluated, while the more conservative approach used for foraminifera assigns fewer OTUs but also reveals a higher diversity of clades (Fig. 5). Although the fractions of globothalamids remain similar, more research is necessary to identify the extent of molecular foraminiferal diversity in the CCZ.

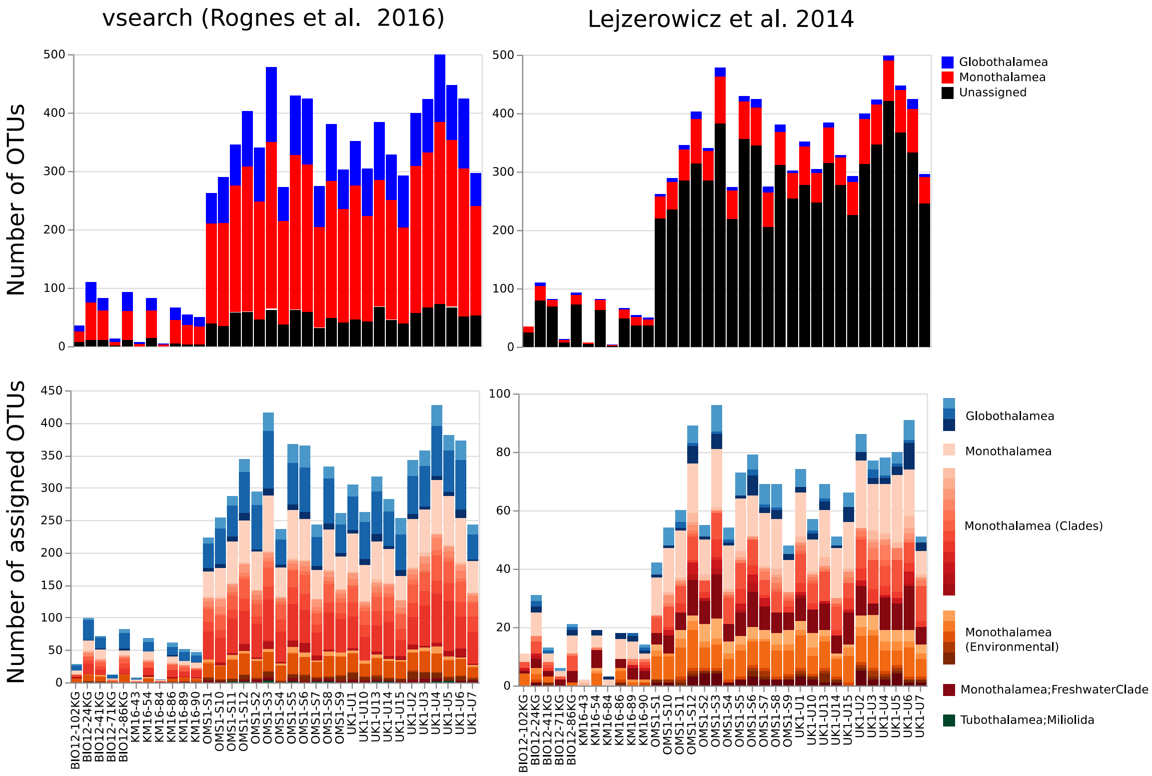

Fig. 5. Taxonomic composition of the OTUs found in samples from CCZ sites using two sequence assignment methods (vsearch - left panels; foraminfera-specific - right panels). The bottom panels include only those OTUs that were assigned to Globothalamea and Monothalamea in the top panels; they show composition at a finer taxonomic level.

On average, across the samples taken at each site, the number of monothalamid OTUs is twice as high as that for Globothalamea. For both groups, the UK1 and OMS1 areas yield significantly higher richness than the thoroughly sampled BGR South area (MANGAN16 cruise) (72 samples, Fig. 5).

Biogeography

Morphological data

Geologically-orientated studies have revealed world-wide ('cosmopolitan') distributions among many well-known 'hard-shelled' multichambered foraminiferal morphospecies at abyssal depths (Gooday and

Jorissen, 2012). Samples from the eastern CCZ include 20 or more such species (Goineau and Gooday, 2019), although wide ranges are supported by SSU rRNA sequences in only two cases (*Epistominella exigua* and *Nuttallides umbonatus*) (unpublished data, see also Lecroq et al., 2009). Some undescribed monothalamid morphotypes from different oceans, including the CCZ and sites elsewhere in the Pacific, are also sufficiently similar to be regarded as conspecific (Gooday et al., 2004).

Most of the macrofaunal foraminiferal morphospecies from epibenthic sledge samples taken in the German, IOM, Belgium, French, and APEI-3 areas are confined to one site, with only 6 (<4%) being found at 4 or 5 sites. Five of these 6 species have been formally described, originally either from the central North Pacific (30°N, 156°W, 6070 m depth) (Tendal and Hessler, 1977) or the North Atlantic (Shires et al., 1994), indicating wide distributions, albeit based solely on morphology. Similarly, some of the nodule encrusting macrofaunal morphospecies in the UK-1 and OMS areas (Gooday et al., 2015; unpublished data) are also recorded from the eastern and western sites of Veillette et al. (2007). These include two species of the komokiacean genus *Chrondrodapsis,* originally described from within the CCZ (15°N, 125°W) (Mullineaux, 1988).

Xenophyophores (described and undescribed) have been collected in the UK-1 and OMS areas and APEI-6 (eastern CCZ), the Russian area (more central CCZ) and APEIs 1, 4 and 7 (western CCZ). However, many species are represented by 1-2 specimens and are known from a single site, so nothing can be said about their wider distributions. One species, *Aschemonella monile,* spans the Russian and UK-1/OMS areas (Gooday et al., 2017b) and APEI-4. *Psammina limbata,* described morphologically from the Russian area (Kamenskaya et al., 2015), may also be present in the UK-1/OMS, although several closely similar stalked species of *Psammina* have been recognized at these more easterly sites (Gooday et al., 2018b) and so whether one of these is the same as *P. limbata* cannot be confirmed without genetic data from Russian specimens. However, wide ranges spanning a distance of 3,800 km (from APEI-4 to UK-1/OMS sites) have recently been confirmed genetically for two xenophyophore species (Gooday et al., unpublished).

All samples from an area spanning several distances of 100s of kilometers in the UK-1 and OMS areas fall within 95% confidence limits in MDS plots, indicating a high degree of assemblage uniformity at this spatial scale (Goineau and Gooday, 2019). A decay-distance analysis based on Bray-Curtis similarity vs distance showed only minor differences in the species composition of samples from the UK-1 and OMS sites seperated by increasing distances of up to ~220 km (Fig. 6). However, there was a general tendency for differences in species composition to increase with distance, and this overall trend was significant ($p = 0.006$). This suggests that, although particular morphospecies may have wide ranges, there are gradual shifts in the species composition of foraminiferal assemblages in relation to environmental gradients within the CCZ.

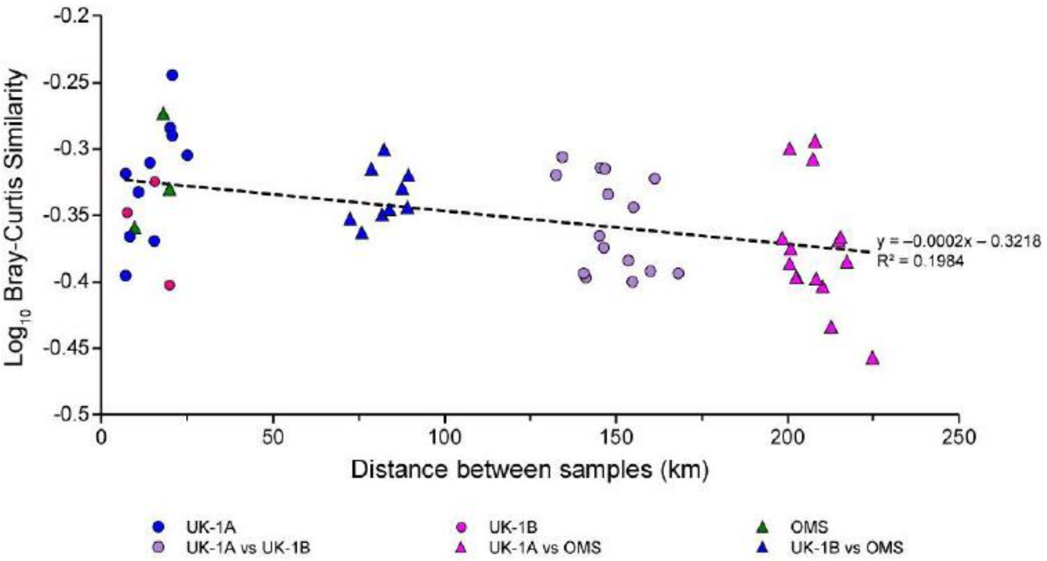

Fig. 6. Similarity between samples compared across increasing distances within the UK-1 and OMS contract areas. Based on Bray-Curtis similarity indices computed with species presence-absence data. The cluster of symbols at the left-hand side refer to comparisons of samples within a 30 x 30 km 'stratum', the other clusters refer to comparisons between strata. Within stratum and between stratum values are significantly different (t-test, p = 0.012) only in the case of the UK-1A vs OMS comparison (right-hand side). However, the overall trend is significant (Spearman's rank correlation, rs = 0.373, p = 0.006). From Goineau and Gooday (2019).

A recurrent difficulty in trying to establish foraminiferal species ranges across the CCZ is that many morphospecies are chronically under-sampled. Around 60% of the 547 species (>150-µm fraction) in UK-1 and OMS samples are confined to 1-2 out of 11 sites but are represented by a relatively small proportion (16.7%) of specimens (Fig. 7). Many species confined to one site are singletons. On the other hand, the few species (6%) that span 9-11 sites are represented by a disproportionally large proportion (~44%) of specimens. Similarly, >80% of macrofaunal foraminiferal species from the Belgium, German, IOM, and French areas and APEI 3 are confined to one area, and 74% are confined to one of the 2 replicate samples analyzed from each area. Thus, to a large extent, restricted distributions appear to often reflect relative rarity combined with under-sampling (i.e. 'pseudo-endemism').

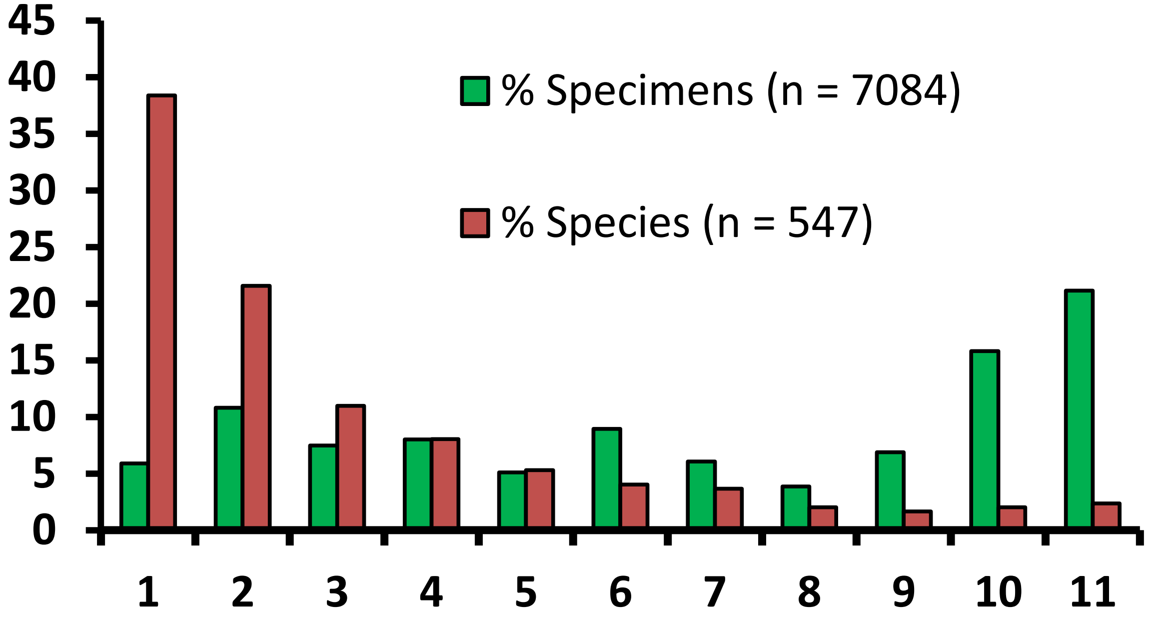

Fig. 7. The percentage abundance of species represented by complete tests (red, n = 547) and specimens (green, n = 7084) distributed across different numbers of sites (1-11) within the UK-1 and OMS areas in the eastern CCZ. On the left-hand side, 60% of species are confined to 1-2 sites but these represent only 16.7% of specimens; many of the species confined to one site are singletons. In contrast, only 6% of species occur at 9-11 sites, but these represent about 44% of specimens. Based on data from Goineau and Gooday (2019).

Environmental DNA and RNA data

The number of OTUs unique to a site is related to the sampling effort. Indeed, more than 500 monothalamid OTUs are unique to the ~240 samples sequenced for each of UK-1 and OMS areas, whereas 10 and 3 samples from BGR_South and IFREMER (BIONOD2 cruise) yielded only 45 and 3 OTUs, respectively, that were unique to these sites (Fig. 8). These data highlight the need for more even sequence sampling between areas, and the likely prevalence of ‘pseudo-endemism’, already evident in morphological data.

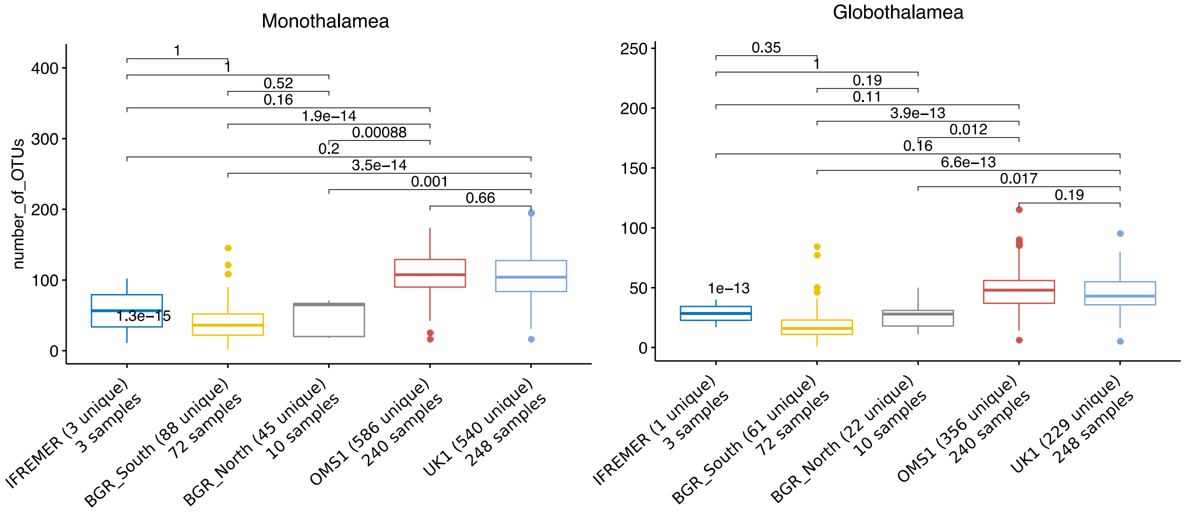

Fig. 8. Foraminifera OTUs richness per area (x axis labels) and between areas of the CCZ (pairwise Wilcoxon test) for the Monothalamea (left panel) and Globothalamea (right panel). The significance levels of the tests are indicated between each pair of sites. For the BGR claim, the BGR North site was visited during the BIONOD2 cruise and the BGR South site during the MANGAN16 cruise.

Genetic Connectivity

As indicated above, there is genetic evidence that some foraminiferal species have wide ranges across the CCZ and beyond, but we do not have sufficient data to conduct a genetic study of any species at a population level. However, LeCroq et al. (2009) analyzed the population genetics of *Epistominella exigua* (a calcareous species found in the UK-1 and OMS areas) from sites in the Arctic, North Atlantic, Southern Ocean, and the western Pacific off Japan, based on ITS rDNA sequences. They found very little divergence between haplotypes from different oceans. Whether or not these results for *E. exigua*, which is an unusually opportunistic species that exploits phytodetritus deposits, are typical for other foraminifera living in the CCZ is an important question that needs to be addressed.

Conclusions

Biodiversity

- Benthic foraminiferal assemblages are highly diverse across the CCZ. Individual megacorer samples yield >100 meiofaunal-sized morphospecies. An estimated 700-900 such species exist in the UK-1 and OMS areas. When macrofaunal and megafaunal species, and those sessile on nodules, are added to the meiofaunal species, total species numbers across all size classes are probably well in excess of 1000, just in the relatively well-studied eastern CCZ. The number of molecular species (OTUs) is even higher – in total, 3 times the number of morphological species in the UK-1 and OMS areas.

- Meiofaunal assemblages are dominated by rare species, with 29% of the 580 species recognized in UK-1 and OMS samples being singletons.

- Limited morphological data suggest that meiofaunal foraminiferal diversity is somewhat lower in the western than the eastern CCZ. eDNA data reveals a similar trend between the eastern end of the CCZ and the French area further west.

- Single-chambered Monothalamea, largely undescribed, constitute the majority of meiofaunal and macrofaunal abundance and diversity, with better-known multichambered Globothalamea constituting a relatively minor component. eDNA reveals similar proportions, suggesting that this approach could be used for rapid evaluations of community composition.

- The CCZ hosts unusually diverse assemblages of megafaunal xenophyophores, many of which are sessile on nodules. Nodules are often densely encrusted with numerous species of macrofaunal foraminifera, the vast majority of them undescribed monothalamids.

Biogeography

- Some morphospecies (mainly multichambered globothalameids) present in CCZ samples are known from literature records to be widely distributed in the deep ocean. Global ranges are confirmed by genetic and eDNA data in a few cases.

- Many morphospecies and molecular species are confined to 1-2 sites, but under-sampling combined with the rarity of many species make it impossible to establish whether any are endemic.

- Foraminiferal assemblages appear to be fairly uniform across claim areas (UK-1 and OMS), but with some indication of gradual change over distances of several 100 kms.

Data gaps

- Foraminiferal data from the western CCZ are very limited. At present, we only have some quantitative data from the JET site, some published information on nodule-encrusting foraminifera from the western French site, and a small collection of xenophyophores from western APEIs.

- eDNA data based on adequate sample numbers and sequencing effort are only available from a restricted region of the eastern CCZ, limiting its use for establishing faunal patterns.

- There are no quantitative data or eDNA data on foraminifera from APEIs. Sampling has been limited to the collection of xenophyophores from APEIs 1, 4 and 6 in the western CCZ, and APEI-6 in the eastern CCZ.

- There are currently insufficient genetic data to analyze the population genetics of any foraminiferal species from the CCZ.

- Acquiring quantitative faunal data on foraminifera is very time-consuming if the very diverse monothalamids are included. Time can be saved in various ways. One possibility is to confine the analyses to the multichambered taxa, which are generally much better known and easier to work with and evaluate the diversity and abundance of monothalamids using eDNA approaches.

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d. SEDIMENT MACROFAUNA –

A synthesis of sediment macrofaunal biodiversity in the Clarion-Clipperton Zone

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A) Introduction

The macrofauna constitute the size class between the meiofauna and the megafauna; in the deep sea including the CCZ, this size class is generally designated as animals retained on 300 (sometimes 250) μm sieves, but too small to be identified in bottom photographs. The macrofauna is comprised of a huge diversity of sediment-dwelling taxa (>1000 species at single CCZ sites; Smith et al., 2008b) including in decreasing order of numerical importance, polychaete worms, tanaid crustaceans, and isopod crustaceans (Borowski and Thiel, 1998; Smith and Demopoulos, 2003). The polychaetes dominate macrofaunal standing crop and species richness, accounting for about 50-65% of macrofaunal abundance, biomass, and number of species in nodule regions (e.g., Borowski and Thiel, 1998; Smith and Demopoulos, 2003). Macrofaunal community abundance in abyssal nodule regions is relatively low compared to shallower, and more coastal, deep-sea regions, typically totaling about 300-500 individuals m^{-2} (Glover et al., 2002; Smith and Demopoulos, 2003). The majority of macrofaunal species, in particular those in the abundant polychaete families Spionidae and Cirratulidae, appear to be surface deposit feeders that consume a very thin veneer of labile organic material depositing on the sediment-water interface (Paterson et al., 1998; Smith and Demopoulos, 2003; Smith, 2008; Bonifacio et al., 2019). Subsurface deposit feeders (such as the paraonid polychaetes) may also be abundant in the CCZ. Other trophic types, including predators and omnivores, are also present within the macrofauna (Smith, 2008b; Bonifacio et al., 2019). At least 95% of macrofaunal abundance in abyssal sediments in nodule regions is concentrated in the top 5 cm of sediment, presumably to facilitate access to labile organic matter (i.e., food for deposit feeders) concentrated at the sediment–water interface (Smith and Demopoulos, 2003). Macrofauna also contribute substantially to deep-sea ecosystem functions, including the respiration and burial of phytodetritus, and bioturbation (Smith et al., 2008a).

Previous nodule-mining impact simulations indicate that the macrofauna is also likely to be highly sensitive to mining disturbance (Borowski and Thiel, 1998; Borowski, 2001; Jones et al., 2017). Because of the extraordinary biodiversity of the macrofauna, and the potential sensitivity of the macrofaunal community to nodule-mining impacts, it is important to protect representative macrofaunal communities (in terms of biodiversity, abundance and community structure) within the APEI network in the CCZ.

The goal of this report is to synthesize patterns of sediment macrofaunal biodiversity in the CCZ region, using published and unpublished data available at the time of the workshop. After assembling the available data, we first evaluated the comparability of data across sampling programs. We then tried to address three initial questions of the workshop, to the extent possible given adequacy of the data. These questions are:

1) *Does macrofaunal species/taxon richness and evenness, and community structure, vary along and across the CCZ? What are the ecological drivers of these variations?*
2) *Do claim areas have similar levels of species/taxon richness and evenness, and similar community structure, to the proximal APEI(s)?*
3) *Are species ranges (based on morphology and barcoding) generally large compared to the distances between APEIs and contractor areas? What is the degree of species overlap between different study locations across the CCZ?*

Based on the answers obtained to the questions above, we then considered (a) whether the current APEIs appear to capture the full range of macrofaunal communities and biodiversity observed along and across the CCZ, (b) whether species ranges appear to bridge APEIs and contractor areas, (c) whether similar levels of community structure are demonstrated in contractor areas and proximal APEIs, and (d) whether the data were simply too limited to address these questions. If the data were too limited, we then considered what data gaps must be filled to fully address these questions.

B) Data Collection and Characteristics of Data Sets

Box-core Data. To assess the key questions addressed by the Deep CCZ Biodiversity Synthesis Workshop, we assembled all available sediment macrofaunal data collected by standard quantitative sample methods, i.e., by box corer, in the CCZ and surrounding abyssal regions. Data were collected from the peer-reviewed scientific literature, and as unpublished data from a variety of sources. The abyssal macrofauna in these data sets consists of animals retained after sieving sediments on 300-µm, or in one data set 250-µm, sieves. Nematodes, harpacticoid copepods, and ostracods were omitted from these macrofauna counts because the vast majority of individuals from these taxa (>90%) pass through 250- and 300-µm sieves, and are thus not quantitatively retained in these macrofaunal samples.

Through direct solicitation from scientists and contractors, sediment macrofaunal data collected by box cores have been obtained from a variety of research projects for areas in the central and eastern Clarion-Clipperton Zone. Abundances of taxa in samples, and area per sample, were provided for use in the Deep CCZ data synthesis workshop. (Figure 1, Table 1).

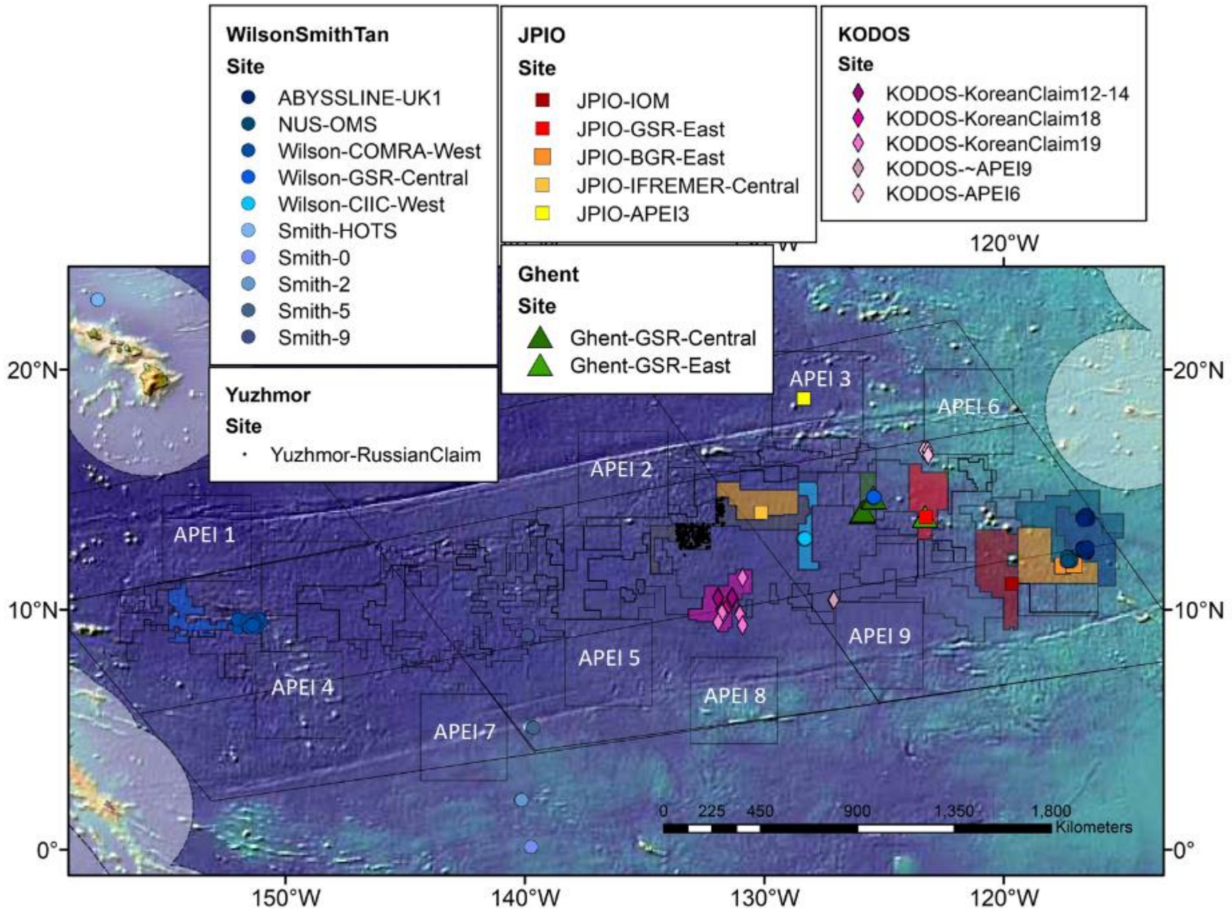


Figure 1. *Map of CCZ showing study sites from which macrofaunal box-core data were used in this workshop were assembled for use in the workshop. The characteristics of the data sets collected at these sites are presented in Tables 1 and 2.*

Table 1. *Sources, numbers of box cores, locations, and depths for macrofaunal box-core data used in this workshop.*

Study	Site	Source of Data (Name and Email or citation)	# of Box Cores	Area of Box Core Sampled (m^2)	Latitude (N) (Decimal Deg.)	Longitude (W) (Decimal Deg.)	Depth (m)
ABYSSLINE	NUS-OMS	Tan Koh Siang - tmstanks@nus.edu.sg	12	0.2267 - 0.2275	12.01 - 12.22	117.18 - 117.38	4041 - 4183
NUS	ABYSSLINE-UK1	Craig Smith - craigsmi@hawaii.edu	24	0.25	12.37 - 13.96	116.46 - 116.72	4036 - 4218
Wilson	Wilson-COMRA-West	Wilson, 2017	54	0.2454	9.25 - 9.61	151.01 - 151.97	4842 - 5283
Wilson	Wilson-GSR-Central	Wilson, 2017	15	0.25	14.62 - 14.71	125.37 - 125.46	4480 - 4567
Wilson	Wilson-CIIC-West	Wilson, 2017	16	0.25	12.91 - 12.98	128.28 - 128.37	4708 - 4854
Smith	Smith-HOTS	Craig Smith - craigsmi@hawaii.edu Glover et al., 2002	4	0.15	22.91 - 22.92	157.83 - 157.84	4843 - 4867
Smith	Smith-0°	Craig Smith - craigsmi@hawaii.edu Glover et al., 2002	3	0.18	0.11 - 0.12	139.73 - 139.74	4300 - 4305
Smith	Smith-2°	Craig Smith - craigsmi@hawaii.edu Glover et al., 2002	4	0.18	2.06 - 2.07	140.13 - 140.15	4408 - 4414
Smith	Smith-5°	Craig Smith - craigsmi@hawaii.edu Glover et al., 2002	3	0.18	5.07 - 5.08	139.64 - 139.65	4320- 4446
Smith	Smith-9°	Craig Smith - craigsmi@hawaii.edu Glover et al., 2002	3	0.18	8.93	139.86 - 139.88	4981 - 4991
JPIO	JPIO-IOM	Lenaick Menot - lenaick.menot@ifremer.fr Bonifacio et al., 2019	8	0.25	11.07 - 11.08	119.65 - 119.66	4414 - 4434
JPIO	JPIO-GSR-East	Lenaick Menot - lenaick.menot@ifremer.fr Bonifacio et al., 2019	5	0.25	13.84 - 13.86	123.23 - 123.25	4503 - 4516
JPIO	JPIO-BGR-East	Lenaick Menot - lenaick.menot@ifremer.fr Bonifacio et al., 2019	8	0.25	11.81 - 11.86	117.05 - 117.55	4118 - 4370
JPIO	JPIO-IFREMER-Central	Lenaick Menot - lenaick.menot@ifremer.fr Bonifacio et al., 2019	6	0.25	14.04 - 14.05	130.13 - 130.14	4921 - 4964
JPIO	JPIO-APEI3	Lenaick Menot - lenaick.menot@ifremer.fr Bonifacio et al., 2019	3	0.25	18.77 - 18.80	128.34 - 128.36	4816 - 4847
Ghent	Ghent-GSR-Central	Ellen Pape - Ellen.Pape@ugent.be De Smet et al., 2017	19	0.25	14.02 - 14.71	125.51 - 125.93	4477 - 4629
Ghent	Ghent-GSR-East	Ellen Pape - Ellen.Pape@ugent.be	5	0.25	13.88 - 13.89	123.28 - 123.31	4535 - 4560
KODOS	KODOS-Central-2018	Se-Jong Ju - sjju@kiost.ac.kr	15	0.23 - 0.25	9.85 - 10.52	131.33 - 131.93	4995 - 5174
KODOS	KODOS-Central-2012-14	Se-Jong Ju - sjju@kiost.ac.kr	36	0.23 - 0.25	10.48 - 10.52	131.29 - 131.94	4772 - 5206
KODOS	KODOS-Central-2019	Se-Jong Ju - sjju@kiost.ac.kr	10	0.25	9.35 - 11.33	130.90 - 131.93	4712 - 5220
KODOS	KODOS-APEI9	Se-Jong Ju - sjju@kiost.ac.kr	2	0.23 - 0.25	10.40 - 10.41	127.09 - 127.12	4784 - 4792
KODOS	KODOS-APEI6	Se-Jong Ju - sjju@kiost.ac.kr	4	0.23 - 0.25	16.44 - 16.64	123.15 - 123.33	4232 - 4290
Yuzhmor	Yuzhmor-Central	Slava Melnik - melnikvf@ymg.ru	214	0.2175 - 0.225	12.26 - 14.66	131.68 - 133.64	4713 - 5254

Table 2*. Macrofaunal taxonomic groups and taxonomic levels of identification, taxonomists and techniques used for identification, for macrofaunal box-core data sets used in this workshop.*

Study	Taxonomic Resolution	Taxonomic Method	Polychaete Taxonomists	Tanaid Taxonomists	Isopod Taxonomists
ABYSSLINE	Species	Morphology	Iris Altamira - irisis@hawaii.edu	Magdalena Blazewicz - madgalena.blazewicz@biol.uni.lodz.pl	Nils Brenke - nils.brenke@ruhr-uni-bochum.de
NUS	Family	Morphology	Chuar Cheah Hoay - tmscch@nus.edu.sg	Chim Chee Kong - tmscck@nus.edu.sg	Helen Wong Pei San - tmswpsh@nus.edu.sg
Wilson	Species	Morphology	Kristian Fauchald & Kirk Fitzhugh - kfitzhugh@nhm.org	Jurgen Sieg, Timothy Ragen, & George D. F. Wilson - gdfw@snhlab.com	George D. F. Wilson - gdfw@snhlab.com
Smith	≥ Order (to species for polychaete rarefaction)	Morphology	Adrian Glover - a.glover@nhm.ac.uk *(Rarefaction Only) Glover et al. (2002)	-	-
JPIO	Species	Morphology (and DNA for polychaetes)	Paulo Bonifacio - bonif@me.com	Magdalena Blazewicz - madgalena.blazewicz@biol.uni.lodz.pl	Stefanie Kaiser - ssm.kaiser@gmail.com
Ghent	Species	Morphology	Lisa Mevenkamp, Bart De Smet, & Paulo Bonifacio - bonif@me.com	Lisa Mevenkamp, Bart De Smet, & Magdalena Blazewicz - madgalena.blazewicz@biol.uni.lodz.pl	Lisa Mevenkamp, Bart De Smet, & Torben Riehl - torben.riehl@senckenberg.de
KODOS	Species	Morphology	Iris Altamira - irisis@hawaii.edu	Jin Hee Wi - sumiae425@hotmail.co.kr.	Ok Hwan Yu - ohyu@kiost.ac.kr
Yuzhmor	≥ Class	Morphology	-	-	-

Datasets were collected from the ABYSSLINE Project **- ABYSSLINE**, the National University of Singapore (NUS) **- NUS**, Wilson (2017) **- Wilson**, Smith et al. (1997) **- Smith**, the Joint Programming Initiative Healthy and Productive Seas and Oceans (JPIO) – **JPIO** (Bonifacio et al., 2019, Blazewicz et al., 2019), JPIO and Global Sea Mineral Resources (GSR) through Ghent University – **Ghent** (partially in De Smet et al., 2017), the Korea Institute of Ocean Science and Technology (KIOST) – **KODOS**, and Yuzhmorgeologiya – **Yuzhmor** (Table 1). Macrofaunal data were compiled at the site level, which was considered to be different exploration areas in this study (Table 1; Figure 1). The majority of studies included macrofaunal data identified to the species level; however, NUS data were at the family level, Yuzhmor data were at the class level, and Smith data included only abundances and polychaete rarefaction values calculated in Glover et al. (2002) (Table 2). All studies collected macrofauna via a 0.25 m^2 box corer, but there were differing amounts of sub-coring among and within studies for other analyses (Table 1). All studies sieved material on a 300-μm sieve except for NUS, which used a 250-μm sieve. The box cores samples in Wilson (2017) were collected from 1977 – 1989, those in Smith et al. (1997) were collected in 1992, and the box cores for the remaining data sets were collected between 2012 and 2019.

It is important to note that the various box-core macrofaunal data sets assembled for the workshop were collected by different research programs (Table 1) using (in some cases) different types of box cores, box-core deployment protocols, and sample-washing procedures, all of which may influence collection efficiency and the ability to resolve macrofauna at the species level. In addition, because the thousands of sediment macrofaunal species collected across the CCZ are mostly undescribed (Smith et al., 2008a and b), the various research programs have worked with a number of separate reference collections of working species developed by different taxonomic experts based on morphological taxonomy and, in some cases, DNA barcoding. Since collections of morphological working species have not been intercalibrated across all research programs, and only a small proportion of all macrofaunal species collected in the CCZ have been barcoded using DNA sequencing, we have mainly conducted between-site species-level comparisons *within* research programs to assure consistency in species-level determinations. While KODOS-KoreanClaim12-14, KODOS-KoreanClaim18, and KODOS-KoreanClaim19 datasets were all collected in the same program and exploration area, they were analyzed as separate data sets. This was done because the percentage of polychaetes identifiable to the species level were different among studies (~70% for 2012-14, ~30% for 2018, and ~95% for 2019) and abundances/core, species/family accumulation curves, and other parameters had large differences between cruises, suggesting differences in sample collection and processing between time periods. To facilitate diversity comparisons more broadly across research programs, we have conducted analyses at higher taxonomic levels (e.g., family level) after merging taxonomy at these higher levels across the data sets.

Macrofaunal data from combined sampling methods. We also used a large data set of polychaete worms, resolved to species level using CO1 barcoding, collected from a variety of locations using a combination of box cores, epibenthic sleds and ROV sampling (data sets from Glover and Dahlgren, and Bonifacio et al., 2019). While this data set is non-quantitative due to the opportunistic nature of sampling and pooling of material from different gear types, it allows comparison of species across different sampling programs in a manner not currently possible using morphological taxonomy.

C) Analytical Methods

First, comparability (i.e., sampling efficiency) of box cores sampling across research programs was explored by comparing polychaete abundance per square meter to annual particulate organic carbon (POC) flux to the seafloor estimated by Sweetman et al. (2017) for the sampling localities using the Lutz et al. (2007) POC-flux model for the period 1998 – 2010 (see Sweetman et al. (2017) for details); we call these estimates of POC flux "Lutz POC flux." Using Excel and 'lmodel2' (Legendre 2018) in R, we conducted Type II regression analyses of polychaete abundance (a metric available from all the assembled box-core studies) versus Lutz POC flux using linear and exponential functions. The functionality (either linear or exponential) with the highest R^2 (i.e., that which explained the greatest amount variance) was selected, and ordinary least squares (OLS) regressions were used for all studies as they produced the best fit to the data. Excel was also used to explore regression relationships between macrofaunal abundance and other individual environmental parameters, in particular nodule abundance estimated from the ISA (2010) Geological Model, and ocean depth, respectively.

Patterns of macrofaunal diversity across abyssal sites where explored with species and family accumulation curves, Chao 1 species richness estimators, rarefaction, and Pielou's evenness, as described in Magurran (2004) using EstimateS (Colwell, 2013), R (Venebales, 2019) or Primer 7 (Clarke and Gorley, 2015). Rarefaction curves with 95% confidence limits were calculated in EstimateS on each site by summing the number of individuals in each taxon in all box cores collected, effectively treating all box cores collected within a study and site as one sample. Rarefaction results are Accumulation curves and Chao 1 richness as well as standard deviations were also calculated in EstimateS; however, these were calculated at the box-core level to examine how these estimators change as more samples are taken. Pielou's evenness for species was calculated in PRIMER 7 for each box core and then averaged within a site. Non-metric multidimensional scaling was also performed in PRIMER 7 at the family level to compare community compositions among sites. Finally, the number of species in each site with abundances of only 1 or 2 individuals (singletons or doubletons) was calculated and compared to the total number of species found within each site.

The amount of variation in macrofaunal abundance and rarefaction diversity explained by a range of environmental variables was explored using Generalized Linear Modeling 'glm2' in R (Marschner and Donoghoe, 2018). Depth, Lutz POC flux, nodule abundance (kg/m^2), bottom water oxygen concentration, bottom-water salinity and bottom-water temperature values averaged across box cores for a particular site were used to explain average polychaete abundance and Chao 1 species estimations. GLMs use different combinations of explanatory variables to determine which combination explains the most variation in different continuous variables.

The number of species shared between sites was explored using UpSet plots in R (Conway et al., 2017). UpSet is a visualization technique, similar to Venn diagrams, which visualizes where datasets intersect. UpSet uses a data matrix to show intersections and sizes of these intersections.

D) **Results**

a) ***Polychaete abundance versus POC flux and the comparability of box-core data sets***

Because polychaetes typically constitute >50% of abyssal macrofaunal abundance, and polychaete abundance was tabulated in all the box-core data sets, we used polychaete abundance to explore comparability (e.g., sampling efficiency) across research programs. The efficiency of macrofaunal sampling using box cores (e.g., sample quality) can vary substantially with box-core deployment protocols, sample washing techniques, preservation protocols, sorting procedures, and sea state (for an accepted set of box-core sampling protocols, see Glover et al. (2016)). Based on previous abyssal studies of the relationships between seafloor POC flux and macrofaunal abundance (e.g., Glover et al., 2002, Smith et al., 2008a; Wei et a., 2010), we expected polychaete abundance across the CCZ to exhibit a positive relationship (exponential or linear) with estimated annual Lutz POC flux (Sweetman et al., 2017). When all box-cores samples were pooled across all studies, polychaete abundance was exponentially related to POC flux (Fig. 2), with approximately 20% of the variation explained with Type II regression ($R^2 = 0.2046$). However, the data sets from individual sampling programs were not evenly distributed above and below the regression curve, as would be expected if they were from the same statistical population, with a number of data sets falling largely above or essentially entirely below the general curve. This suggests that individual data sets may have different relationships between POC flux and polychaete abundance, as might be expected if sampling protocols (and sampling efficiency) varied across research programs.

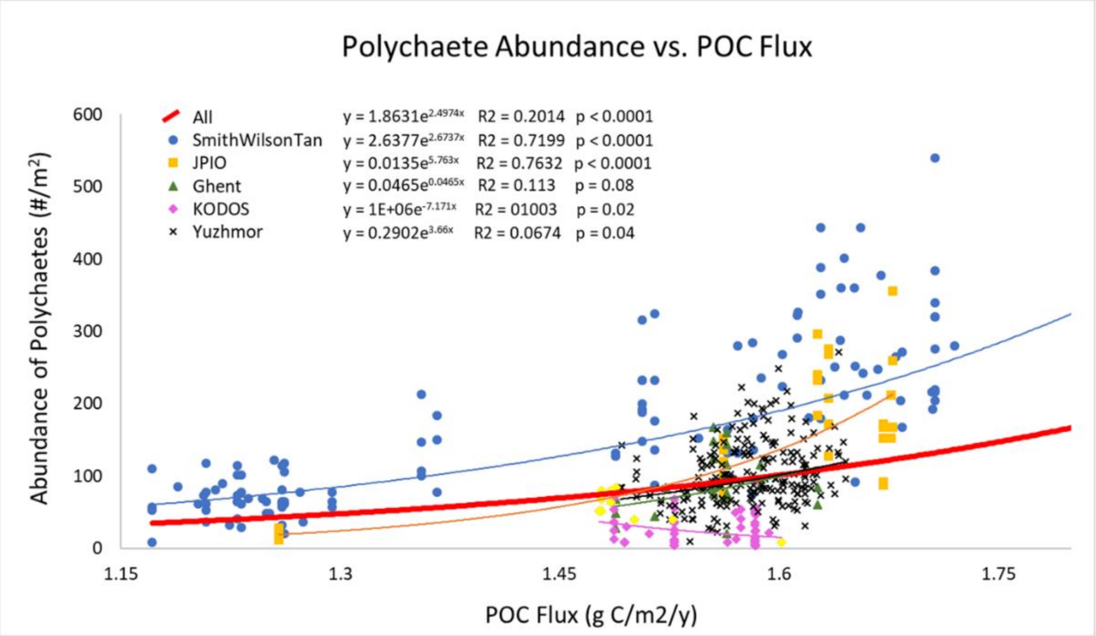


Figure 2. *Polychaete abundance in individual box cores versus Lutz POC flux (Sweetman et al., 2017). Curves are Type II regressions conducted in R, with regression equations,* R^2 *values, and p levels of regressions indicated. The red line is the exponential regression for all sampling programs combined. The pink regression line is for the KODOS data, indicated by pink diamonds for samples collected from 2012-1028, and yellow diamonds for those collected in 2019. Regression lines for the other data sets match the colors of their symbols in the upper left.*

We then conducted POC versus polychaete abundance regressions for individual research programs, i.e., studies that were conducted by investigators trained within the same laboratory and thus expected to use similar sampling protocols. It should be noted that the box-core data sets contributed by Wilson (2017), Smith (ABYSSLINE and EqPac), and Tan (NUS) were collected and processed with a similar set of protocols (first described in Hessler and Jumars, 1974; and more recently in Glover et al., 2017) by personnel trained in a single laboratory (that of R. R. Hessler), so these samples were considered to be a single Wilson-Smith-Tan data set for regression analyses. The Wilson-Smith-Tan and JPIO studies exhibited positive exponential relationships with high R^2 values (>0.7), nearly all studies showed positive exponential relationships, and one (KODOS) exhibited a significant negative exponential relationship versus Lutz POC flux (Fig. 2). The negative relationship in the KODOS data set was driven largely by relatively low values in box cores collected prior to 2019 (Fig. 2), potentially due to differences in sampling protocols, sea states, and/or seasonal/temporal trends in the KODOS area. The Wilson-Smith-Tan data, which covered the broadest ranges of longitude, latitude and POC fluxes (Table 1), exhibited a strong, significant exponential relationship between polychaete abundance and Lutz POC flux (n=138, $R^2 = 0.72$), providing robust support for the importance of POC flux as an ecosystem driver across the CCZ (cf. Wedding et al., 2013; Bonifacio et al., 2019). We conclude that the most robust data sets assembled for the workshop indicate that Lutz POC flux is a good predictor of polychaete (and macrofaunal) abundance in the CCZ, a result consistent with expectations of macrofaunal food limitation in this region based on direct measurements of POC flux versus macrofaunal parameters at many different abyssal sites (Smith et al., 2008a), and with the reasonable match of Lutz POC fluxes (within 20%) with results from sediment diagenetic models (Volz et al., 2018). *Thus, POC flux is likely a major driver of polychaete abundance across the CCZ, and evidently an important contributor to habitat quality. Thus, POC flux is an important variable to consider when setting up and evaluating of APEIs across the CCZ (as in Wedding et al., 2013).*

Nodule abundance and depth, when assessed individually with Type II regression, exhibited weaker negative exponential relationships with polychaete abundance, but still explained a substantial amount of variation in the Wilson-Smith-Tan data set (Fig. 3). This suggests that on regional scales across the CCZ, nodule abundance and depth are also likely to be important drivers of macrofaunal habitat quality.

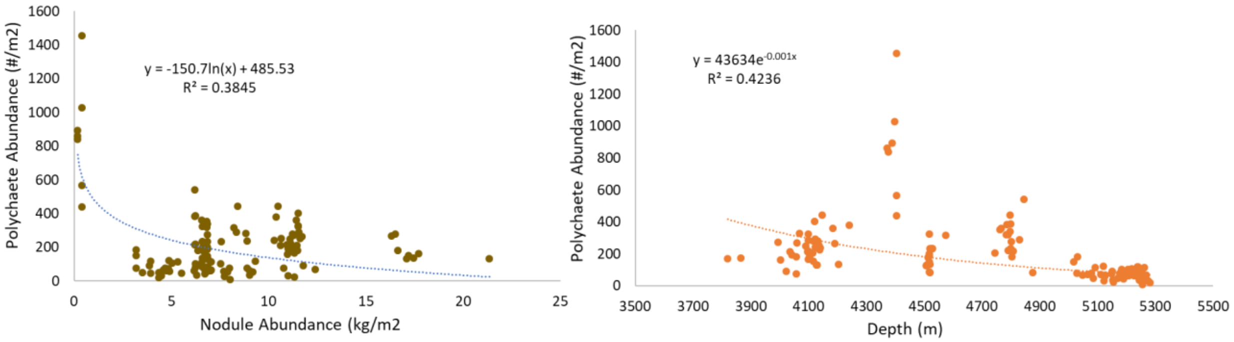


Figure 3. *Nodule abundance and depth versus polychaete abundance in the Wilson-Smith-Tan box cores. Lines shown are Type II regressions conducted in R. Both regressions are highly statistically significant (*$p < 0.0001$*).*

We also explored the relationship between average polychaete abundance at all sites sampled across the region (Fig. 1) versus a variety of potentially explanatory variables using Generalized Linear Models (GLM). The explanatory variables used were depth, Lutz POC flux, nodule abundance (kg/m^2), bottom-water oxygen concentration, bottom-water salinity and bottom-water temperature. The model explaining the most variance in mean polychaete abundance (numerical density) included POC flux and nodule abundance, which explained 84.7% and 2.6% of the variation, respectively. At an alpha level of 0.05, the influence of POC flux was highly statistically significant (Fig. 4) but the influence of nodule abundance was not (although nearly so, $p = 0.06$). *Thus, this GLM also indicates that POC flux is an important variable to include in the siting and evaluation of APEIs across the CCZ.*

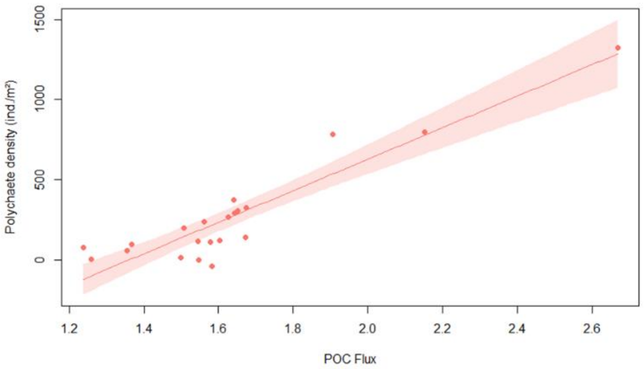


Figure 4. *Relationship between Lutz POC Flux and polychaete numerical density (abundance) when the variance due to the other explanatory variables has been removed, based on GLM analysis (ANOVA,* $p < 0.001$*).*

b) *Macrofaunal biodiversity along and across the CCZ*

In this section, we address key **biodiversity** questions of the workshop for sediment macrofauna.

> ***<u>Question 1:</u> For macrofauna, does species/family richness and evenness, and community structure, vary along and across the CCZ? What are the ecological drivers of these variations?***

Regional Patterns of Polychaete Abundance. Polychaete abundance showed strong variations along and across the CCZ, including within data sets (e.g., the Wilson-Smith-Tan data set in blue and the JPIO data set in yellow)(Fig. 5). Many of the between-site differences are clearly statistically significant, as indicated by the small size of within-site standard errors compared to between-site differences. As noted above (Figs. 2 and 3), these variations in polychaete abundance across the region are strongly related to Lutz POC flux, supporting the use of POC flux in dividing the CCZ management area into ecological subregions (Wedding et al., 2013)(Fig. 5).

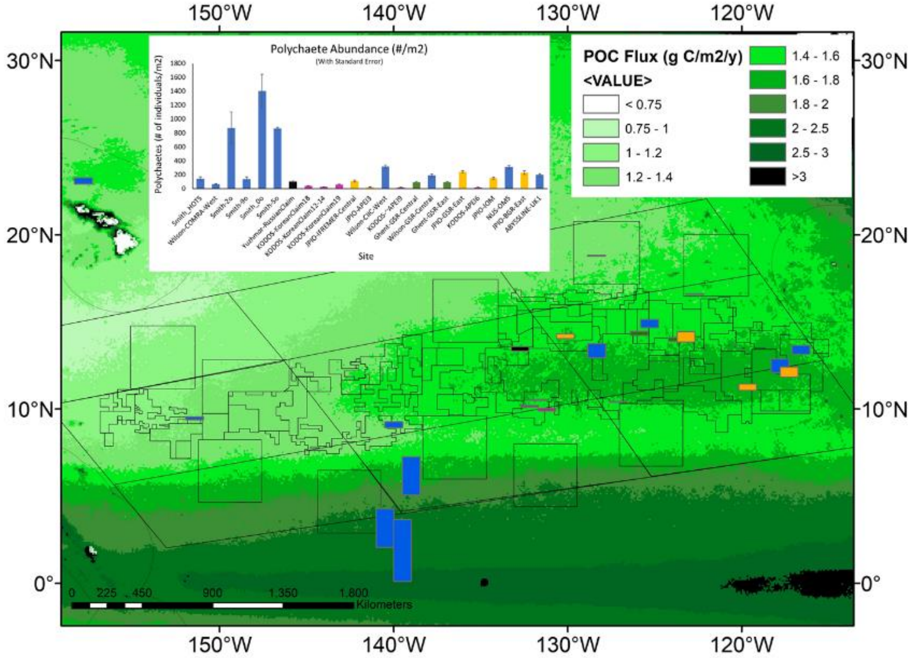


Figure 5. *Mean (<u>+</u> s.e.) polychaete abundance per box core at 21 sites across CCZ region, plotted with Lutz POC flux, and outlines of exploration contract and reserve areas, APEIs, and the nine subregions (large rectangles) used delineate similar ecoregions within the CCZ (Wedding et al., 2013). Sites considered to have been sampled with similar protocols (and thus have similar sampling efficiencies) have the same colored bars. The bottom of bars in the map indicate site location, with some offsets to allow all bars to be visible. Sites in the bar-chart insert are ordered from west to east.*

Biodiversity Patterns at the Species Level. We have evaluated diversity patterns for the major macrofaunal taxa (polychaetes, tanaids and isopods) separately, because there are differences between data sets in the taxa identified (only one data set, ABYSSLINE UK1, has distinguished all macrofaunal taxa at the species level). In addition, because different research programs have used different taxonomists, varying levels of bar coding, and different sets of working species, we explore regional variations in species diversity primarily *within* data sets that have used a consistent taxonomy (e.g., the same taxonomists).

Polychaetes at the Species Level. All sites with species-level, box-core, polychaete data exhibit rising species accumulation curves, in many cases with steep slopes (Fig. 6). *These curves indicate that polychaete species richness at all sites remains under-sampled, i.e., species are still rapidly accumulating and additional sampling at any site will collect previously unsampled species, even when large numbers of box cores have already been collected* (e.g., > 50 at COMRA-West)(Table 1). The rapidly rising curves reflect the fact that many/most species at each site are rare; ≥ 49 % of species are singletons or doubletons, i.e., represented by only one or two individuals, in the pooled samples from any site (Fig. 7). Within internally consistent data sets (e.g., within the Wilson and within the JPIO data sets), there are substantial between-site differences in the slopes and apparent asymptotes of species accumulation curves (Fig. 6).

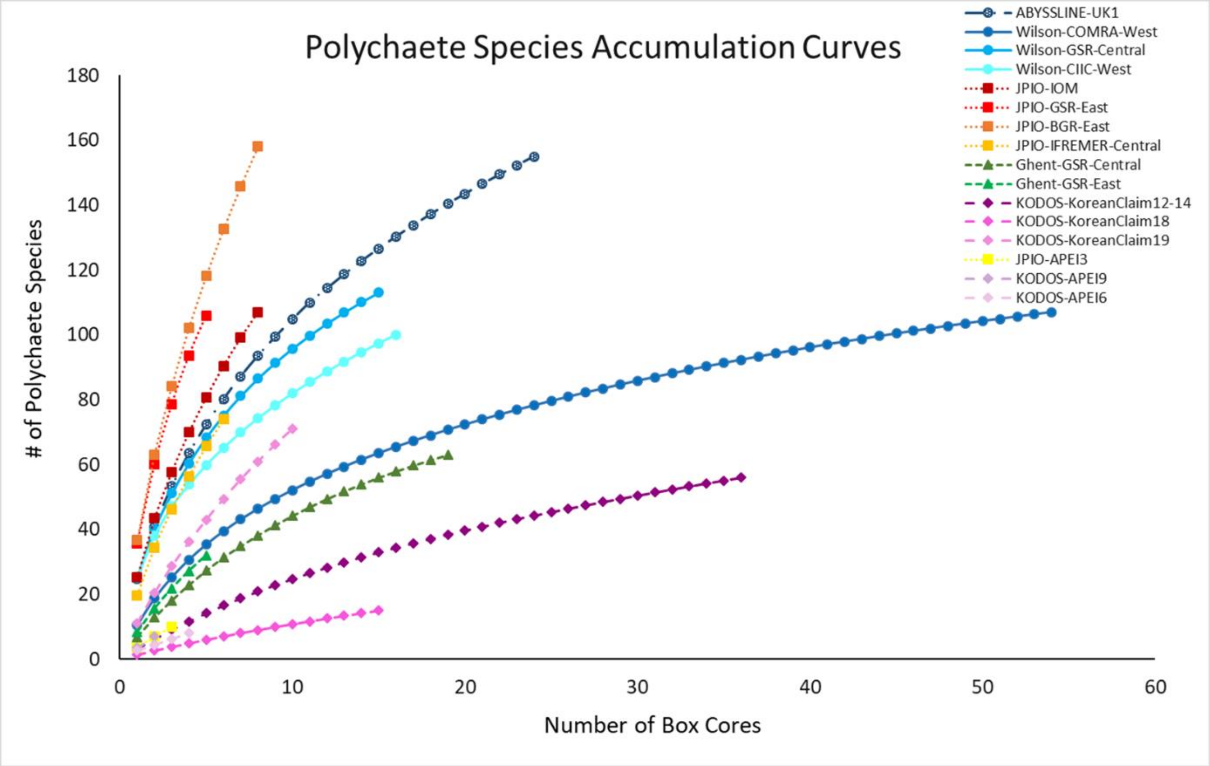


Figure 6. *Mean polychaete species accumulation versus number of box-core samples (UGE plot from EstimateS, 100 permutations) at different sites in the CCZ region. Note that the Korean Claim data come from a single site sampled in different years. Note that the Korean Claim data come from a single site sampled in different years. Data sets considered to have been sampled with similar protocols and to have used a consistent taxonomy, are indicated by similar symbols.*

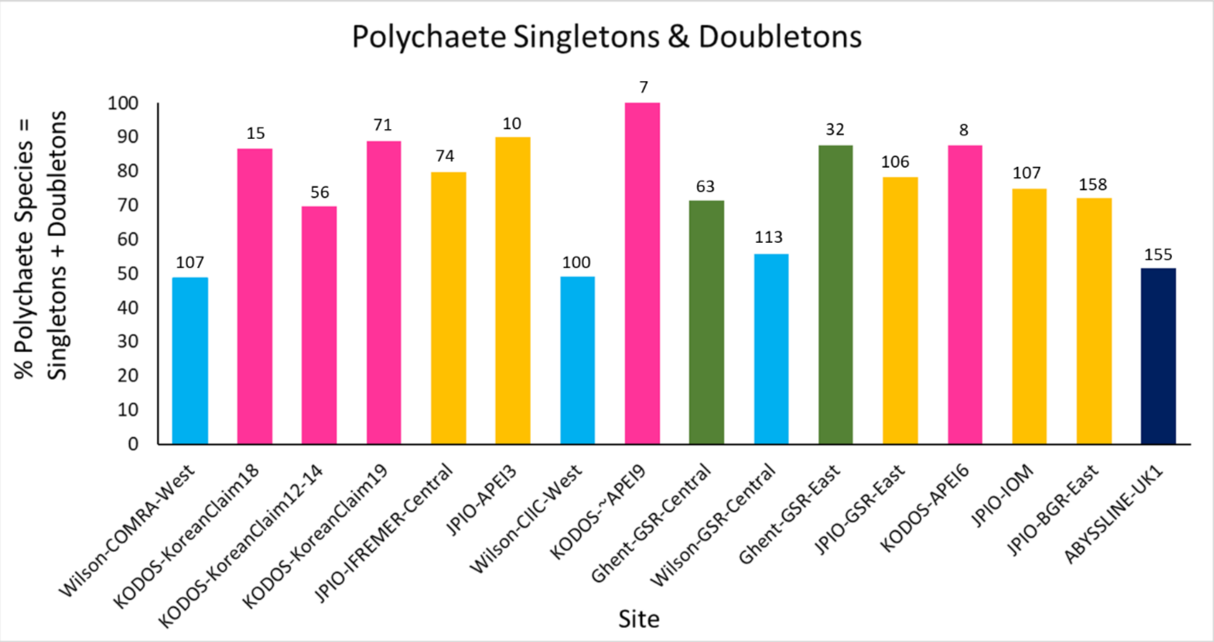


Figure 7. *Percentage of total polychaete species represented by singletons + doubletons in pooled collections from each site and study. The total number of polychaetes collected for each data set is indicated at the top of each bar. Note that the Korean Claim data come from a single site sampled in different years, and potentially with different efficiencies. Data sets considered to have been sampled with similar protocols and to have used a consistent taxonomy, are indicated by similar colors*.

Because species are still accumulating at all sites, we used the Chao 1 statistic to estimate the total number of species expected to be collected at each site if the polychaete assemblage were fully sampled (Magurran, 2004)(Fig. 8). Chao 1 estimates range from ~25 to ~370 species, with all the well sampled sites estimated to have >100 species of polychaetes. Note that for all sites, estimated total species richness substantially exceeds the number of species collected, i.e., only 25 – 73% of estimated polychaete species richness has been recovered at any site (Fig. 8). It is also important to note that for many sites (ABYSSLINE-UK1, Wilson-CIIC-West, all five JPIO sites), the Chao 1 curve is rapidly increasing with additional box cores (Fig. 8) suggesting that at these sites, estimated species richness will increase substantially with additional sampling (i.e., the current Chao 1 number is an underestimate). Species diversity (including richness) can only be directly compared between those sites with a common polychaete taxonomy (i.e., internally consistent species identifications), and only one internally consistent box-core data set, JPIO, has sampled > 3 sites (n = 5) across a substantial range (1400 km) of the CCZ (Fig. 1)((Bonifacio et al., 2019). *The JPIO data (based on morphological and molecular differentiation of species) indicates substantial variability in species richness across sites (Fig. 9), which appear to be driven by differences in POC flux and nodule abundance (Bonifacio et al., 2019).* It should be noted that the relatively high species richness estimated for most JPIO sites is likely related to use of molecular approaches that distinguish a substantial number of "cryptic" species lumped together with current morphological taxonomy (Bonifacio et al., 2019). *We infer that use of molecular approaches for polychaete identification at all sites across the CCZ is likely to also reveal significant numbers of currently undetected, cryptic polychaete species.*

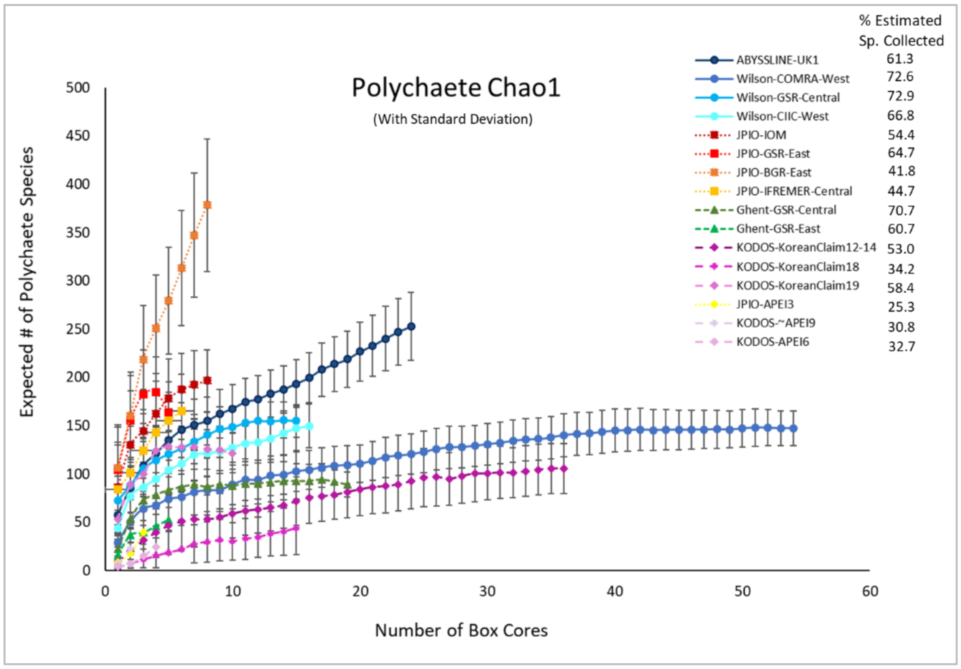


Figure 8. *Chao 1 (+ s.e.) estimates of polychaete assemblage species richness, as a function of number of box cores collected, at 16 sites across the CCZ region. Note that the Korean Claim data come from a single site sampled in different years. Data sets considered to have been sampled with similar protocols and to have used a consistent taxonomy, are indicated by similar symbols.*

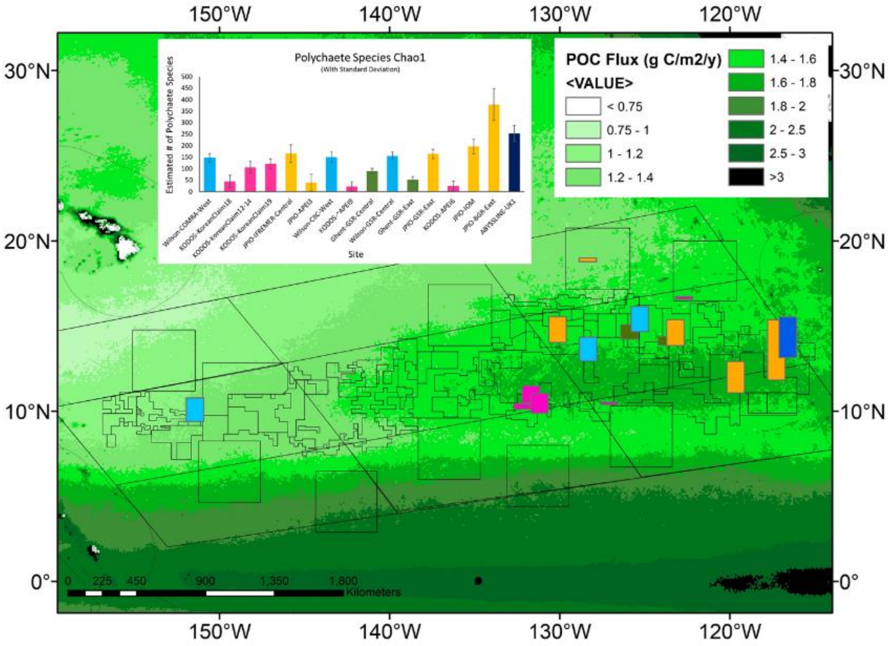


Figure 9. *Chao 1 (+ s.e.) estimate of polychaete species richness at 16 sites in the CCZ region, plotted with Lutz POC flux, and outlines of contract and reserve areas, APEIs, and the nine subregions (large rectangles) used for locating APEIs (Wedding et al., 2013). Sites with similar polychaete taxonomy have the same bar color. Sites in the bar-chart insert are ordered from west to east.*

Individual-based species rarefaction curves for all sites exhibit similar initial slopes (with overlapping 95% confidence limits) suggesting similar, high levels of species evenness across sites. However, rarefaction diversity at higher numbers of individuals, e.g., towards the right ends of curves and at $Es_{(130)}$, exhibit significant variability across sites within data sets (Fig. 10). These between-site differences in rarefaction diversity were not strongly related to POC flux (Fig. 11), in agreement with the findings of Bonifacio et al. (2019).

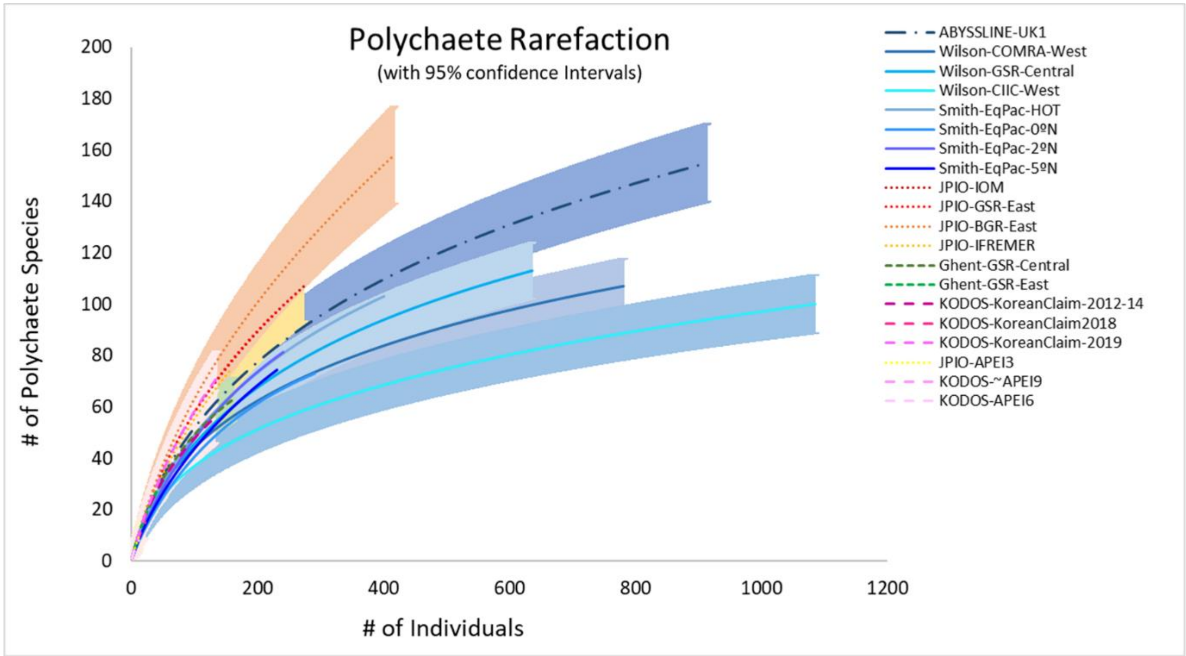


Figure 10. *Individual-based polychaete species rarefaction curves by site. Envelopes indicate 95% confidence limits for curves, centered around the respective curves. Note that the Korean Claim data come from a single site sampled in different years. Data sets considered to have been sampled with similar protocols and to have used a consistent taxonomy, are indicated by similar symbols.*

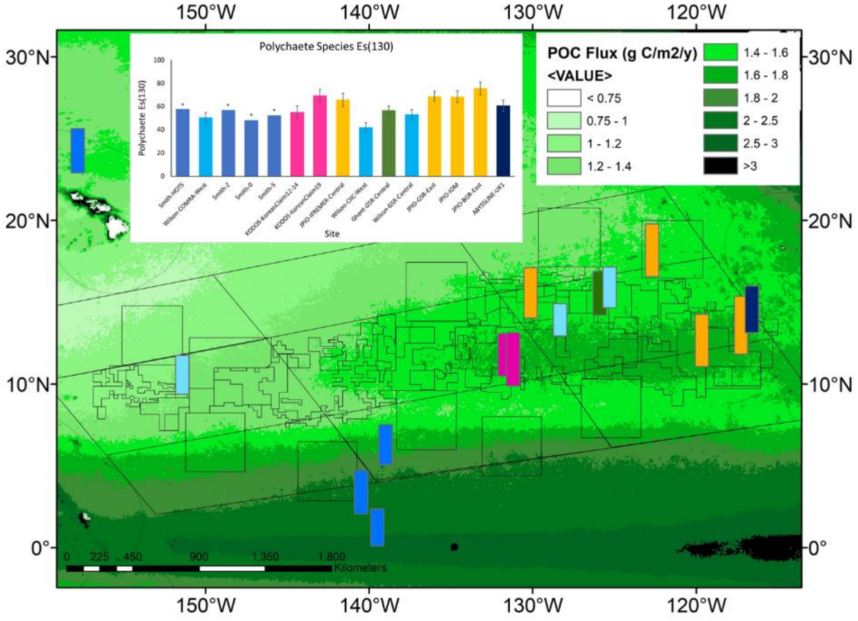


Figure 11. *$Es_{(130)}$, i.e., polychaete species rarefaction diversity at 130 individuals (± standard deviation) plotted on the regional map of POC flux. Sites considered to have been sampled with similar protocols (and thus have been sampled with similar efficiencies) have the same color in the bar chart. Sites in the bar-chart insert are ordered from west to east. The bottom of bars in the map indicate site location, with some offsets to allow all bars to be visible. Standard deviations could not be calculated for bars with asterisks.*

Mean Pielou Evenness J', calculated at the box core level, was generally high (near 1.0) and showed little variation across sites, except that Wilson-CIIC-West site value was unusually low (Fig. 12). Overall, this result is consistent with the similarity of initial slopes of species rarefaction curves in Figure 10.

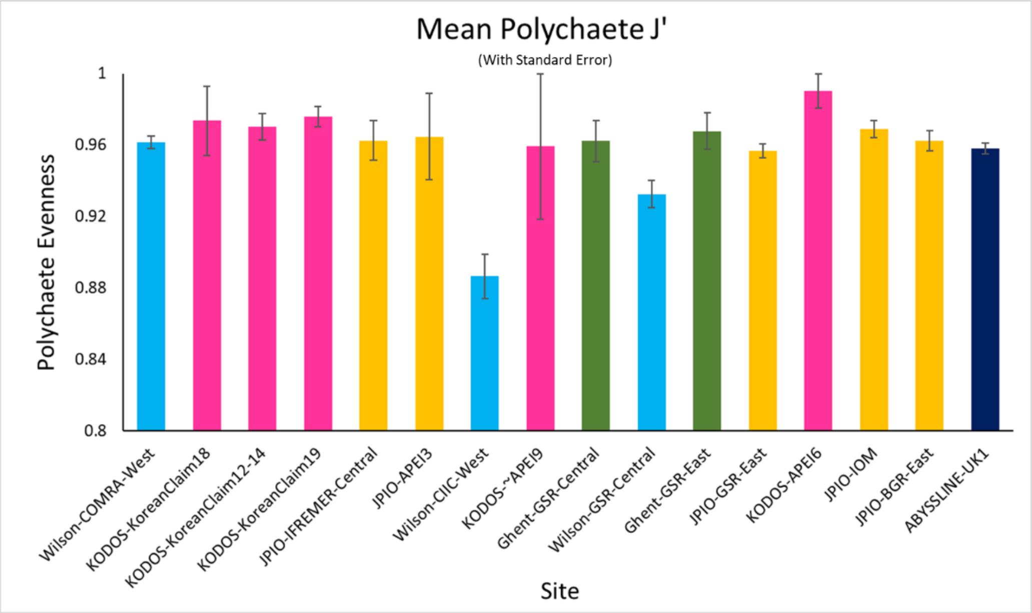
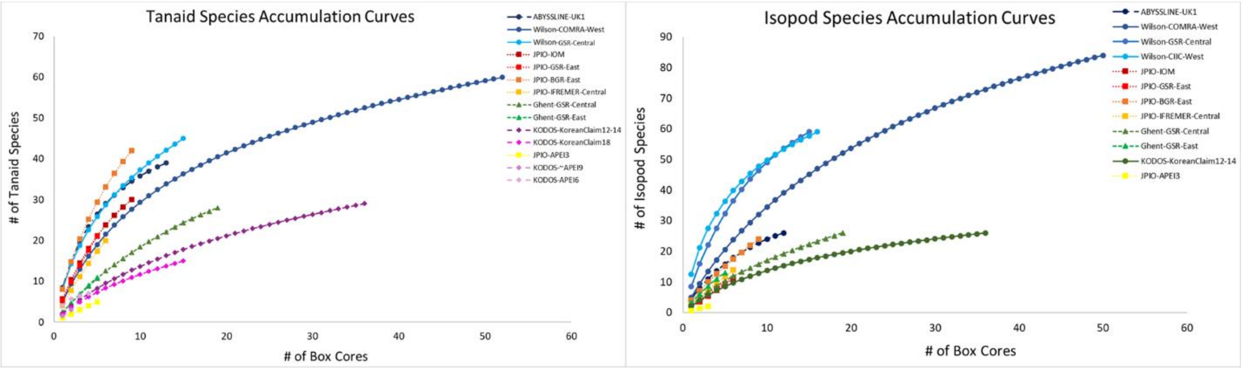


Figure 12. *Mean Pielou Evenness J’ (± s.e.) for individual box cores, by site. Sites considered to have been sampled with similar protocols and taxonomy have the same color in the bar chart.*

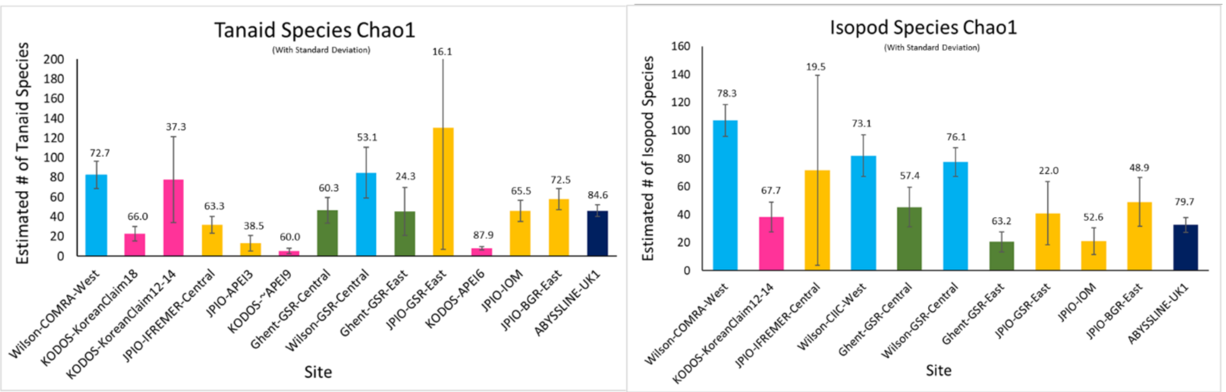
Tanaids and Isopods at the Species Level. Rapid rates of species accumulation where observed across all sites for tanaid and isopod crustaceans, again indicating that these crustacean assemblages remain poorly sampled (Fig. 13). As for polychaetes, large proportions of the species at all sites (>45%) were represented by singletons + doubletons, and a substantial percentage of estimated species richness remains uncollected (>15%), indicating that these assemblages remain incompletely sampled, even where >50 box cores have been collected (Wilson-COMRA-West). Within data sets, there was some heterogeneity between sites in accumulation curves and estimated species richness (Figs. 13-14) but the data sets are too small (in some cases < 20 individuals at a site) to warrant further comparisons.



Tanaid Species Accumulation Curves

Isopod Species Accumulation Curves

Figure 13. *Mean tanaid and isopod species accumulation versus number of box-core samples (UGE plot from EstimateS, 100 permutations) at different sites in the CCZ region. Note that the Korean Claim data come from a single site sampled in different years. Data sets considered to have been sampled with similar protocols and to have used a consistent taxonomy, are indicated by similar symbols.*



Tanaid Species Chao1

(With Standard Deviation)

Isopod Species Chao1

(With Standard Deviation)

Figure 14. *Chao 1 (± s.e.) estimate of species richness for tanaid and isopod assemblages at the various sites sampled across the CCZ. Numbers over bars indicate the percentage of estimated species richness that has been collected at each site.*

Family Level Comparisons. In an effort to minimize differences in taxonomy among data sets, we also explored patterns of diversity and community structure at the family level. Identifications at the family level are generally standardized across taxonomists and sampling programs, and the sampling of families is usually more complete and less biased than sampling of many hundreds of rare species. For older data sets (e.g., Wilson, 2017), we updated family classifications to the current family taxonomy on WORMS.

For most sites with >10 box core samples, polychaete family accumulation curves are leveling off (Fig. 15), and the number of families collected is within >80% of Chao 1 family richness estimates (Fig. 16), suggesting that most sites are well sampled for polychaete families. Nonetheless, there is substantial across-site variability in estimated family richness, both within and across sampling programs. A GLM exploring the relationship between polychaete family Chao 1 and six explanatory environmental variables (depth, Lutz POC flux, nodule abundance in kg/m^2, bottom-water oxygen concentration, bottom-water salinity and bottom-water temperature) found that only nodule abundance was statistically significant ($p < 0.001$), explaining 35.6% of the deviance. *This suggests, once again, that nodule abundance is an important habitat characteristic in the CCZ region.*

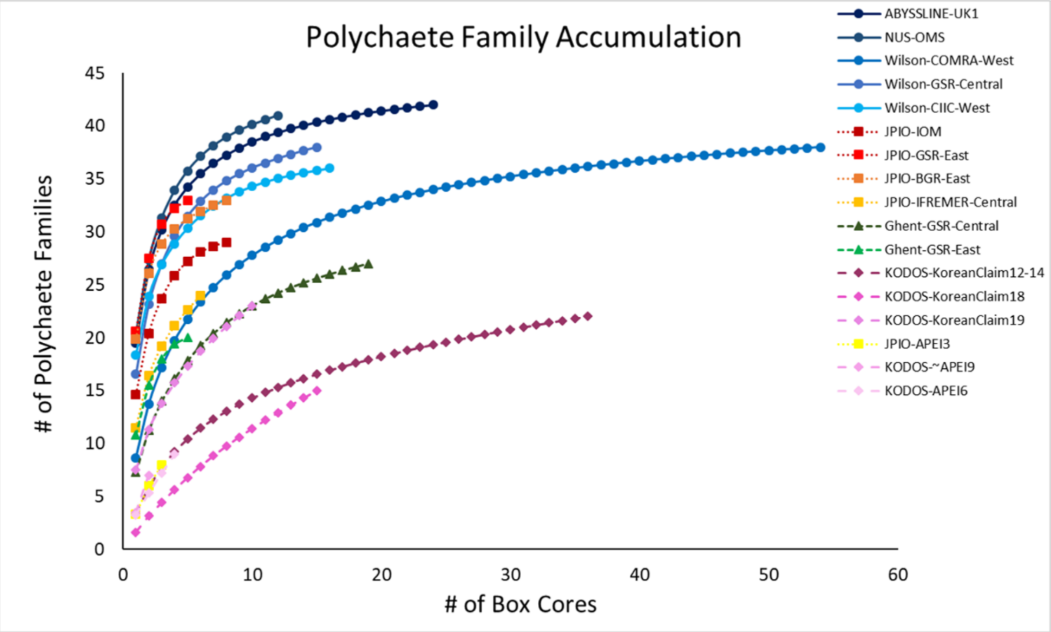


Figure 15. *Mean polychaete family accumulation versus number of box-core samples (UGE plot from EstimateS, 100 permutations) at different sites in the CCZ region. Note that the Korean Claim data come from a single site sampled in different years. Data sets considered to have been sampled with similar protocols and to have used a consistent taxonomy, are indicated by similar symbols.*

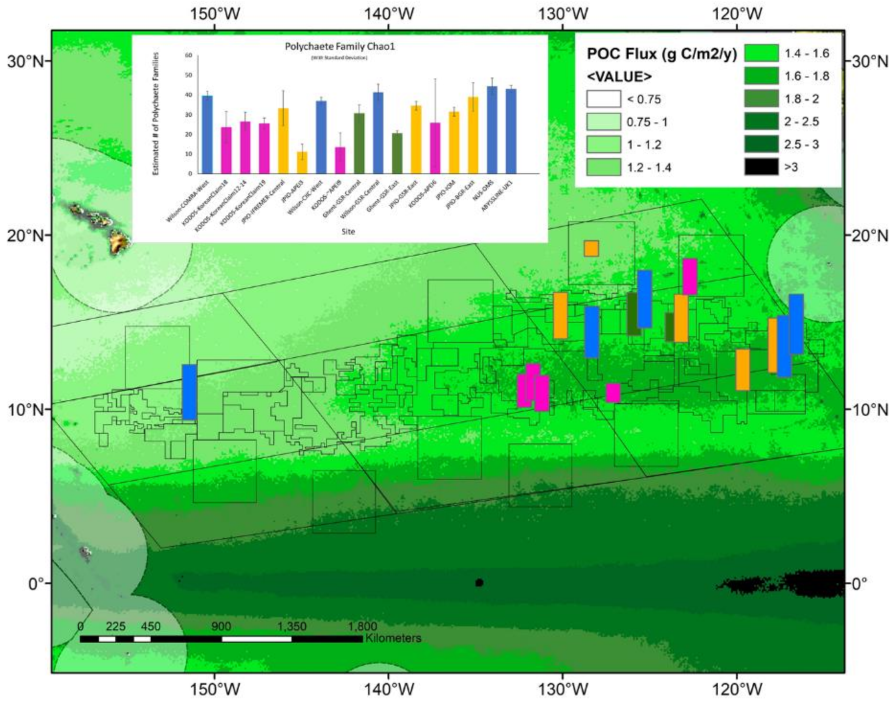


Figure 16. *Sample-based Chao 1 family richness estimates (± standard deviation) plotted on the regional map of POC flux. Sites considered to have been sampled with similar protocols (and thus have been sampled with similar efficiencies) have the same color in the bar chart. Sites in the bar-chart insert are ordered from west to east. The bottom of bars in the map indicate site location, with some offsets to allow all bars to be visible.*

Community structure at the family level also differed across sites, with some carnivorous families (e.g., lumbrinerids and goniadids) being relatively common at sites with higher POC flux and rare or absent from sites with low POC flux (Fig. 17). *These regional changes are consistent with previously documented variations in polychaete community composition, e.g., reduction in the abundance of carnivorous polychaete families, with declining POC flux* (Smith et al., 2008b; Bonifacio et al 2019).

<u>Question 2:</u> Do claim areas have similar levels of species/taxon richness and evenness, and similar community structure, to the proximal APEI(s)?

The sediment macrofaunal data from APEIs are extremely limited, with only one APEI 3 sampled within its core region (at a single site) with 3 box cores, and single sites on the edges of APEIs 6 and 9 sampled with 4 and 2 box cores, respectively. Polychaete community abundance and Chao 1 species richness and family richness were substantially lower in the core of APEI 3 than in contract areas (IFREMER Central and GSR-West) sampled during the JPIO program 600-900 km away (Figs. 5 and 8). These differences have been related to lower POC flux and nodule abundance in APEI 3 (Fig. 3)(Bonifacio et at., 2019). Polychaete abundance and Chao 1 species richness were also lower on the edges of APEI 6 and 9 than in the KODOS area 600-1200 away sampled during the same cruise (Fig. and 8). These differences may also be related to differences in POC flux.

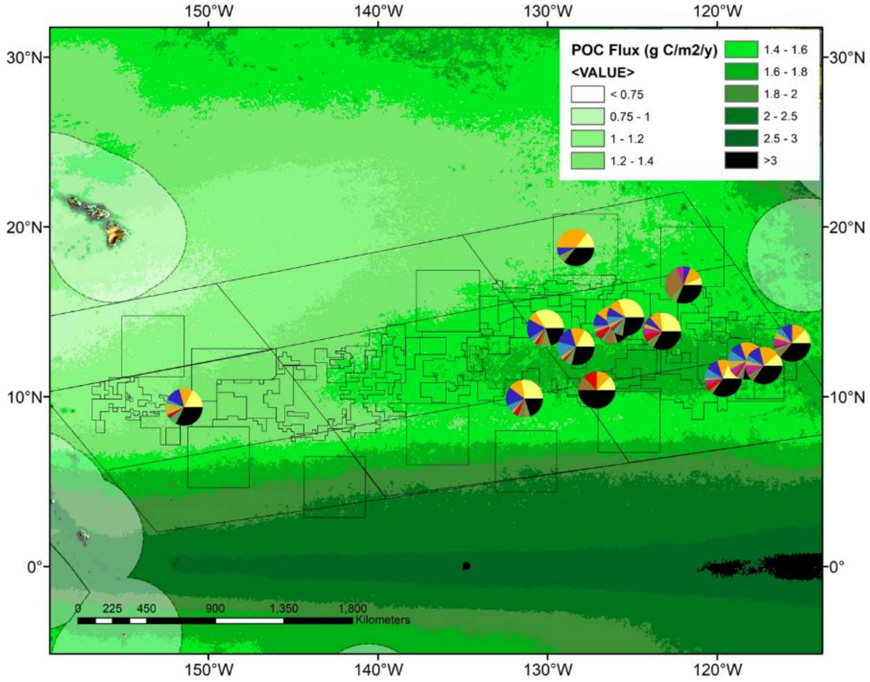


Figure 17. *Percent composition of polychaetes by family plotted on the regional map of POC flux. The percent abundance of the 10 most common families is shown, with the size of wedges of circles proportional to percent abundance. Sites considered to have been sampled with similar protocols (and thus have been sampled with similar efficiencies) have the same color in the bar chart. Sites in the bar-chart insert are ordered from west to east. The bottom of bars in the map indicate site location, with some offsets to allow all bars to be visible. Standard deviations could not be calculated for bars with asterisks*.

At the polychaete family level, NMDS analyses suggest that all three sites inside or near APEIs are outliers in community structure compared to most sites sampled within contract areas (Fig. 18).

However, these differences could well be caused by the very limited number of box cores (3 – 4) and animals (≤ 16) collected in or near the APEIs. It is noteworthy that KODOS 2018, which also has very few polychaetes identified to family level (n = 28), is an outlier compared to the sites with larger samples. For tanaid families, APEIs 3 and 9 are again outliers, but these sites had ≤ 4 tanaids identified to family. *Thus, we have low confidence that the currently available data can be used to meaningfully compare polychaete or tanaid community structure between exploration contract areas and any APEI.*

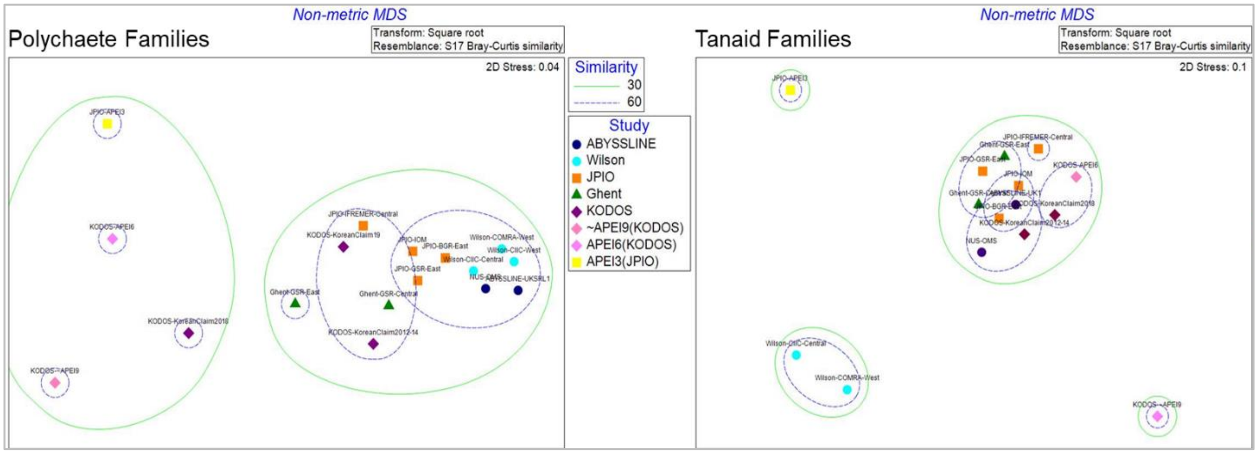


Figure 18. *NMDS plots of polycheate and tanaid family community structure for contractor contract areas and in or near APEIs. Dashed lines bound sites with 30% and 60% similarity.*

<u>Question 3:</u> Are species ranges (based on morphology and/or barcoding) generally large compared to the distances between APEIs and contractor areas? What is the degree of species overlap between different study locations across the CCZ?

The JPIO study provides the most extensive data set to explore the known distributions of macrofaunal species compared the scale of contractor contract areas. Note that all these data come from the eastern CCZ. Within the polychaetes, tanaids and isopods, which constitute the bulk of macrofaunal abundance and species richness in the CCZ, some common species identified in the JPIO data set range over 600-1200 km, with a few occurring in APEI 3 and contract areas (Figure 19). However, the vast bulk of species, which typically are rare, have been found only at single sites. These results are consistent with those of Bonifacio et al (2019), who found high polychaete species turnover among sites sampled in the JPIO program, with average species ranges estimated to be 25 km. These results suggest that the ranges of many species could be small compared the size of contractor contract areas (up to 75,000 km^2) and the distance from contractor areas to the nearest APEIs (often 100s of kilometers). However, because most macrofaunal species sampled are rare, it is very difficult to distinguish whether species typically are endemic to single sites (i.e., have small ranges compared the spacing of samples across the region (Fig. 1)), or are present but not yet sampled at multiple sites.

The broadest data set to address macrofaunal species ranges in the CCZ comes from Glover, Dahlgren, Bribiesca-Contreras et al. (in prep.), as well as Bonifacio and Menot (2018) and Bonifacio et al. (2019), who have barcoded polychaetes sampled opportunistically with a broad range of methods (box core, megacore, EBS, ROV) from sites spanning the eastern to western CCZ. Among the 297 molecular taxonomic units (MOTUs) identified, approximately 50 MOTUs are shared across distances of 500-800 km, and 2 are shared from western APEIs to the eastern CCZ, over a span of 3000 km. However, most MOTUs (~240) have been found so far only at a single site, again raising the possibility that the ranges of many species could be relatively small. Once again, because of the long list of rare species, and the relatively small numbers of samples collected at any site, the effect of small species ranges cannot be distinguished from the effects of under-sampling.

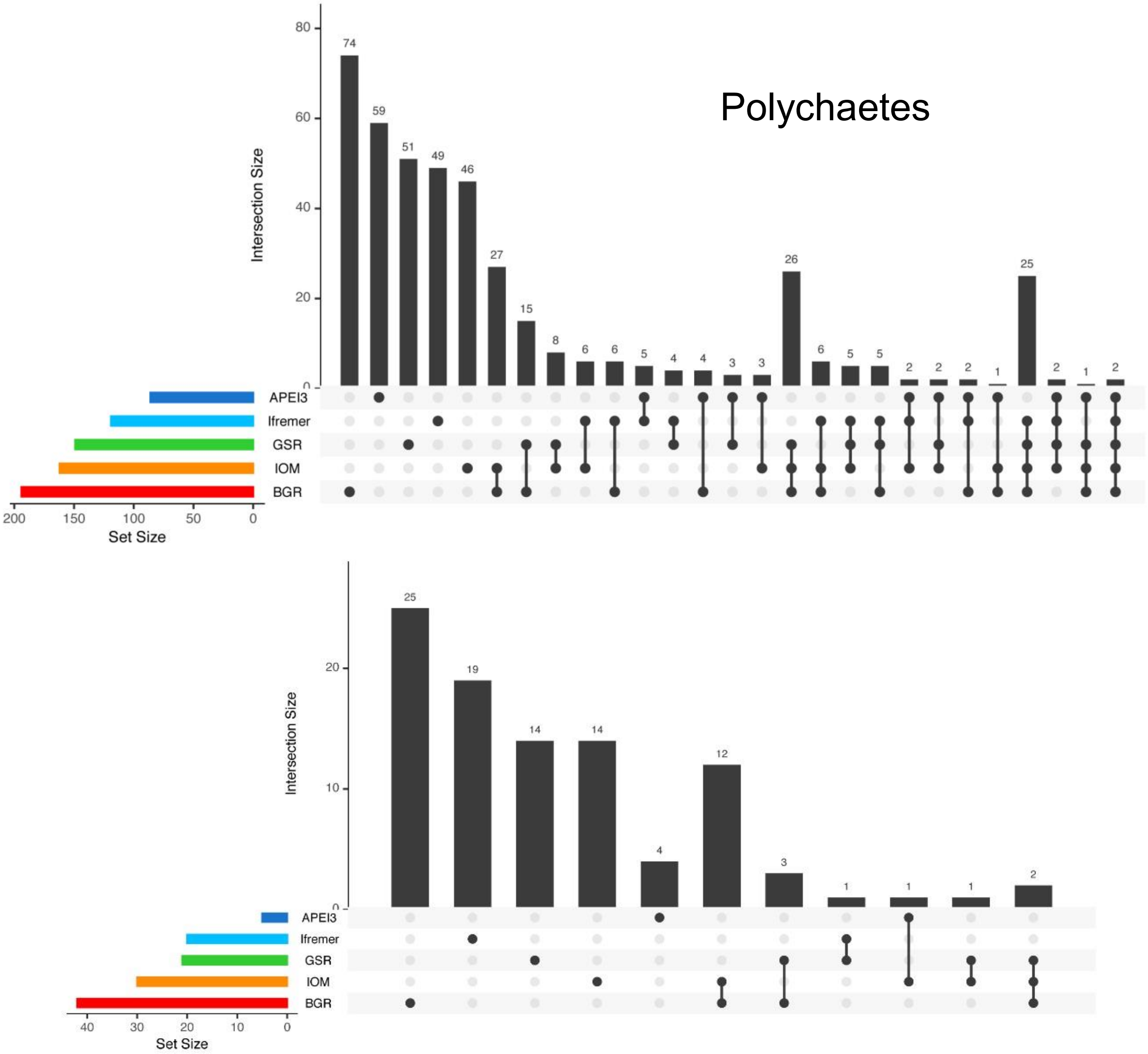


Figure 19. *UpSet plots showing the intersection of sediment macrofaunal species resolved by morphological and/or molecular approaches across the five JPIO sites (i.e., sites with a common species-level taxonomy). The polychaete data at top includes box cores, epibenthic sleds and ROV samples. The tanaid and isopod data in the bottom two panels are from box core samples. Vertical bars on the main plots represent the number of the unique species in each area or shared between the sites (dots) connected by lines. Bars on the left are the total number of MOTUs identified in each of the 5 areas.*

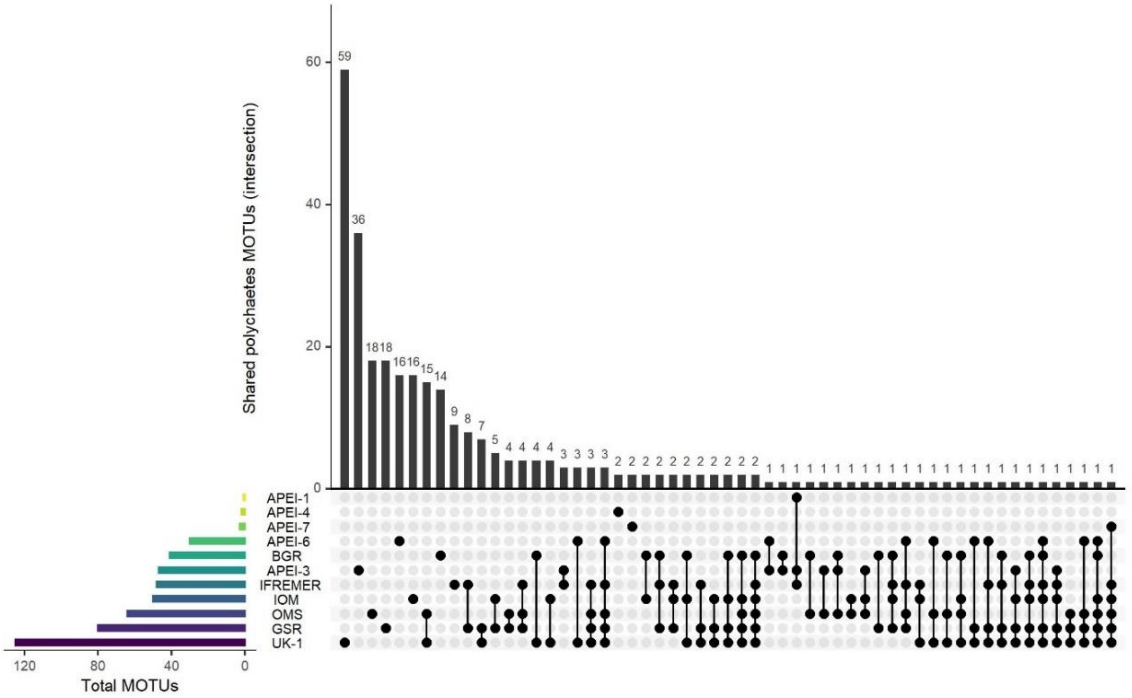


Figure 20. *UpSet plot of the intersection of polychaete molecular operational taxonomic units (MOTUs) identified using barcode gap at sites distributed across the CCZ (Glover, Dahlgren, Bribiesca-Contreras et al., in prep; Pape et al., in prep., Janssen et al., 2015, 2019; Bonifacio and Menot, 2018; Bonifacio et al., 2019). These data come from box cores, epibenthic sleds and ROV samples. Vertical bars on the main plot represent number of the unique species in each area or shared between the sites (dots) connected by lines. Bars at left represent the total number of MOTUs identified in each of the 11 areas.*

E) Conclusions

1) Although quantitative box-core samples for macrofauna have been collected at widespread sites in the North Pacific, there are huge, unsampled gaps within the CCZ, particularly in the central and western portions (Fig. 1). Macrofaunal diversity data are available from the core of area only a single APEI (APEI 3, only three box cores). Thus, in much of the central and western CCZ, and in all of the APEIs, sediment macrofaunal biodiversity patterns remain poorly studied or unevaluated.

2) Based on relationships between POC flux and macrofaunal abundance in box cores, sampling efficiencies vary across data sets and sampling programs in the CCZ**.** Varying sampling efficiencies, plus differences between sampling programs in the identification of working species, means that quantitative comparisons of macrofaunal biodiversity are best made within research programs, or across programs with similar sampling protocols and consistent taxonomies.

3) Strong positive relationships between polychaete abundance and the explanatory variables POC flux and nodule abundance in multiple data sets indicate that POC flux and nodule abundance are important variables to include in abyssal habitat mapping, and in designing and evaluating APEIs across the CCZ (as in Wedding et al., 2013).

4) Macrofaunal species accumulation curves are rising rapidly at all sites, indicating that species diversity at every site remains under-sampled, even where large numbers box cores (>50) have already been

collected. Use of molecular techniques is likely to reveal even more undetected macrofaunal diversity in the form of morphological cryptic species.

5) Macrofaunal abundance, diversity and community structure vary substantially across the CCZ very likely in response to variations in POC flux and nodule abundance.

6) Very limited data suggest lower abundance and diversity, and different community structure, in APEI 3 compared to contractor contract areas 600-900 km away, but we cannot say whether it differs from nearer contract and reserve areas. No other direct comparisons can be made between APEIs and contractor areas.

7) Some common species (identified with morphology and/or DNA techniques) range over 600-900 km, and a few range over 3000 km, with a species shared between APEIs 1,3,4, and 7 and contractor contract areas. However, the vast majority of identified macrofaunal species are rare and so far collected only at single sites. The effect of small species ranges cannot be distinguished from the effects of under-sampling in creating this pattern.

8) Because rarity is often correlated with small species ranges in better known ecosystems (Pimm et al., 2014), we cannot assume that the numerous rare species in the CCZ are widely distributed, and simply under-sampled.

F) Data Gaps

In brief, key data gaps include the following:

1) No quantitative macrofaunal sampling in eight APEIs, and extremely limited sampling in the ninth (APEI 3).

2) A lack of quantitative macrofaunal sampling in near ally of the central and western CCZ, i.e., over >50% of the management area.

3) Very limited understanding of full macrofaunal diversity at any site, and thus in the degree to which species are distributed across the CCZ.

4) The hundreds of macrofaunal species collected from the CCZ are mostly undescribed fauna, there has been little intercalibration of morphological taxonomy, and DNA barcoding of macrofauna has been very limited.

5) There are no available time-series measurements of seafloor macrofaunal parameters, or the key ecosystem driver POC flux, anywhere in the CCZ, making baseline temporal variability impossible to assess.

G) References

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e. INVERTEBRATE MEGAFAUNA –

DeepCCZ Synthesis Workshop - Invertebrate Megafauna

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(Note: Tables and Figures follow the References in this section)

INTRODUCTION

The benthic invertebrate megafauna are defined here as metazoans over 1 cm in maximum dimension (sensu Grassle et al. 1975) that live on or just above the seafloor. They include animals such as cephalopods (e.g. octopus and squid), scavenging amphipods and shrimp, large deposit feeders such as holothurians (sea cucumbers) and asteroids (sea stars), and suspension-feeding sponges, anemones, corals and other cnidarians. They constitute an important component of the biodiversity in the abyss. Patterns in megabenthic density, diversity and community composition are related to the benthic habitat, and are driven by variation in environmental parameters such as depth, sediment quality, presence/type of bathymetric features, food availability, and patterns associated with previous disturbance (e.g. Simon-Lledó et al. 2019 a,b,c,d; Smith et al. 1997; Cuvelier et al. in review; Durden et al. in prep). Individual fauna may require hard substratum, in the form of nodules (Vanreusel et al. 2016), and the effects of these species-specific responses are visible at the community level (Simon-Lledó et al., 2019b). Megafauna play a significant role in deep-sea ecosystem function, in terms of phytodetritus consumption and bioturbation (Smith et al., 2008) and carbon flow through the abyssal Pacific food web (Stratmann et al. 2018).

Knowledge about Clarion Clipperton Zone (CCZ) megafaunal abundance and diversity in the published literature is mostly restricted to the eastern CCZ (e.g. Amon et al. 2016, Vanreusel et al. 2016, Simon-Lledó et al. 2019a), where about 50% of all megafauna were shown to depend on nodules (Amon et al. 2016). While higher numerical densities have been observed in locations with higher nodule coverage (Vanreusel et al., 2016), the precise role of nodules and other local environmental factors in the ecology of CCZ megafauna is still poorly understood and based on relatively small areas sampled (Simon-Lledó et al. 2019a, b).

In this study, we aim to compile and synthesize the available data on the invertebrate megafauna across the CCZ. This includes published and unpublished studies. We use these data to describe regional-scale patterns in megafaunal density, diversity and community structure.

METHODS

For the purposes of regional assessment, megafauna were examined using several methods that facilitated specimen collection and quantification over large seabed areas. Specimens were collected using trawls, epibenthic sledges and by remotely operated vehicles (ROVs), facilitating robust taxonomic inspection to species level and genetic studies (e.g. Amon et al. 2017, Glover et al. 2016). Quantitative spatial assessments were done using seabed photography (Durden et al. 2016b), with cameras mounted on ROVs, autonomous underwater vehicles (AUVs) and towed cameras. As this approach makes identification to species level difficult for some taxa, animals are categorized into distinct 'morphotypes' in lieu of true species identification.

Invertebrate megafauna were assessed using data inferred from seabed-image surveys conducted across the CCZ and nearby locations (Table 1, Figure 1). A total of 22 areas were evaluated in this synthesis report. These included data from six APEIs, contractor areas and areas to the periphery of the CCZ (Kiribati EEZ and an area to the east of UK-1; Figure 1). These data were originally collected using a range of different methodologies (e.g. ROV, AUV, and towed-camera systems) and above-seabed altitudes. Datasets with the highest resolution and lowest above-seabed altitude of collection were re-analyzed in accordance with a CCZ standardized morphotype catalogue (see section below). Available invertebrate megafauna data (see Table 1) can be split into the following categories:

- Standardized data (Std-analysis): imagery collected at above-seabed altitudes ranging from 2-5 m, with precise measurements of the seabed area encompassed by sampling, a minimum total sampling effort of 4,000 m^2 per study area, and (re)annotated using the standardized taxonomic catalogue. As such, these data enable density-based comparative analyses of faunal abundance, diversity, and assemblage composition. These include data from: eastern Kiribati EEZ (Nautilus Minerals, Simon-Lledó et al, 2019d); western CCZ APEIs 1, 4, and 7 (DeepCCZ, Durden et al, in prep); TOML areas B, C, and D (Nautilus Minerals, Simon-Lledó et al, in prep); eastern CCZ sites (APEI3, GSR, BGR; Cuvelier et al, in review); APEI-6 (Simon-Lledó et al, 2019b); and UK-1 area (Amon et al, 2016). Notes on Std-analysis:
    - Despite being geographically peripheral to the CCZ, data from the eastern Kiribati EEZ were included in Std-analyses (e.g. faunal density and diversity) as these formed a geographically close comparison with the western APEIs.
    - Additional data from the DISCOL area in the Peru Basin (Simon-Lledó et al, 2019c) were not included in any analyses owing to the large geographic separation from the CCZ.
    - Data from UK-1 area (Amon et al, 2016) were only used in a set of the Std-analyses (e.g., faunal density), as at the time of writing, the dataset was under reannotation in order to incorporate the most recent update of the unified catalogue (i.e. lacking abundance at the morphotype level; only taxa presence data was available for diversity assessment).
    - Eastern CCZ (APEI3, GSR, BGR) data from video transects collected during SO239 (Cuvelier et al in review; with taxa aligned with standardized catalogue) were only used in semi-quantitative assessments of fauna distribution, given the different nature of the collecting platform (see below).

- Unstandardized data (meta-analysis): imagery collected at above-seabed altitudes ranging from 2-5 m, with relatively precise measurements of the seabed area encompassed by sampling (e.g. video transects), and/or only partially aligned with the standardized taxonomic catalogue.

Original data were obtained from these studies and initial standardization was carried out to broadly align the taxonomic groups included. These allowed quantitative comparisons of (total) faunal abundance, but no consistent evaluation of diversity or taxonomic composition variations at the regional scale.

Std-analysis

Standardized morphotype catalogue

All metazoans were sorted into taxa and identified to the lowest taxonomic hierarchy possible in image-based identification (i.e. morphotype: typically genus or family level). Faunal catalogues created for the different areas were combined, morphotype identifications were aligned and a common megafaunal morphotype catalogue for the CCZ was created. At the time of writing, this catalogue encompassed and classified all metazoan megafaunal taxa encountered in the Std-datasets, some of which are recently described in CCZ taxonomic publications (e.g. Amon et al., 2017a and b; Dahlgren et al., 2016; Glover et al., 2016; Kersken et al., 2019; Kersken et al., 2018; Molodtsova & Opresko, 2017; Purser et al., 2016). A list of distinct and characteristic taxa was compiled and their presence was reassessed at all the locations described in Table 1.

Semi-quantitative analysis of taxa distribution

A selection of morphotypes was derived from those abundant morphotypes in the Std-datasets that could be identified with the most confidence (e.g. by non-experts). A total of 22 characteristic morphotypes were selected and their presence across Std-dataset locations from west to east in the CCZ was assessed.

Morphotype dominance / rarity

To investigate patterns in morphotype abundance, all the individuals encountered were pooled across all the Std-datasets, except UK-1, to perform a rank morphotype abundance assessment. Note that full datasets with varying total seabed area surveyed (see Table 1) were pooled together for this analysis to explore dominance/rarity patterns for the CCZ region, while regional variations of these parameters were explored in the area-controlled analyses described below.

Area-controlled bootstrapped analyses

Regional patterns in metazoan abundance, diversity and composition were quantitatively assessed using all the picture-based Std-datasets. Since the number and size of replicate transects, as well as the total seabed area covered in each of the surveyed locations, were variable, we chose to apply a modified form of ecological bootstrapping (Davison and Hinkley, 1997) with a fixed sampling effort unit resulting from the resampling of image data, to control the impact of the physical sample size and the potential inclusion of local variations on the evaluation of each parameter at the regional scale. Resampling techniques provide robust estimates of standard errors and confidence intervals of sample parameters (see e.g. in Crowley 1992) and are particularly well suited to analyze data derived from survey designs that lack true sample replication (e.g. Simon-Lledó et al 2019b, d). To implement the bootstrap, each study area image data subset was randomly resampled with replacement until a minimum of 1000 m^2 of seafloor were encompassed (bootstrap-like sample), and that process was repeated 1000 times for each area. Numerical densities (ind. m^{-2}) and a range of diversity metrics were assessed in each of the bootstrap-like samples. Mean values of these 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).

The SO239 datasets were not included in the bootstrap analysis at this stage, since the bootstrap approach used images as a base unit for data resampling. The potential conversion from video-transect data into image/polygon data units is being explored for future incorporation.

UK-1 data were included in assessments of total or phylum-specific faunal abundance but excluded from analyses based on morphotype abundance (e.g. all diversity metrics)

Morphotype accumulation curves

Rarefied morphotype accumulation curves (e.g. Colwell et al., 2012) were calculated using the Std-datasets to infer the representability of each dataset and explore the potential effect of the varying sampling effort sizes in the assessment of diversity patterns. Image data for each area, as well as pooled data for all APEIs and the rest of CCZ locations (excluding the Kiribati EEZ dataset), were randomly resampled without replacement to generate subsamples with fixed coverage of 500 m^2 of seabed. Subsamples for each separate area (or combined locations) were randomly resampled 100 times without replacement forming increasingly larger sampling units, and the total number of different morphotypes was calculated for each randomization, along with mean values and 95% confidence intervals, using Estimate S v.9.1 software (Colwell, 2013). Sampling unit size was quantified as both number of individuals and seabed area surveyed.

Environmental drivers

Broad-scale, modelled environmental data were used to explore potential drivers of megafauna morphotype richness and density. Both particulate organic carbon (POC), a measure of food input to the benthic community, and nodule abundance have been shown to influence megafaunal density, community composition and/or diversity in the CCZ and were thus included in the analyses (Simon-Lledó et al 2019b; Smith et al 1997; Vanreusel et al. 2016). Estimates for POC in the CCZ were obtained from a global model produced by Lutz et al. (2007), and estimates of nodule abundance were obtained from 'ISA Technical Study No. 6: A Geological Model of Polymetallic Nodule Deposits in the Clarion Clipperton Fracture Zone' (ISA 2010) (see Habitat Mapping section for more detail). POC and nodule abundance estimates were interpolated to 1 km^2 resolution. Megafaunal parameters obtained from the meta-analysis (e.g. density) and the bootstrapped analyses on Std-datasets (e.g. density and taxa richness) were compared, where possible, by nonparametric correlation (Spearman's rank: r_s) with POC and nodule abundance of each data location.

RESULTS

Standing stocks and biodiversity of CCZ megafauna

Standing stocks

The geographic spread of faunal density derived from the meta-analysis presented few obvious patterns (Fig. 3A), except for a general reduction in density to the west and a possible trend of higher density in the central CCZ compared with the peripheral APEIs. Variations in megafauna (> 1 cm) density obtained from bootstrap analyses performed on Std-datasets (Kiribati EEZ, APEIs 1, 4, 6, 7; TOML B, C, D; and UK-1 areas) described a similar east to west trend of faunal density decrease (Fig. 3B). This pattern was also observed at the phylum level (Fig. 4), with cnidarians and sponges (predominantly composed by sessile taxa) describing a gradual decrease in abundance towards the west (Fig 4A,C), and a much sparser distribution of density in echinoderms and arthropods, though generally also unbalanced towards the easternmost sites (Fig. 4B,D). Arthropod density was exceptionally high within the Kiribati area, driven by a large presence of sessile barnacles, most of which were observed attached to dead sponge stalks.

Biodiversity

Overall taxa richness (gamma diversity)

A total of 632 invertebrate megafaunal morphotypes from 11 phyla were identified from imagery and specimens collected across the CCZ (including seamount areas). At the time of writing, these encompassed a total of: 198 echinoderms, 169 cnidarians, 121 sponges, 50 arthropods, 33 annelids, 12 ctenophores, 16 molluscs, 12 tunicates, 11 bryozoans, 5 hemichordates (class Enteropneusta), and 3 nemerteans. These are likely underestimates owing to poor image resolution, difficulty identifying fauna from images, and the presence of cryptic species. Furthermore, to ensure robust identification across imagery datasets, some of the catalogued morphotypes with complex taxonomic determination were combined into generic morphotypes (eg. sp to Genus spp), reducing the total number of effective taxa down to a total of 587 morphotypes. From these, a total of 351 morphotypes were observed in imagery collected in abyssal plain sites across the Std-datasets (Kiribati EEZ, APEIs 1, 4, 3, 6, 7; TOML B, C, D; BGR; and GSR areas), after exclusion of tube-living and shelled taxa (e.g. some of the annelid and gastropod mollusc taxa). The echinoderms (123), cnidarians (88), poriferans (83), and arthropods (28) were the most diverse phyla in the Std-datasets.

Variations in (alpha) diversity

Taxa richness, as obtained from Std-dataset bootstrap analyses, ranged between 14 and 67 morphotypes per c. 1000 m^2 of seabed across the CCZ (Fig. 5A). Western APEIs 1, 4 and 7 showed a comparably lower mean richness per unit of seabed, but this result was strongly driven by the lower faunal density found in these locations. TOML D area exhibited a substantially higher richness than the rest of the locations, partially driven by the much larger density in this area (i.e. in comparison with the rest of Std-datasets included in this analysis) but also resulting from the presence of 29 morphotypes (12 poriferans, 9 echinoderms, 3 tunicates, and 4 cnidarians) only found in this area (see beta-diversity section). In turn, no substantial variations were found across areas in Chao1 index (Fig. 5B) nor heterogeneity diversity metrics (Shannon and Simpson indexes; Fig. 5C-D), as these metrics smothered the differences observed in the richness-only assessment.

Morphotype accumulation patterns

Morphotype accumulation curves showed significant variations in taxa richness between some of the areas (Fig. 6A), some of which were only perceptible in sample sizes >10,000 m^2. For instance, while differences between the most taxon rich location (TOML B) and most of the other areas were statistically detectable in samples >2000 m^2, differences between western APEIs required samples >5000 m^2 to become perceptible, while detection of differences between TOML B and C required samples >10,000 m^2. However, individual-based assessments revealed a relatively similar taxa accumulation pattern in all the areas assessed, except for the APEI6 dataset, which exhibited a lower upper range in taxa accumulation (Fig. 6 C). Curves suggested that the total megafauna richness at the APEI6 was about 30% lower than in TOML D, the only comparable dataset to that collected at the APEI6 (both reach a plateau and have a comparable number of individuals in the sample).

Environmental controls

Megafaunal density and morphotype richness were investigated in relation to modeled nodule abundance and POC flux to the seafloor but the biological data were of insufficient ranges for statistically robust trends to be drawn in some cases (e.g. taxa richness obtained from Std-analyses, 8 points only), particularly given the coarse resolution of the environmental data grids (see below). Consequently, only preliminary trends were explored in this analysis.

Megafaunal density appeared to increase with increasing nodule abundance in the seabed (Fig. 7 A). We found a statistically significant correlation (Spearman's rank) between density and nodule availability in the meta-analysis data ($r_s = 0.60$, $p < 0.05$) although this correlation was non-significant when using only data from the Std-analysis ($r_s = 0.63$, $p > 0.05$).

Biogeography of megafauna across the CCZ

Exploration of both taxa abundance and beta-diversity patterns in this section was performed upon collation of pooled data available from all the Std-datasets, which presented the following issues: 1) some of the datasets greatly differed in the total sample size surveyed (e.g. Table 1), which may have conditioned the detectability (presence and absence) of taxa across areas, 2) many locations included in these analyses were represented by only a single – or very few – data points, sometimes from within a small area of seafloor targeted by sampling. Some data presented here may therefore not be representative of all habitats encompassed within each area. And 3) despite the use of a unified taxa catalogue, different annotators processed the image data, and only some of the datasets could be taxonomically cross-validated at the time of writing. For all these reasons, results presented in this section should be considered preliminary.

Taxa abundance and rarity

The majority of megafaunal morphotype distributions in the CCZ are poorly resolved, partly because there are a high number of morphotypes with only a handful of known occurrences. Morphotype abundance data across all Std-datasets (Kiribati; APEIs 1, 3, 4, 6 7; TOML sites B, C, D; GSR; and BGR areas) resulted in a rank abundance curve with an extremely long tail (Fig. 9); only 60 morphotypes (17 % of all taxa) accumulated more than 50 specimen detections, whereas 157 morphotypes (44 % of all taxa) accumulated only 3 or less. Note also that colors in Figure 9 bars are an artifact of the total sample size of each dataset. On the other hand, a much-reduced number of taxa appears to be sufficiently abundant across the CCZ to be encountered in several of the Std-dataset locations evaluated (Table 2). Distributions observed in the 22 selected morphotypes (e.g. all highly abundant and relatively easy to detect in seabed imagery) appear to be highly variable with no obvious patterns by phyla or mobility, with some morphotypes occurring from Kiribati to the east CCZ, and in both APEIs and contract areas (Table 2).

Beta-diversity (shared and unshared taxa)

The number of morphotypes shared between Std-dataset locations with sample sizes >4000 m^2 ranged from 22 to 118 (Figure 10), while the number of unshared morphotypes between these areas was similar (ranging from 15 to 102). The greatest numbers of shared morphotypes were between TOML areas, which were the most proximate areas, but also those with the largest sampling size. Morphotypes were shared to a lesser extent between TOML areas and APEI6, but these also had high numbers of unshared morphotypes. The TOML and Kiribati areas shared an intermediate number of morphotypes, despite their great separation. TOML D and Kiribati also exhibited the highest rate of unshared taxa, with 29 and 20 unshared morphotypes respectively, followed by TOML B (18 unshared taxa), APEI6 (11 unshared taxa), and APEI3 (9 unshared taxa). The Kiribati sampling area is proximate to APEIs 1, 4 and 7, but these areas had a high number of unshared morphotypes. APEI 7 did not share many morphotypes with other sites and had a high number of unshared morphotypes with other areas. Many (102) morphotypes were only found in a single location (Figure 10).

Comparison of APEIs and claim areas

Comparison of megafaunal diversity between the APEIs and mining claim areas is difficult because of the paucity of data generally and particularly from multiple claim areas. However, these preliminary data show that many morphotypes are only found in TOML-B/D and Kiribati areas (129) and are not represented in other areas or in the APEIs. A total of 25 morphotypes were only found in the APEIs, of which most were only found in a single APEI. Furthermore, taxa accumulation curves with combined data (Fig. 6 B,D) from APEIs 1,4, 6 and 7 (large spatial spread) showed a much reduced trend in total taxa richness than combined data from the rest of claim areas (relatively small spatial spread).

Taxa accumulation curves highlighted that the most reliable paired comparison of shared / not shared taxa across Std-datasets possible was between the TOML D and APEI6 datasets, given the higher representativity exhibited by the sufficiently large numbers of individuals surveyed in each of these datasets (Fig. 6C). This comparison is also highly relevant since the APEI6 is the spatially closest APEI to the TOML D area. From the total of 189 morphotypes found in the TOML D dataset, only 87 (46 %) were also found in the APEI6 dataset, and from the 119 morphotypes found at the APEI6, despite the inherently lower richness, 32 taxa (26 %) were not found in TOML D.

DISCUSSION

Synthesis of available invertebrate megafaunal data across the CCZ reveals that huge advances have been made in sampling the CCZ region. Our meta-analysis shows that a total of 481,797 m^2 of seabed from photographs and 7963 m of video transect has been evaluated quantitatively, most of this since 2013. Improvements in technology have meant that, with the exception of the 1979 OMCO photographic data, much of the older survey data had to be excluded from the quantitative analysis, mostly because of issues in standardizing the areal coverage. Our standardized image-dataset extends over 134,550 m^2 and includes 28,990 individual animals. The invertebrate megafauna have been evaluated in six of the nine APEIs. Despite this effort however, the total area surveyed represents only a tiny fraction of the 6,000,000 km^2 (Lodge et al., 2014) of seabed area in the CCZ (roughly equivalent to the area of a coin in a soccer field) and our evaluations may not represent the areas we aimed to assess. Despite these limitations, our analyses demonstrate spatial structure in the invertebrate megafauna at regional scales (also suggested by Vanreusel et al., 2016), in addition to the landscape scale patterns (Simon-Lledó et al., 2019a) and fine scale patterns with nodule abundances (Simon-Lledó et al., 2019b) already documented.

Environmental Controls

Morphotype richness appeared to slightly increase with increasing nodule abundance (Fig. 7 C), but this relationship was weak and not statistically significant ($r_s = 0.47$, $p > 0.05$). However, nodule abundance can be extremely patchy; abrupt changes (in < 50 m) from high coverage to nodule-free patches are common in the CCZ seabed (see e.g: Peukert et al. 2018; Simon-Lledó et al. 2019b), but the nodule data used here was modelled at a coarse resolution (1 km^2) based on relatively sparse input data, and therefore did not reflect local-scale variability. Comparisons based on directly measured nodule abundance from seabed photographs to megafaunal density, also at regional scale, may be best suited to explore such patterns.

Nodule cover has previously been shown to be an important factor in the structuring of megafaunal communities (Simon-Lledó et al. 2019b), as some taxa are known to require nodule habitat (Vanreusel et al. 2016, Amon et al 2016). However, these patterns are not necessarily linear. Simon-Lledó et al (2019b) showed how local megafaunal densities increased describing a rapid asymptote (i.e. stabilising in mid-low nodule abundance levels) over a gradient of nodule coverage (Figure 8). This study also showed that local taxa richness appeared to be invariable across the nodule gradient unless this metric was calculated upon fixed-areal sampling units, as this effectively incorporated faunal density as a factor in the calculation of taxa richness (e.g. see differences between taxa richness and taxa density in Simon-Lledó et al (2019b; Fig. 8).

No clear relationships between POC flux and either megafaunal density or morphotype richness were found (Fig. 7 B,D). Again, this may be related to an issue of scale, since the POC data is integrated over several years and modelled across the CCZ without spatially extensive sampling in this area. Furthermore, megabenthic biomass has not been investigated, and this parameter is typically related to food supply (e.g. Billett et al. 2010). The density and diversity of megabenthic fauna are known to vary at interannual timescales in relation to POC flux at other abyssal sites (e.g. Smith et al. 2009; Ruhl and Smith 2004), and patterns with megafaunal community parameters in the CCZ may emerge if measured POC (or sediment community oxygen consumption, SCOC) at the sites is employed in the comparison, rather than coarse-resolution modelled data.

Biogeography

The geographic distribution of megafaunal morphotypes is not consistent. Many morphotypes show broad geographic ranges across the CCZ, which is supported by high connectivity in common taxa (Taboada et al. 2018). However, some distinctive taxa display clear east / west differentiation. There are also considerable differences in the occurrence distribution of common morphotypes between sites (Table 2). At the phylum level (at least the 4 main phyla examined), there are clear differences in relative composition and densities between sites (Figure 4).

Although for the purpose of this regional synthesis only megafauna data collected in abyssal plain habitats were assessed, the landscapes of the CCZ are punctuated by seamounts and smaller bathymetric features such as abyssal hills, ridges and troughs (Harris et al. 2014; Simon-Lledó et al. 2019a), which provide a variety of habitats. Seamount summits investigated in BGR, GSR and APEI3 areas showed differences in megafaunal composition when compared to nodule fields, and thus appear to represent a unique habitat (Cuvelier et al. in review).

Biodiversity

No regional syntheses of invertebrate megafauna are available for abyssal plains to compare to the CCZ. However, the biodiversity of the invertebrate megafauna assessed in the CCZ (632 from catalogue) is considerably higher than other extensively evaluated abyssal plain sites. Even the species richness of most individual sites exceeds morphotype counts for a long-term monitoring station in the eastern Pacific, Station M (102 taxa; Kuhnz et al., 2014), and the Porcupine Abyssal Plain in the northeast Atlantic (43 morphotypes; Durden et al., 2015). Biodiversity patterns across the CCZ are not obvious. There was no clear correlation with any environmental drivers evaluated here, although there are scale-mismatches between datasets.

APEIs

The density of taxa is thought to be lower in APEIs across the CCZ (Vanreusel et al., 2016). Our data provide some weak support for this, although the variation in density from west to east of the CCZ is much greater in magnitude. There appear to be fewer megafaunal taxa observed at the APEI sites than the contractor areas, when standardized by area or individuals, and there are relatively few shared morphotypes between contractor areas and the nearby APEIs. Comparable patterns of beta-diversity are poorly known in the deep sea, most studies focus on depth-related patterns. APEIs appear to contain fewer nodules (and smaller nodules in many cases), which may explain the differences in megafaunal density and diversity (Simon-Lledó et al 2019b), although further study is needed.

Possible temporal patterns

Two long-term (~30 year) time-series locations on abyssal plains provide useful temporal context for our spatial analysis of the CCZ region: the Porcupine Abyssal Plain in the northeast Atlantic (4850 m water depth) and Station M in the eastern Pacific (4000 m water depth). Significant variations in the megafaunal communities at these two sites have been observed over time. Total density at each site ranged over an order of magnitude, and the densities of some morphotypes changed by three orders of magnitude (Billett et al 2010; Kuhnz et al. 2014). Diversity also varied inter-annually. At Station M, dominance changed over time, both in terms of the identity and order of magnitude changes to the density of the most dominant fauna. The combination of such large changes in both total densities and individual densities with alterations to diversity resulted in major changes to the community structure. Such large temporal variations in density, diversity and community structure suggest that potential temporal variation should be evaluated in the CCZ, to ensure that the spatial differences in densities found in this regional analysis (which range from ~0 to 0.6 ind m^{-2}, less than an order of magnitude) are not confounded by temporal variation.

Qualifications and caveats

There are a number of important qualifications and caveats associated with the data used in this work.

- Many of the data are preliminary and may not be extensively error-checked.
- More detailed data checking and analysis is planned prior to subsequent publication in the scientific literature.
- The number of transects and images were low in some cases.
- There may be differences in the levels of detection and identification between studies using different annotators.
- The fauna are not well known taxonomically and identifications may be preliminary
- The use of morphospecies may over or underestimate true biodiversity as well as impact our assessment of community patterns and biogeography

Gaps

The megafaunal data synthesis identified a number of data gaps:

1) There is a paucity of data from the contract areas located in the central and west CCZ, as well as APEIs 2, 5, and 8.
2) While there are a large number of seamount features located across the CCZ, there have been very little data collected from these habitats (both summits and flanks), despite indications of limited faunal overlap and connectivity with abyssal areas. This prevents conclusions being drawn about the potential use of seamounts as refuge areas. Based on the possible uniqueness of their inhabiting fauna, seamounts should be considered within the CCZ environmental management plan.
3) Temporal data are also severely lacking from the CCZ, and as a result the level of natural variability and fluctuations in morphotype composition over time cannot currently be evaluated.
4) The image data are from a small number of transects. While these transects can be long, and cover a considerable area, there needs to be more image stations in different directions and across different substrate/topography to improve our characterization of megafaunal diversity and distribution. The current analysis was limited by insufficient data on substrate type (e.g., nodule cover) for a number of data sets.
5) The functional role of megafauna in the ecosystem is not well quantified. Trophic dynamics are poorly known. There is limited information on interactions between megafauna and other size classes, it should be considered that there may be ontogenetic movements of taxa through the size classes.

Conclusions

- Spatial variability is relatively high between sites across the CCZ. There are considerable differences in the distribution of common morphotypes between sites (Table 2). At the phylum level (at least the 4 main phyla examined), there are clear differences in relative composition and densities between sites (Fig 6). There is also high spatial variability at finer spatial scales.
- Temporal variability could not be addressed but is known from other abyssal plain sites to be relatively high on annual scales.
- There is a trend of increasing density from west to east across the CCZ, with comparatively low density levels in the western and central CCZ and high in the eastern areas (Fig 3, Fig 4).
- There are many rare morphotypes, reported to date as singletons or in small numbers (< 3 occurrences). This could be in part an artefact of the low density of taxa characteristic of the CCZ, coupled with insufficiently large sample sizes collected in image-based assessments (see egs: Simon-Lledó et al 2019a; Ardon et al 2019), and thus the real level of rarity or endemism is still uncertain in most areas investigated.
- Overall, although morphotype composition varies, morphotype diversity and evenness are consistent between sites across the EEZ (Fig 5). Morphotype rarefaction curves (Fig 6) indicate that the rate of discovery of morphotypes is lower in APEIs than in other areas, and that morphotype composition is not fully described for all sites across the CCZ.
- There are over 50 shared morphotypes between the most distant sites (APEI6 and Kiribati, 5000 km). There are always at least 20 morphotypes shared between sites surveyed with sample sizes > 4000 m^2 (Fig 10). However, many morphotypes are only found at one site, particularly in the TOML B/D and Kiribati areas.

- Our ability to compare between APEIs and adjacent contract areas is limited by sampling. APEIs 1, 4 and 7 have no nearby sites for comparison. APEI6 has nearly 90 morphotypes in common with TOML D sites and many morphotypes in common with UK-1.
- Nodule cover has been shown in published studies to be an important determinant of megafaunal composition and abundance (Fig 8). It is a key environmental driver for invertebrate megafauna. The modelled nodule data show a weak relationship with megafaunal morphotype richness and density. Individual morphotypes can be exclusively found on nodules. We would like to compare nodule counts from photographs to megafaunal counts directly to look for broader patterns but have not yet done this.
- There is no clear relationship between any megafaunal community parameters and POC flux to the seafloor (Fig 7). Patterns may emerge if we use measured POC (or sediment community oxygen consumption) for the sites rather than coarse resolution modelled data. We have also not investigated biomass, which is typically more closely related to food supply.

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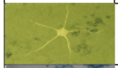
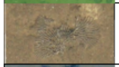
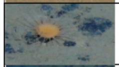
Tables

Table 1: Summary of invertebrate megafaunal data available from the Clarion Clipperton Zone. (S) indicates data used in the standardized assessment. Asterisk indicates that the dataset is based on video transect data. Data from APEIs are highlighted in bold.

Area	**Transects (n)**	**Area sampled (m^2)**	**Source**	**Reference, DOI**
Kiribati (s)	6	14,666	Nautilus Minerals / NOC	Simon-Lledó et al. 2019b, 10.3389/fmars.2019.00605
APEI1 (s)	2	6,539	Univ. Hawaii	Durden, In prep
APEI4 (s)	2	9,139	Univ. Hawaii	Durden, In prep
APEI7 (s)	2	7,021	Univ. Hawaii	Durden, In prep
OMCO-B	2	1,599	UK Seabed Res. / Univ. Plymouth	McQuaid, In prep
OMCO-A	1	800	UK Seabed Res. / Univ. Plymouth	McQuaid, In prep
OMCO-C	1	802	UK Seabed Res. / Univ. Plymouth	McQuaid, In prep
TOML-B (s)	4	24,955	Nautilus Minerals / NOC	Simon-Lledó, In prep
KODOS 2018	7	156,348	KIOST	In prep
KODOS 2019	5	131,064	KIOST	In prep
OMCO-D	1	800	UK Seabed Res. / Univ. Plymouth	McQuaid, In prep
TOML-C (s)	4	29,246	Nautilus Minerals / NOC	Simon-Lledó, In prep
OMCO-E	1	801	UK Seabed Res. / Univ. Plymouth	McQuaid, In prep
OMCO-F	2	1,600	UK Seabed Res. / Univ. Plymouth	McQuaid, In prep
APEI3 (s)	2	*3,300 m	MARE/IMAR/Okeanos-Univ. Azores	Cuvelier, in review, 10.5194/bg-2019-304 initial evaluation in Vanreusel et al. 2016
APEI9	3	52,632	KIOST	In prep
OMCO-G	1	799	UK Seabed Res. / Univ. Plymouth	McQuaid, In prep
TOML-D (s)	3	20,200	Nautilus Minerals / NOC	Simon-Lledó, In prep
GSR (s)	2	*1,200 m	MARE/IMAR/Okeanos-Univ. Azores	Cuvelier, in review, 10.5194/bg-2019-304 initial evaluation in Vanreusel et al. 2016
GSR	4	860	GSR (from Patania-I) / U.	unpublished

			Ghent	
APEI6 (s)	12	18,582	NOC	Simon-Lledó et al. 2019b 10.1002/lno.11157
BGR	1	*1,600 m	MARE/IMAR/Okeanos-Univ. Azores	Cuvelier, in review, 10.5194/bg-2019-304 initial evaluation in Vanreusel et al. 2016
UK-1 (s)	4	4,204	NHM/Univ. Hawaii	Amon et al. 2016, 10.1038/srep30492

Table 2: Presence of characteristic species (e.g. most abundant and identifiable with the most confidence from imagery) across Std-sites from west to east in the CCZ. M=mobile, S=sessile. X denotes presence.

	MOBILE/ SESSILE	KIRIBATI EEZ	APEI 1	APEI 4	APEI 7	TOML B	TOML C	APEI 3	TOML D	GSR	APEI 6	BGR	UK 1
	M	X	X			X	X	X	X	X	X	X	X
	M		X			X	X		X	X	X	X	X
	S		X	X		X	X		X	X	X	X	X
	M	X	X	X		X	X		X	X	X		
	S	X				X	X	X	X	X	X	X	X
	M	X	X	X	X	X	X	X	X	X	X	X	X
	S						X		X	X	X		X
	S	X	X	X	X	X	X	X	X	X	X		X
	M	X				X	X	X			X		
	S					X			X		X	X	X
	S	X	X	X	X	X	X	X	X		X		X
	S		X			X	X		X		X		X
	S		X			X	X		X		X		X
	M	X				X	X		X				
	S	X	X	X	X	X	X	X	X	X	X	X	X
	S		X	X		X			X		X		X
	S					X	X		X		X		X
	M	X	X	X	X	X	X		X	X	X	X	X
	S	X	X		X	X	X		X		X		X
	S	X	X	X									
	S				X						X		X
	S	X	X	X		X	X	X	X		X		X

Figures

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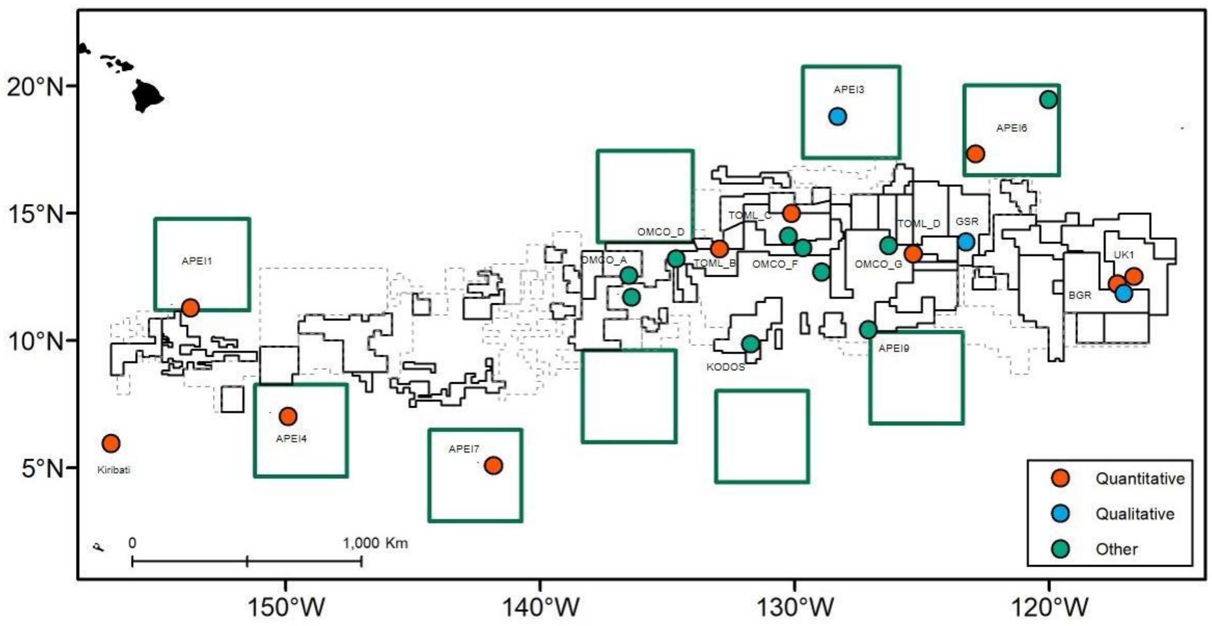

Figure 1: Map of sites from where invertebrate megafaunal data were available for this study from the Clarion Clipperton Zone. The standardized datasets are locations depicted in orange, while the "meta-analysis" set includes some of the other (green) and qualitative (blue) sites.

Figure 2: Megafaunal taxa recorded in the CCZ. Images include examples of the **Porifera**: a) *Saccocalyx* sp. and b) *Sympagella clippertonae*; the **Cnidaria**: c) a sea anemone (Actiniaria), d) cf. *Relicanthus* sp. from a newly erected cnidarian order, e) a soft coral (Alcyonacea, closer view of polyps in inset); the **Echinodermata**: f) a stalked crinoid, g) the ophiuroid *Ophiosphalma glabrum* (oral view), h) an asteroid (Pterasteridae), several holothurians i) *Psychropotes longicauda*, j) *Psychropotes semperiana*, and k) *Peniagone* cf. *leander*; the **Arthropoda**: l) an isopod (Munnopsidae), m) the decapod *Hemipenaeus* cf. *spinidorsalis*; the **Mollusca**: n) the cirrate octopus *Cirroteuthis* sp. and; the **Hemichordata**: o) an acorn worm (Enteropneusta). Image credits: *a,c,e,f,h,l*- SO239, ROV Kiel 6000 (GEOMAR Helmholtz Centre

for Ocean Research Kiel); *b*- Herzog et al 2018; *d,j*- Smith & Amon (University of Hawai'i and Natural History Museum London, ABYSSLINE Project); *g*- Laming (CESAM, REDEEM project); *i*- Durden & Smith (University of Hawai'i, DeepCCZ Project); *k,n,o*- Ju (Korea Institute of Ocean Science & Technology); *m*- Drazen (University of Hawai'i, DeepCCZ).

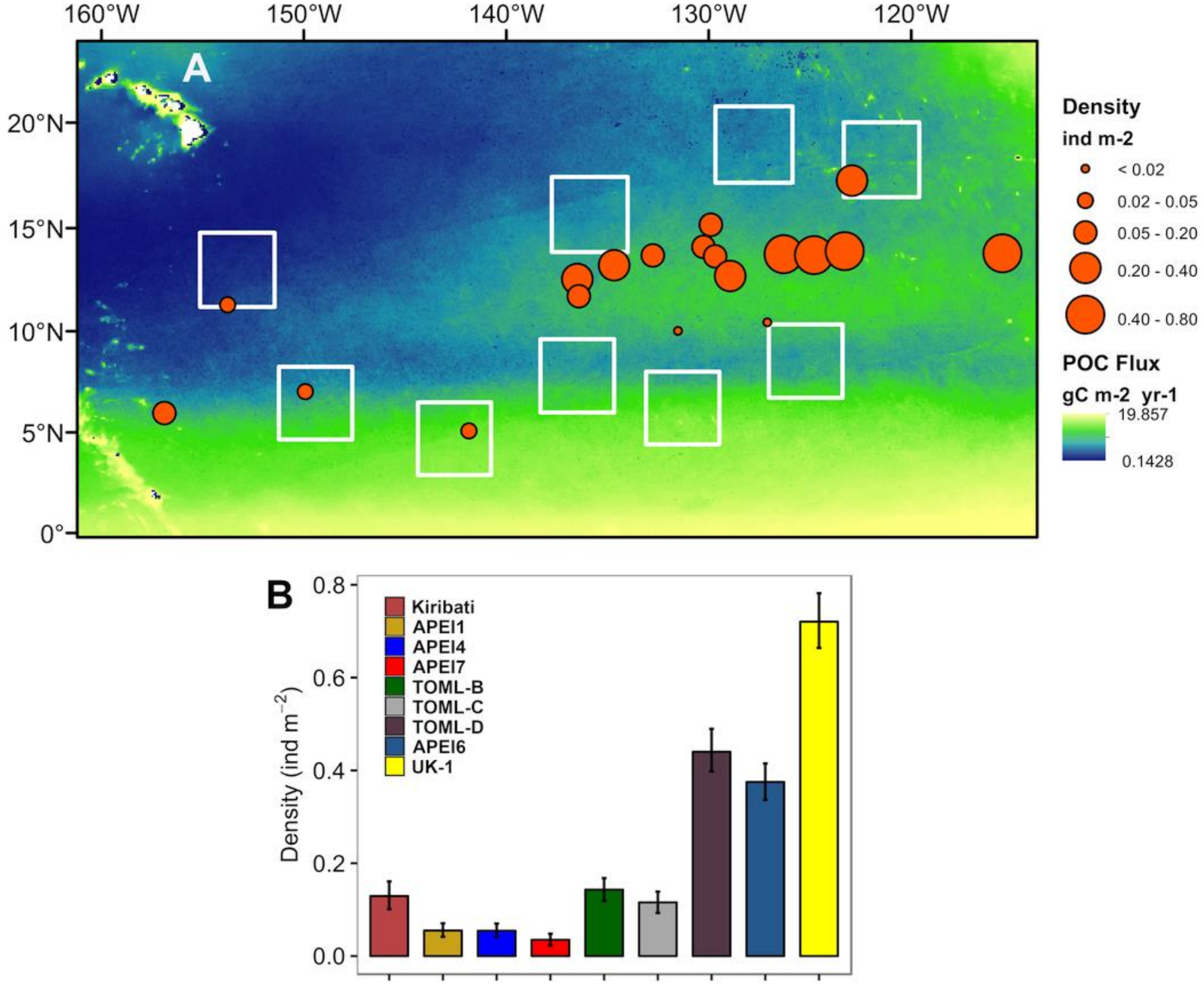

Figure 3: Variations in standing stock, i.e. megafaunal density, across the CCZ. **A)** Bubble plot of megafaunal density in each of the meta-analysis datasets showing POC flux to the seafloor (Lutz et al., 2007). **B)** Megafaunal density in each of the Std-datasets, ordered west to east in the x-axis. Bars indicate mean density calculated from the bootstrap-like sample (c. 1000 m^2) set (n=1000) generated for each area. Error bars represent 95% confidence intervals across randomizations.

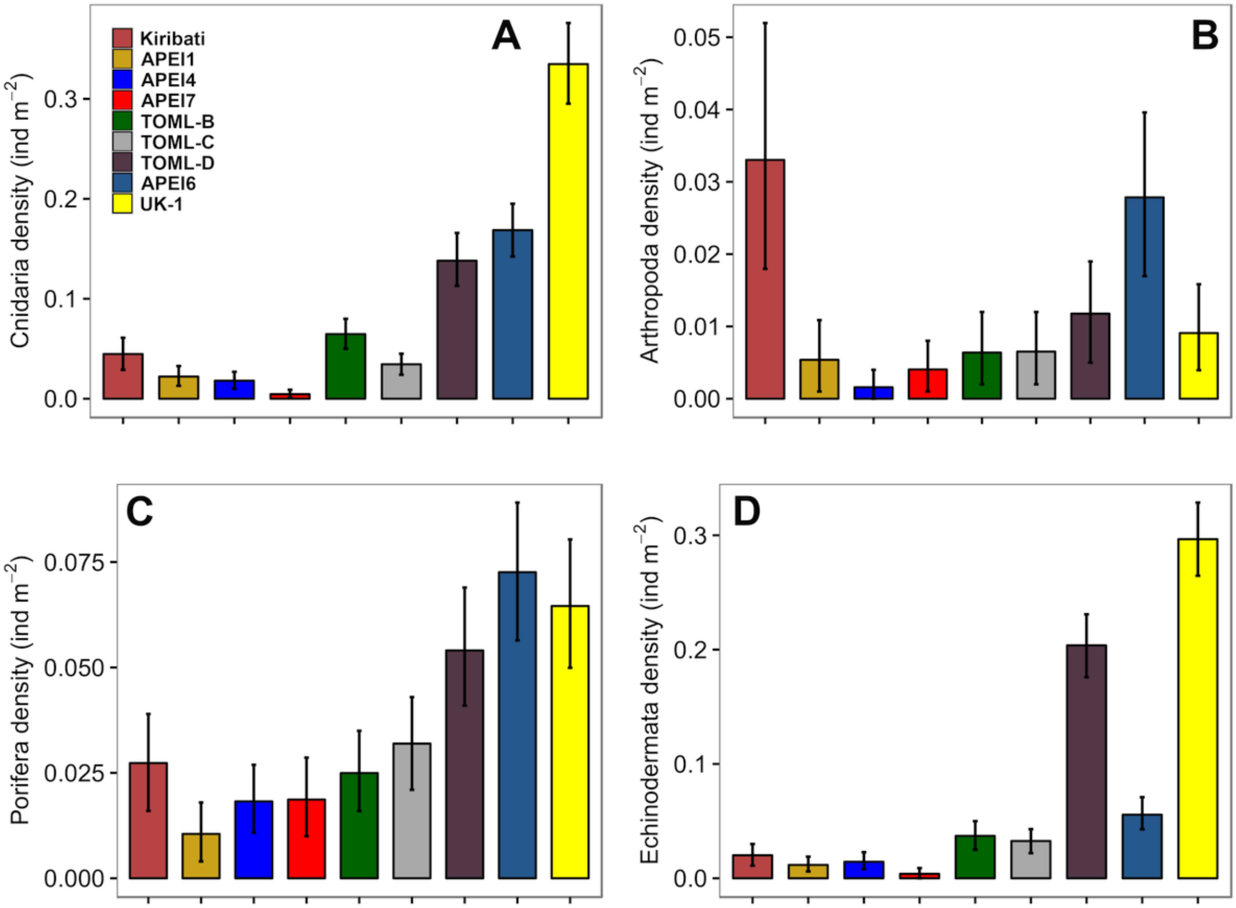

Figure 4: Variations in megafaunal density of the most abundant metazoan taxonomic groups in CCZ megafauna across the Std-dataset locations ordered west to east in the x-axis. Note the different ranges of the y-axis in each panel. Bars indicate mean density in each of the Std-datasets calculated from the bootstrap-like sample (c. 1000 m^2) set (n=1000) generated for each area. Error bars represent 95% confidence intervals across randomizations. **(A)** Cnidaria density. **(B)** Arthropoda density. **(C)** Porifera density. **(D)** Echinodermata density.

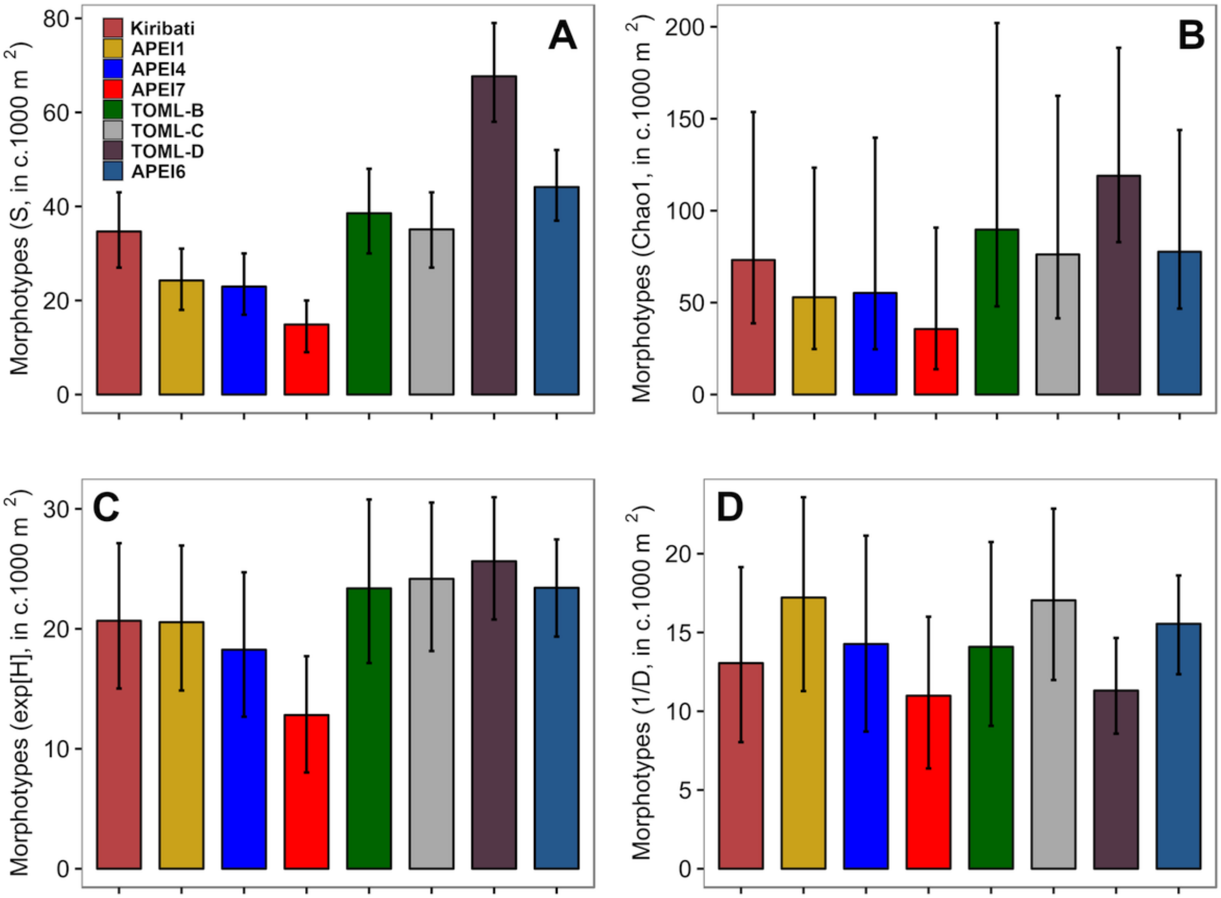

Figure 5: Variations in megafaunal diversity across the Std-dataset locations. Ordered west to east in the x-axis. Bars indicate mean density in each of the Std-datasets calculated from the bootstrap-like sample (c. 1000 m^2) set (n=1000) generated for each area. Error bars represent 95% confidence intervals across randomizations. Note that Shannon and Simpson indices have been transformed to match the units of the other indices (effective numbers of taxa). **(A)** Morphotype richness. **(B)** Chao1 index. **(C)** Exponential Shannon index. **(D)** Inverse Simpson index.

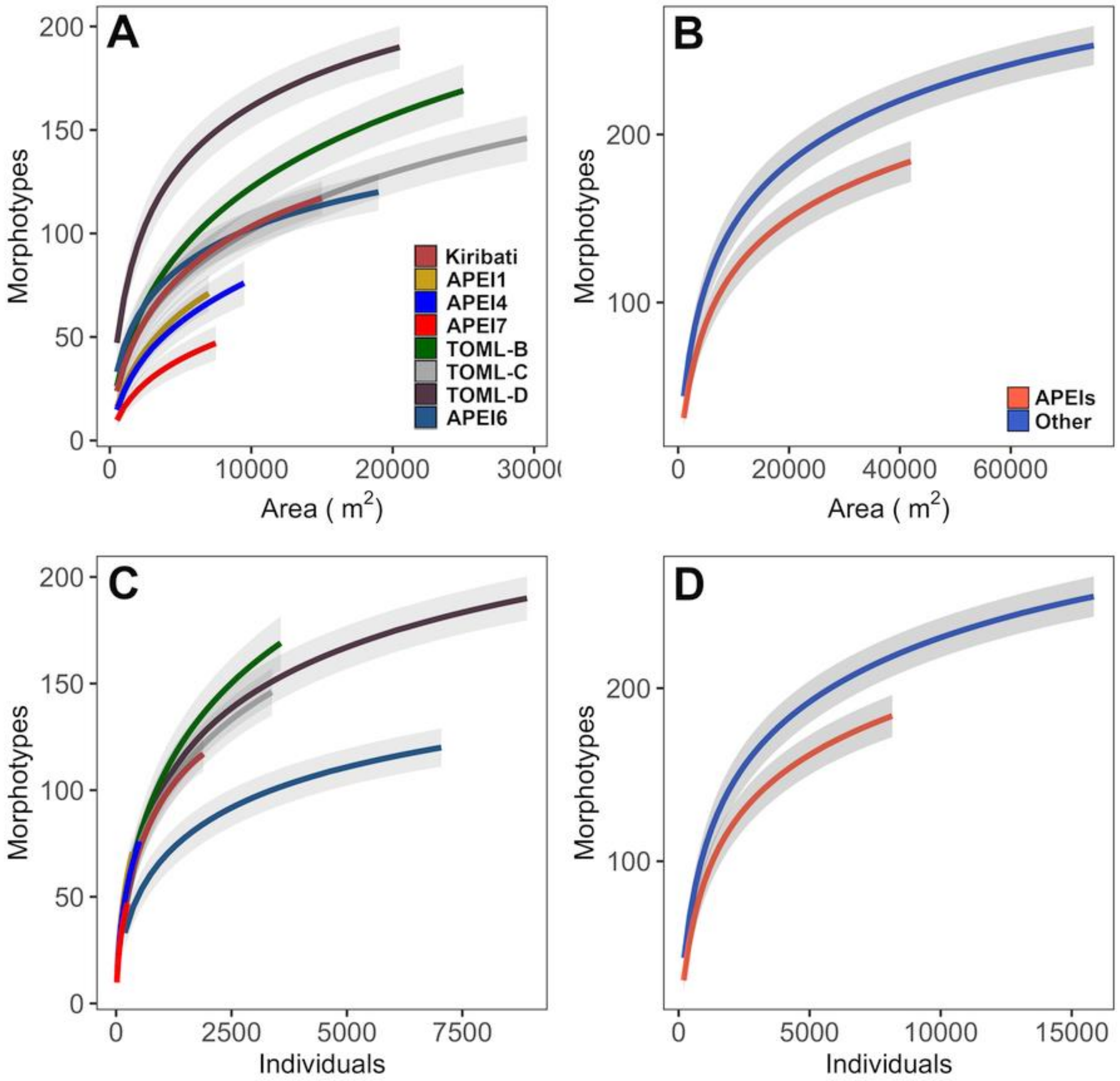

Figure 6: Rarefied morphotypes accumulation curves calculated across and combining Std-datasets. Curves were calculated as a function of the seabed area (A-B) or the number of individuals encompassed (C-D) in image-based sampling. Lines represent mean values across the 100 randomizations performed at each sample unit size increase, for each separate Std-dataset location (A and C) and combined for all APEIs and rest of locations within the CCZ (B and D) and hence excluding the Kiribati EEZ dataset. Shadowing representing 95% confidence intervals.

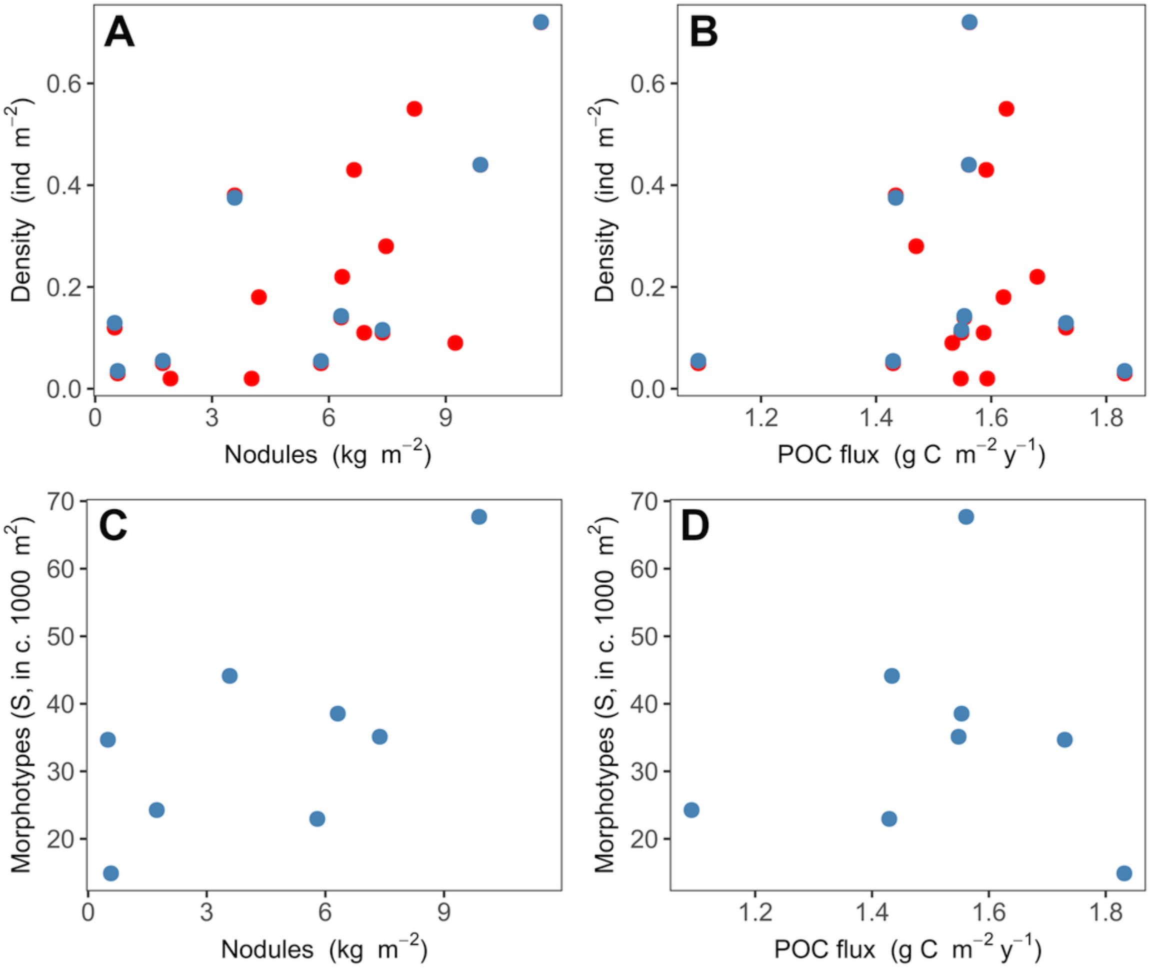

Figure 7: Relationships between megafaunal density and morphotype richness with modelled nodule abundance (ISA 2010, interpolated) and particulate organic carbon (POC) flux to the seabed (Lutz et al. 2007, interpolated). *Red dots*: results from meta-analysis: *blue dots*: results from bootstrap analysis on Std-datasets. (A) Mean megafaunal density along a spectrum of seabed-nodule abundance. (B) Mean megafaunal density along a spectrum of POC flux to the seabed. (C) Mean megafauna taxa richness along a spectrum of seabed-nodule abundance. (B) Mean megafauna taxa richness along a spectrum of POC flux to the seabed.

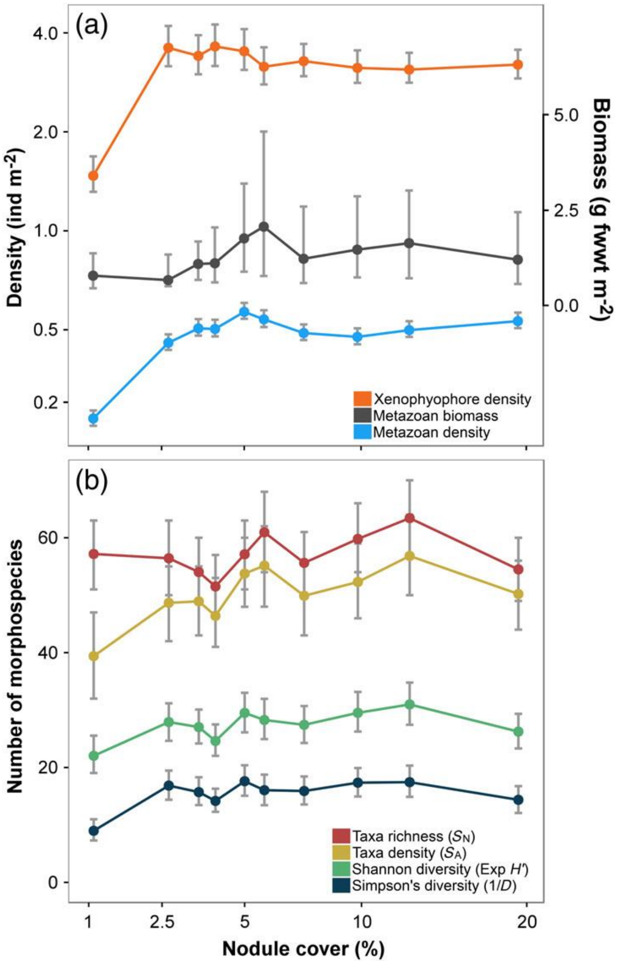

Figure 8: Variation in (a) standing stock and (b) diversity with seafloor nodule cover. Points indicate mean (median for metazoan biomass) values of each parameter calculated from each nodule-cover class, error bars represent 95% confidence intervals. (a) Numerical density of metazoans and xenophyophores (left axis) and metazoan biomass density (right axis). (b) Metazoan diversity measures: morphotype richness (SN), morphotype density (SA), exponential Shannon index (exp H0), and inverse Simpson's index (1/D). From Simon-Lledó et al., 2019b.

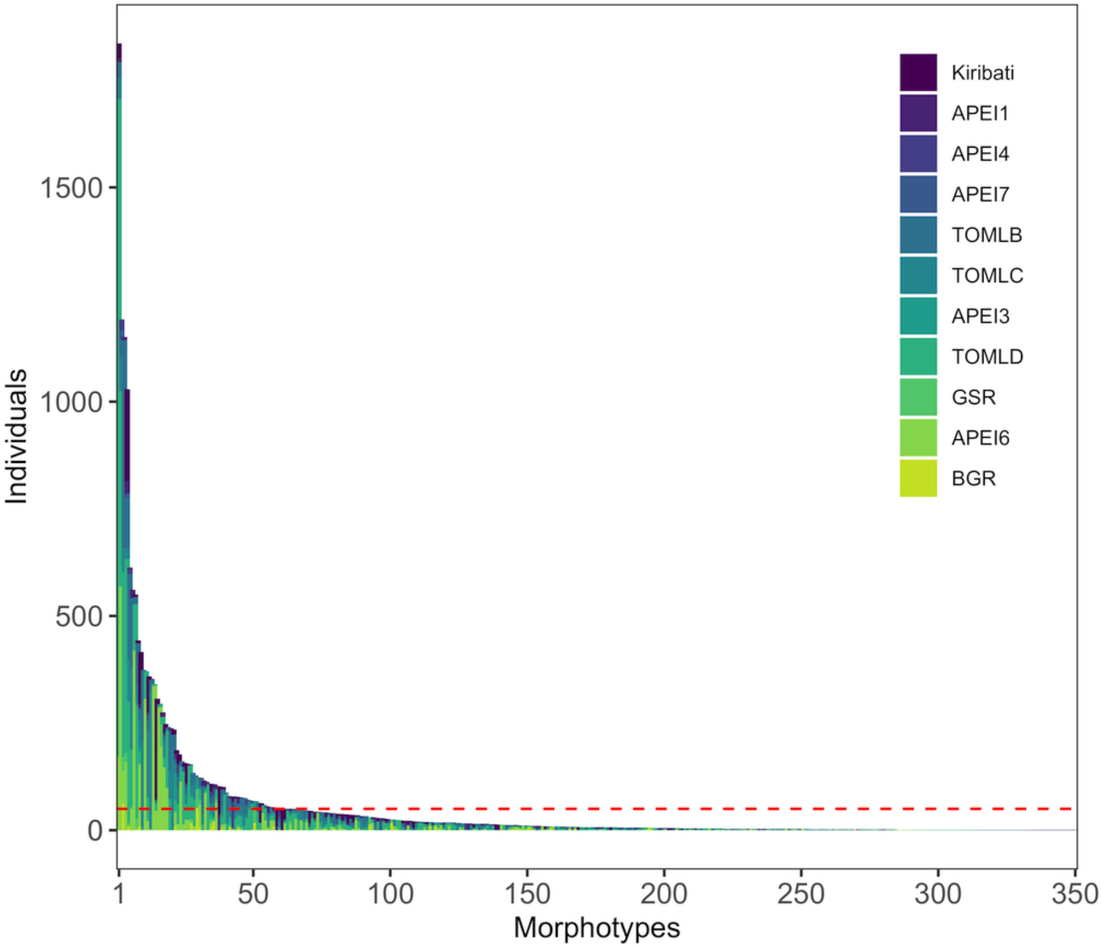

Figure 9: Ranked abundance plot combining all the morphotype abundance data from the Std-datasets. Red dashed line represents 50 occurrences and data locations ordered from west to east (in legend: top to bottom). Note that this ordination combines total pooled data from datasets differing in the total seabed area coverage surveyed.

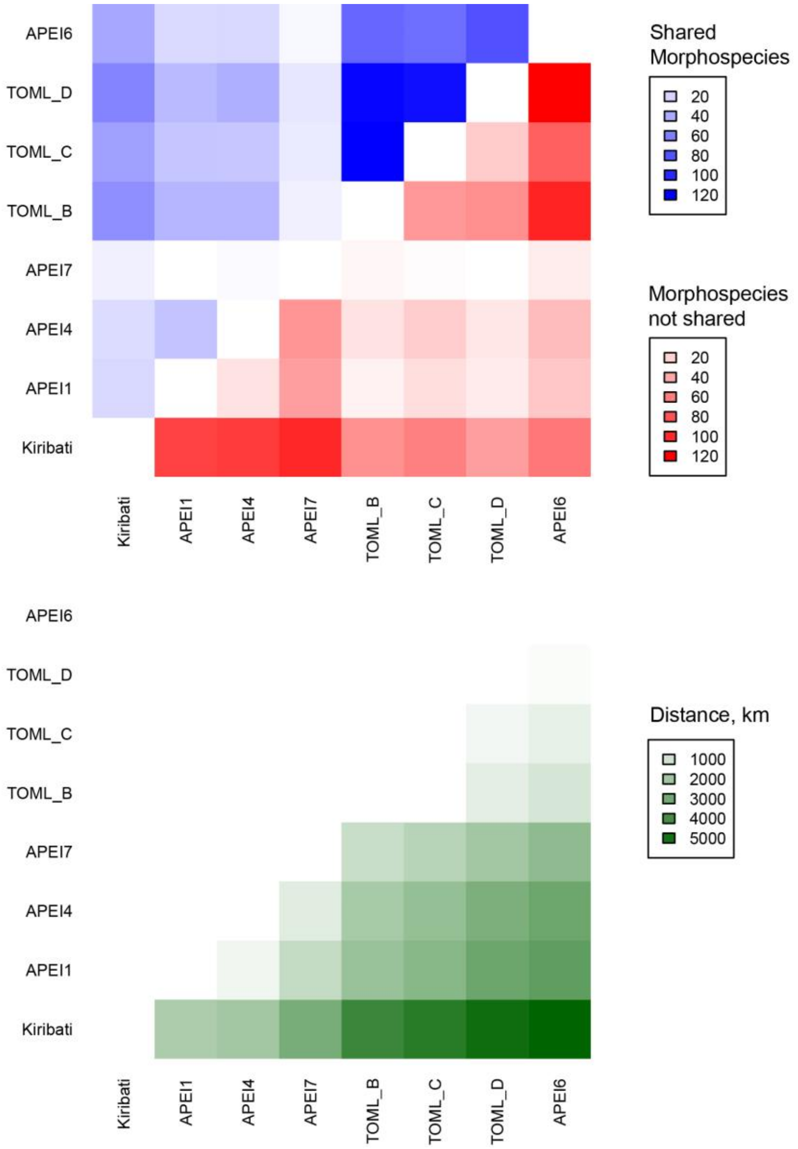

Figure 10: Heat map of shared and unshared morphotypes between Std-dataset locations with sample sizes > 4000 m^2. Note that this classification combines total pooled data from datasets differing in the total seabed area coverage surveyed.

f. FISHES AND SCAVENGERS –

Fishes and Scavengers Working Group Report

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Introduction

Top trophic level animals can have important influences on communities and ecosystems. They have been shown to control prey biomass or abundance, exert selective pressures, and alter the behavior and habitat choices of potential prey (Drazen and Sutton, 2017; Estes *et al.*, 2011; Myers *et al.*, 2007; Polovina *et al.*, 2009). Therefore, it is vital to include studies on the abundance, diversity, and community structure of top predators when conducting baseline surveys, assessing possible environmental impacts of mining activities, and planning spatial management strategies. Top predators are also highly susceptible to both direct and indirect anthropogenic changes, particularly habitat alteration, because many have large habitat requirements (Fosså *et al.*, 2002; National Research Council, 2002). For example, changes in the abundance, composition, and size distribution of large fishes and invertebrates have been documented in response to direct activities such as fishing and to indirect activities such as habitat alteration (Jennings and Kaiser, 1998; Morato *et al.*, 2006). In abyssal regions including the Clarion-Clipperton Zone most of the top predators are large mobile fishes, shrimps, and amphipods (Fig. 1), and the majority of these are also opportunistic scavengers. Because top predators are highly mobile and sensitive to light, noise, and other vibrations they can be a challenge to study with remotely operated vehicles (ROVs) and to some extent also autonomous underwater vehicles (AUVs) because they tend to avoid the vehicles well before they are imaged. For example, in an extensive underwater survey of the Porcupine Abyssal Plain in the Atlantic, only 0.29% of images from oblique camera surveys and only 0.11% of downward looking images contained fish (Milligan *et al.*, 2016). This can lead to underestimations of top predator densities, especially when less than 100,000 images are available for analysis in an area (yielding only ~100 images with individuals) (Durden *et al.* 2016, Trenkel *et al.* 2004). Therefore, it is most useful to combine ROV or AUV visual survey techniques with baited camera techniques that can attract these elusive animals to the camera system where they can be quantified (Durden *et al.* 2016, Leitner *et al.* 2017). In this report we synthesize and analyze data from both visual camera transecting approaches and baited camera studies to evaluate the diversity, abundance, distribution and community structure of fishes and scavengers across the CCZ region.

Data sources and methodology

There are limited data for fishes and scavengers in the CCZ. Two primary data sources were available. Baited camera systems have been used to census fish populations from shallow to deep water and to evaluate the diversity and efficacy of spatial management plans (Bailey *et al.*, 2007; Priede and Bagley, 2000; Sackett *et al.*, 2017). Baited cameras are deployed as free vehicle moorings for short (12 – 48 hrs) deployments to the seabed. Bait attracts fishes and scavengers which are mobile and can generally avoid transecting equipment, to the area in front of the camera for a census. Still photographs or video clips are recorded in time lapse over the duration of the deployment from either an overhead vertical position or a horizontal or oblique position. The latter is much better for identification of the attracted fauna.

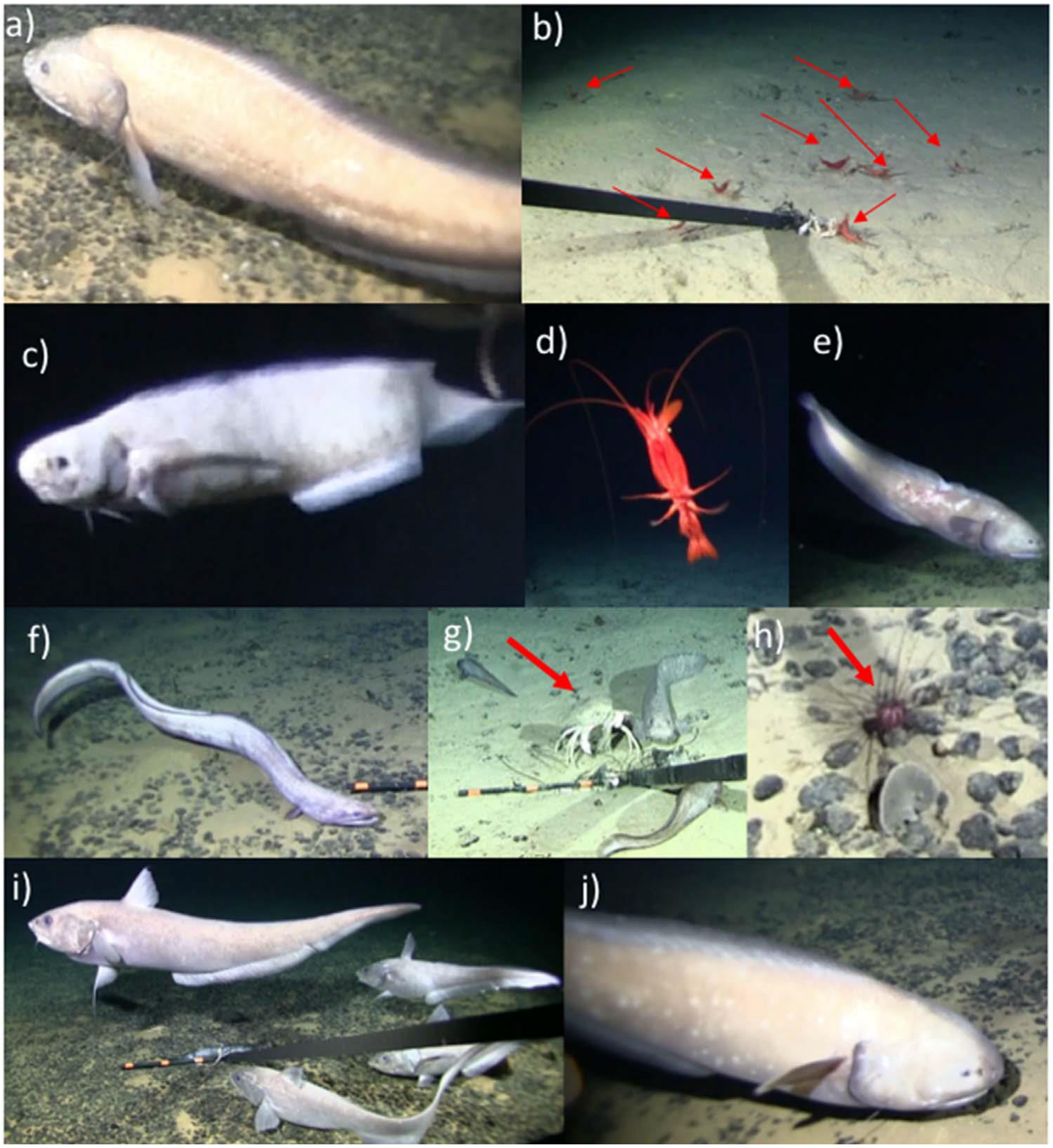

Figure 1. Representative photographs of eastern CCZ scavengers reproduced from Leitner et al (2017). a) *Bathyonus caudalis* b) group of *Hymenopenaeus nereus* c) *Barathrites iris* d) *Plesiopenaeus armatus* e) *Bassozetus* sp. f) *Histiobranchus bathybius* g) *Munnidopsis* sp. (arrow) and three zoarcids including *Pachycara nazca* h) *Plesiodiadema* sp. (arrow) and plate like Xenophyophore i) *Coryphaenoides armatus/yaquinae*. j) *Bassozetus* sp. light morphotype.

Given the mobile nature of the abyssal fish and scavenger fauna, all the baited camera deployments available across the Pacific Ocean, including those in the CCZ, were assembled (Table 1; Fig. 2). Data were gathered from the literature and by contribution of raw data by lead scientists of past studies. A total of 12 studies representing 157 deployments were compiled (see Table 1). Three studies representing 43 deployments were directly from the CCZ region: OMS and UK1 (Leitner *et al.*, 2017), APEIs 1,4, 6 and 7 (Leitner *et al.* 2017; Leitner and Drazen, unpublished data) and BGR (Harbour and Sweetman, unpublished data). The remainder ranged from New Zealand to California and south to the Peru Basin (DISCOL Site). This geographic coverage will enable evaluation of biogeographies of these very mobile animals. Data included camera viewing angle and area, duration of deployment and sampling interval to facilitate sample intercomparison. Only studies that occurred at abyssal depths (3000-6000 m) and presented the maximum number of animals visible during deployment (MaxN) were included. Many studies also presented the time of first arrival for each taxon, though our current analysis did not evaluate it. Both metrics have been used as indicators of relative abundance and enable estimation of diversity and community composition.

Table 1. Summary of available published and unpublished baited camera datasets from across the Pacific Ocean between 3000-6000 m depth (abyssal) by region and data source. # of camera deployments illustrates the samples evaluated/samples used in analysis after methodological standardization.

Region	**Data Source**	**# camera deployments**
BGR	Sweetman et al unpublished	10
OMS	Leitner et al. (2017)	6
UK1	Leitner et al. (2017)	6
western CCZ	Leitner et al. (2017)	2
APEI1	Leitner et al. unpublished	7
APEI4	Leitner et al. unpublished	7
APEI6	Leitner et al. (2017)	1
APEI7	Leitner et al. unpublished	4
CCZ total		**43**
California, Sta. F	Priede et al. (1990)	14/0
California, Sta. M	Priede et al. (1994)	11/0
Central California	Yeh and Drazen (2011)	3/2
central North Pacific Gyre	Priede and Smith (1986)	6/0
	Priede et al. 1990	9/0
DISCOL	Drazen et al. (2019)	6
Hawaiian Islands - Main	Leitner et al. unpublished	2
	Yeh and Drazen (2009)	3
Hawaiian Islands - Northwestern	Yeh and Drazen (2009)	4/3
	Drazen, unpublished	1
Kermadec	Jamieson et al. (2009)	1/0
	Jamieson et al. (2011)	4/0
	Linley et al. (2017)	28/26
Marianas	Jamieson et al. 2009	2/0
	Linley et al. (2017)	10
New Hebrides	Linley et al. (2017)	7/6
South Fiji Basin	Linley et al. (2017)	4
Pacific wide total		157/**107**

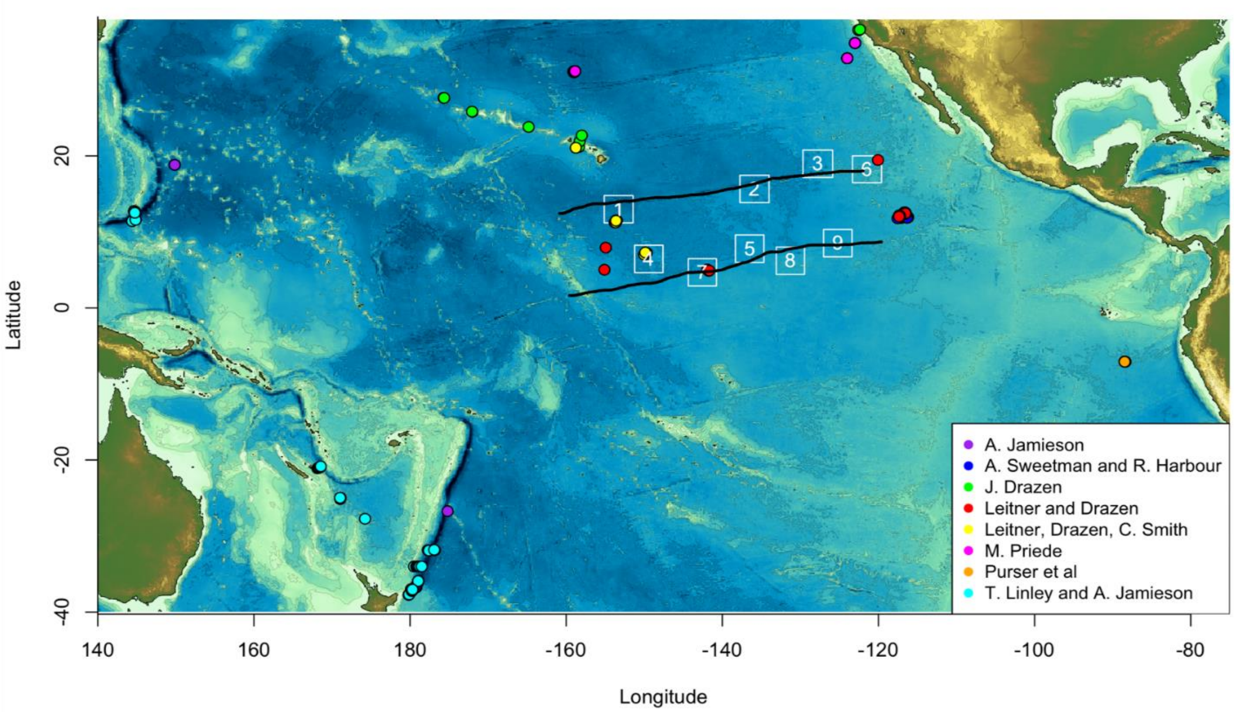

Figure 2. Baited camera sample locations across the Pacific and CCZ region by study.

These data were further evaluated and standardized. Considerable effort was applied to ensure that all of the data used the same taxonomy and identified taxa consistently. A number of earlier studies from off California and in the central North Pacific had been conducted for purposes other than biodiversity assessment and taxa were all identified as *Coryphaenoides armatus/yaquinae*. These studies had to be omitted from the analysis. For the remaining studies there was variability in a few key taxa (Macrouridae, *Bassozetus*, and Synaphobranchidae) as these groups have few obvious visual characteristics to confirm identifications. In a few cases higher taxa (genus, family) had to be used for consistency across studies.

Several methodological issues were also evaluated. First, deployment durations varied considerably across the dataset from <5 to 67 hours. Examination of this data showed that deployments <5 hours had much lower MaxN compared to regionally co-occurring deployments, and so they were eliminated (n=4). One deployment used non-standard bait which is also known to affect survey results (Fleury and Drazen, 2013; Wraith *et al.*, 2013) and was eliminated. Finally, six deployments used a very small viewing area (<0.5m^2), MDS results suggested different community structure for these deployments compared to other deployments in their area/depth, so they were excluded. The final data set for analysis included 107 deployments across the Pacific of which 43 deployments where from the CCZ (Table 1).

Data for fish were also compiled from video and photo transecting methods (including ROV, AUV, and towed cameras) in the CCZ and neighboring regions (Table 2). A full discussion of the methods can be found in the Invertebrate Megafauna section of this report (Jones et al.). Data were collected from 2-5 m above the seafloor with quantified (and occasionally estimated) seafloor area. Data were annotated with a standardized abyssal fish atlas (Drazen and Leitner, unpublished) for consistency. Fish abundance, diversity, and community composition were then extracted. Data exists for 5 contract areas: UK-1 area (Amon et al, 2016); Tonga Offshore Mining Ltd. areas B, C, and D (Nautilus Minerals, Simon-Lledo et al, in prep); OMCO A-G (Kirsty McQuaid and Kerry Howell), eastern Kiribati EEZ (Nautilus Minerals, Simon-Lledo et al, in review); as well as 4 APEIs: western APEIs 1, 4, and 7 (Deep CCZ, Durden et al, in prep), eastern APEI 6 (Simon-Lledó *et al.*, 2019).

Table 2. Areas for which camera transects were available for quantitative community analysis of fishes.

Area	# transects	# of fish	Fish density (# ha^{-1})
APEI 1	4	12	11.4 ± 1.8
APEI 4	6	46	30.3 ± 2.9
APEI 6	12	74	64.5 ± 4.6
APEI 7	4	59	39.6 ± 2.3
DISCOL	48	649	30.2 ± 40.3
Kiribati_A	4	3	3.8 ± 1
Kiribati_B	1	8	18.4
Kiribati_C	1	19	42.7
TOML_B	4	46	16.3 ± 8
TOML_C	4	83	27.3 ± 13.4
TOML_D	3	79	36.5 ± 18.8
UK1	4	7	16 ± 1.7
Grand Total	**95**	**1085**	29.8 ± 28.7

Data standardization across this suite of studies was very challenging. We developed a single CCZ abyssal photographic fish guide (Drazen and Leitner, unpublished) that included inputs from taxonomists, from the University of Hawaii video lab who had worked on observations elsewhere in the Pacific as part of the CAPSTONE project, and from the Hawaii Undersea Research Laboratory database. Voucher specimens from trapping studies were available for a small number of taxa. For instance, morphology and/or barcoding approaches were used for the most common and widespread taxon *Coryphaenoides armatus/yaquinae* (Gaither et al. 2016; Leitner et al. 2017) and for a few other species such as *Pachycara Nazca*, *Illyophis arx*, *Cerataspis monstrosus*, and *Hymenopenaeus nereus*. All transects were annotated using this consistent photo guide, most in the annotation platform BIIGLE. This ensured a high degree of taxonomic consistency which is required for a broad synthesis of the data. These data were used to evaluate community composition and diversity across the CCZ.

We also evaluated the towed camera data from OMCO A-G (Kirsty McQuaid and Kerry Howell, unpublished data) but this data came late in the workshop and while quantitative, observed very few fishes. Data were also made available from towed cameras from KODOS and APEI 9 (Se-Jong Kim et al., unpublished data) and the ROV data from BGR, GSR and APEI3 seamounts (Daphne Cuvelier et al., unpublished data). The former could be quantitative, but not all photos were available for us to identify in time for this report, so at this stage they can be used in a qualitative fashion for presence data only. The latter was not taken in transect fashion, and so it too is used in a qualitative fashion.

To evaluate species ranges and biogeographies of fishes and scavengers, we assembled all presence data (from both transects and baited cameras) by survey area to estimate how widespread these animals are both across the CCZ and across the Pacific.

Results and Discussion

Camera Transect Data

During the workshop several camera transect datasets for fishes were made available, but there was insufficient time to add them to analyses in a completely comprehensive fashion. Thus only preliminary camera transect data analysis is provided in this report and further analysis will occur in the near future.

The camera transect data frequently included very few fishes (Table 2) with the strong suggestion that fishes avoided camera systems towed close to the seafloor. For instance, four transects in Kiribati A observed only 3 fish. Avoidance of fishes by ROVs and towed systems is not uncommon (e.g. Trenkel *et al.*, 2004), though such systems do often provide higher density estimates than trawls if flown at ~4-5m (Cailliet *et al.*, 1999; Milligan *et al.*, 2016) and if they survey large enough areas of seafloor. Some of the OMCO stations only sampled ~800m^2 and observed no fish at all.

Rarefied diversity curves suggested that diversity was highest in two areas of Kiribati and lowest in APEI6 and the DISCOL site (Fig. 3). Generally most of the rarefaction curves were not yet nearing an asymptote suggesting that additional sampling is required.

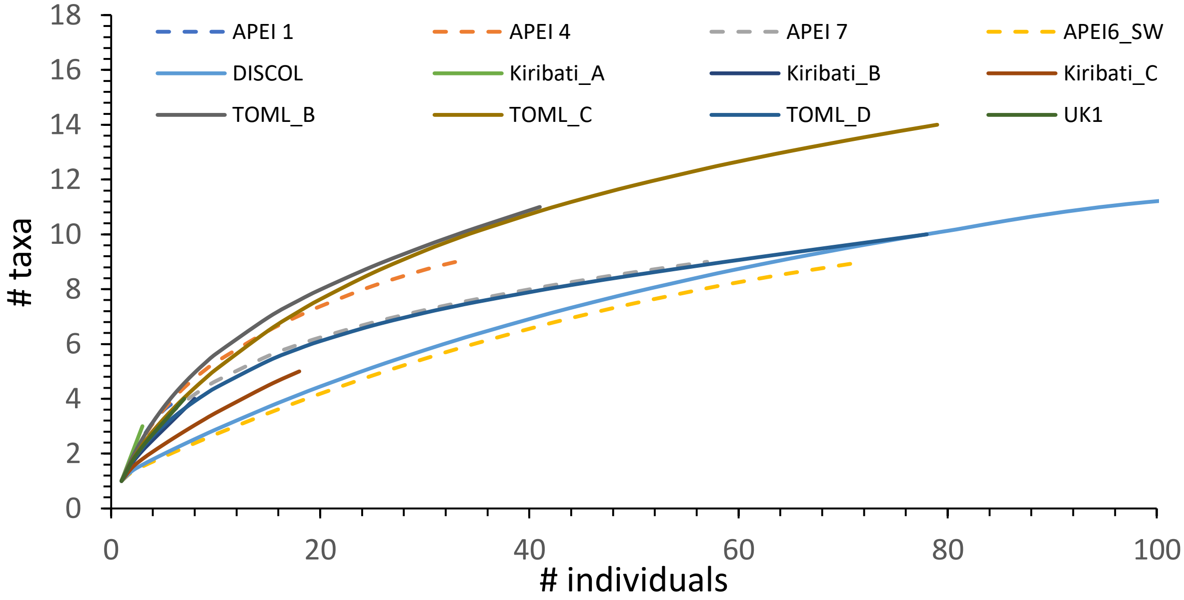

Figure 3. Rarefied fish diversity from quantitative camera transect data by region in the CCZ.

Fish community composition varied across the CCZ (Fig. 4). Samples from within individual areas clustered together with a few exceptions such as a transect in APEI7 that occurred on a seamount rather than the abyssal plain (point in top left corner of Fig. 4). From left to right there is also some structuring that generally concurs with east-west position across the CCZ (longitude).

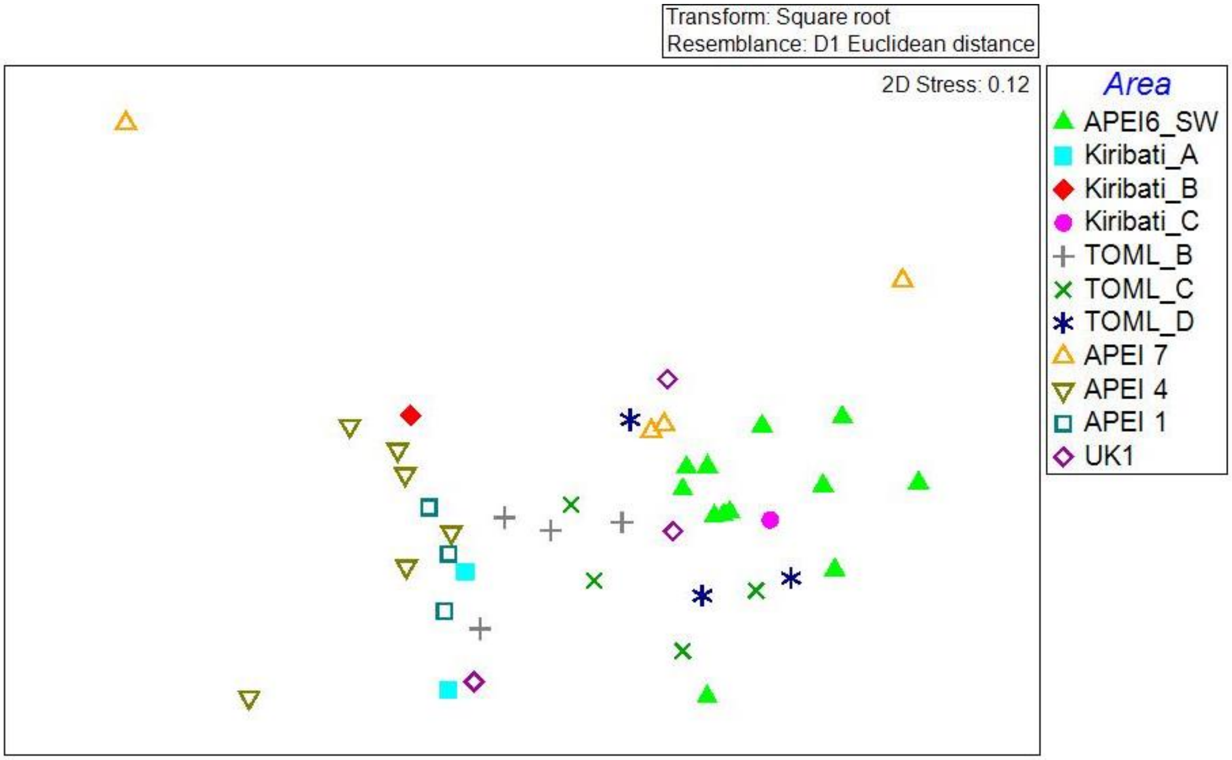

Figure 4. Fish community composition (MDS plot) from camera transect data across the CCZ.

Baited Camera Data

Baited camera studies provide a solid first examination of fish and scavenger communities across the CCZ. For instance, in a comparison of the fish taxa overlap between the CCZ baited camera studies (16 taxa) and the CCZ transect studies (31 taxa) that were available, the baited camera studies observed 42% (13 of 31) of the total fish fauna observed. Furthermore, the baited cameras observed 3 taxa that were not observed in the camera transects. Baited cameras also observed a total of 52 taxa across the Pacific when invertebrates were included.

Scavenger assemblages varied substantially across the Pacific (Fig. 5). The CCZ communities clustered by sampling area and were distinct from those found off California, the Northwestern Hawaiian Islands, and the western Pacific (Kermadec and Marianas sites for instance). The CCZ communities were similar to deeper abyssal samples off the main Hawaiian Islands and those sampled in the DISCOL region, however.

The scavenger assemblages also varied significantly within the CCZ (Fig. 5). There was a clear east to west variability as has been observed before (Leitner *et al.*, 2017). It was also evident that the seamount samples from the western APEIs had a distinct community from their surrounding abyssal plains (see points with shallower depths and lying towards the bottom of the MDS space in Fig. 3). Based on these statistical analyses, macroscale habitat (seamounts vs abyssal plain) has a greater influence on scavenger assemblages that sampling region.

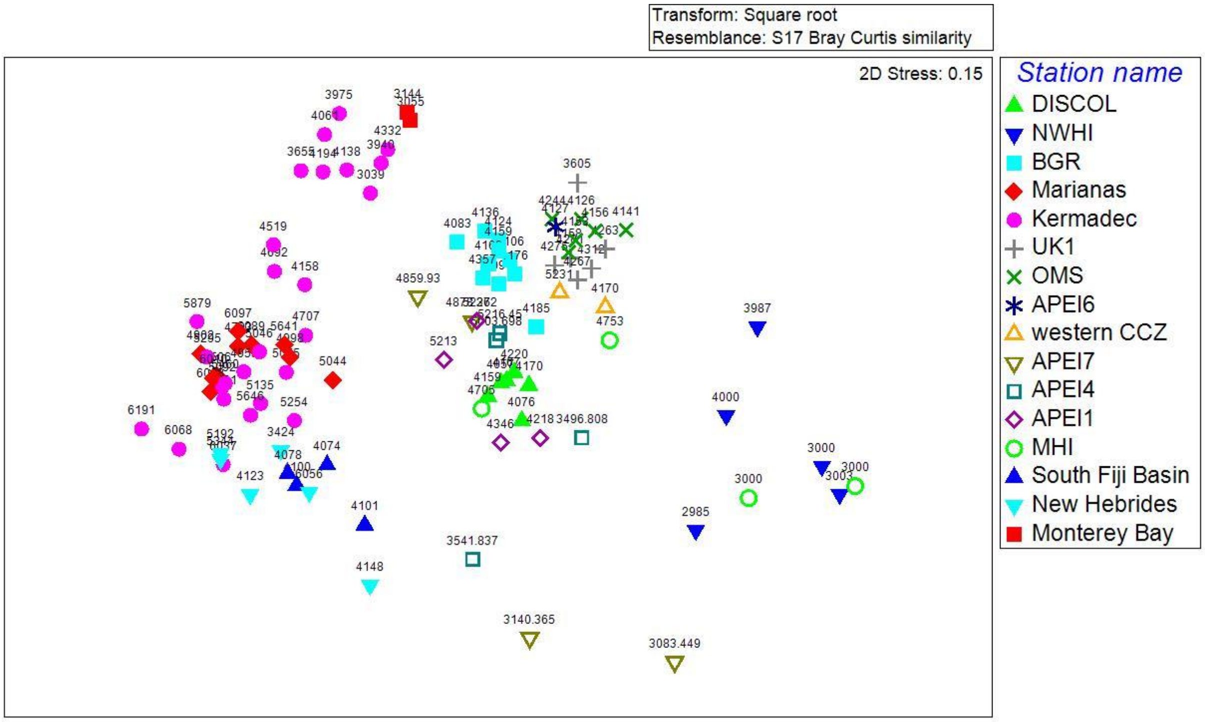

Figure 5. MDS analysis of scavenger MaxN data across the Pacific. Symbols indicate the station and numbers above the symbols are depths.

Diversity also varied across the Pacific and CCZ. Rarefied diversity (ES45) and Shannon diversity (Fig. 6) were slightly higher on the abyssal plains in the western CCZ compared to the contract areas in the eastern CCZ. These diversities were comparable to those found in Hawaii and DISCOL and higher than those from California and the western Pacific. Looking only at the samples from within the CCZ, the eastern CCZ seems to have a significantly lower estimated species richness than the west (Chao estimated species richness 21 versus 29); however, excluding the additional diversity that the western seamount deployments give to the west, the estimated number of species is no longer significantly different between the regions (21 in the east versus 23 in the western abyssal plains, overlapping confidence intervals) (Fig. 7). This finding again highlights the importance of macroscale habitat.

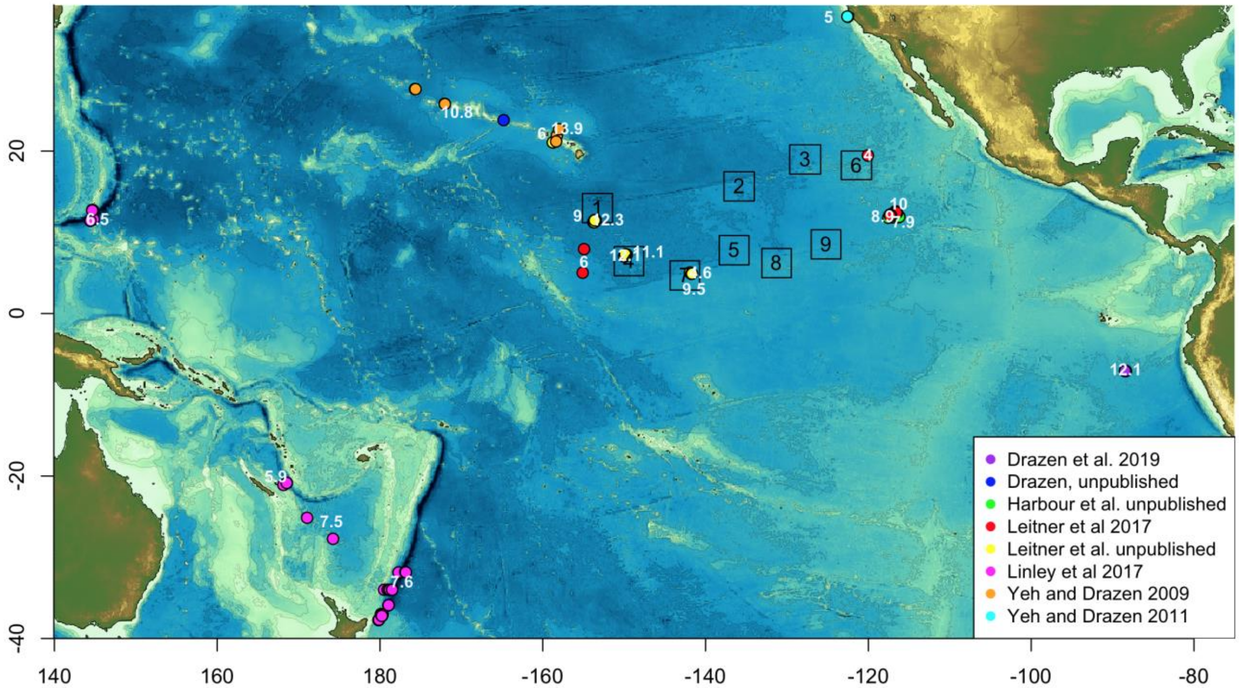

Figure 6. Rarefied diversity (E_S45) for each sampling region across the Pacific.

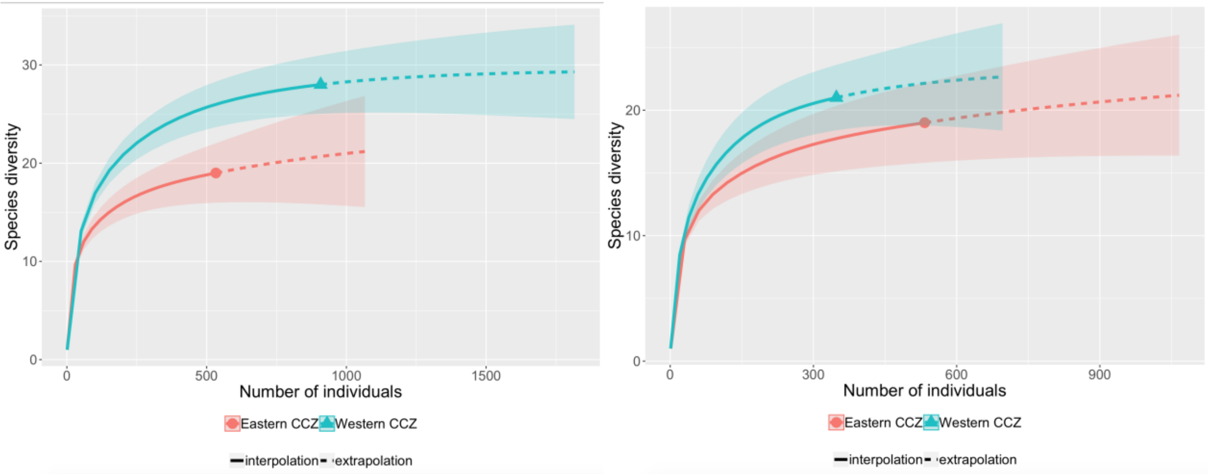

Figure 7. Diversity as estimated species richness (hill number q=0) compared between the eastern and western CCZ including all CCZ baited camera deployments (left) and excluding all seamount deployments (right).

For the APEIs to function as conservation zones they should preserve a fauna that is similar to the neighboring contract areas (Wedding *et al.*, 2015). However, we are not able to conclude whether this is true at this point in time because we still lack data. In those regions where we have data from APEIs, we are missing data from neighboring contract areas (western CCZ), and in those regions where we have data from claim areas, we are missing data from neighboring APEIs (eastern CCZ). Additionally, there is no currently available data from the central CCZ. Looking only at abyssal plain sites, a cluster analysis confirmed the MDS analysis results of clear differences across contract and APEI areas (SIMPROF

$p<0.05$). However, it must be restated that all of the sampled contract areas are in the eastern CCZ and all of the APEIs sampled are in the western CCZ.

To evaluate the drivers of the spatial variability in **diversity** described above, we used generalized linear models to compare the response of these biological variables to habitat variables that included food supply (seafloor POC flux), nodule abundance, and a measure of seafloor height relative to the surrounding area (BPI). None of these environmental variables explained variation in diversity metrics (H', ES45, Chao 1 diversity) across the CCZ or across the Pacific (multiple linear regression, $p>0.05$). There was a significant negative relationship between H' versus POC flux within CCZ stations (linear regression, $p<0.05$). However, this was dominated by the APEI7 seamount site (n=2) which had very low diversity and high seafloor POC flux. When this data point was removed the relationship was no longer significant. This highlights the need for more data across a POC flux gradient where the sampling design takes macroscale habitat into account (sampling elevated features like abyssal hills, ridges, and seamounts as well as troughs and abyssal plains equally). Such additional data would allow for a robust test for the importance of POC flux as a driver of the CCZ scavenging community.

We also evaluated environmental drivers of the large variation in **community composition** that was evident across the Pacific and CCZ and described above (Fig. 5), using principal coordinate analysis (PCoA, Fig. 8). The first axis was largely related to longitude (east to west across the Pacific), again pointing to regional variations, but without much power to discern why this variation exists environmentally. Depth was also correlated with this axis which explained some variation and generally the western sites were deeper than the eastern ones. The second axis is largely aligned with variations in POC flux and to a lesser extent, with depth. The cluster of points representing the CCZ, the top left quadrat (Fig. 8), is largely distributed along this axis. To test whether the available environmental parameters significantly explained this community structuring, a PERMANOVA stratified by region to account for spatial-autocorrelation was run on the Pacific-wide baited camera dataset. Significance tests showed that longitude was marginally significant ($P=0.057$), POC flux was non-significant ($P>0.05$), and both depth and habitat type (abyssal plain, seamount, island flank, trench, and basin) were highly statistically significant ($P<<0.01$) in structuring scavenger communities. From this test we can conclude that both depth and macroscale habitat type are very important to structuring scavenging communities and that scavenger community structure changes significantly across the Pacific. When evaluating only the CCZ deployments, a second PCoA showed dramatic clustering by BBPI (significant marginal test, $P<0.05$), which indicates that macroscale habitat is the most important driver in determining the structure of scavenger communities within the CCZ only. Other factors included in the CCZ-only analysis were non-significant (modelled POC flux, model predicted nodule abundance, depth).

It should be noted that for these analyses data is very limited (e.g. n=10 regions/habitats for CCZ) and environmental data layers are often model predictions rather than empirically derived variables, suggesting low statistical power to detect environmental patterns.

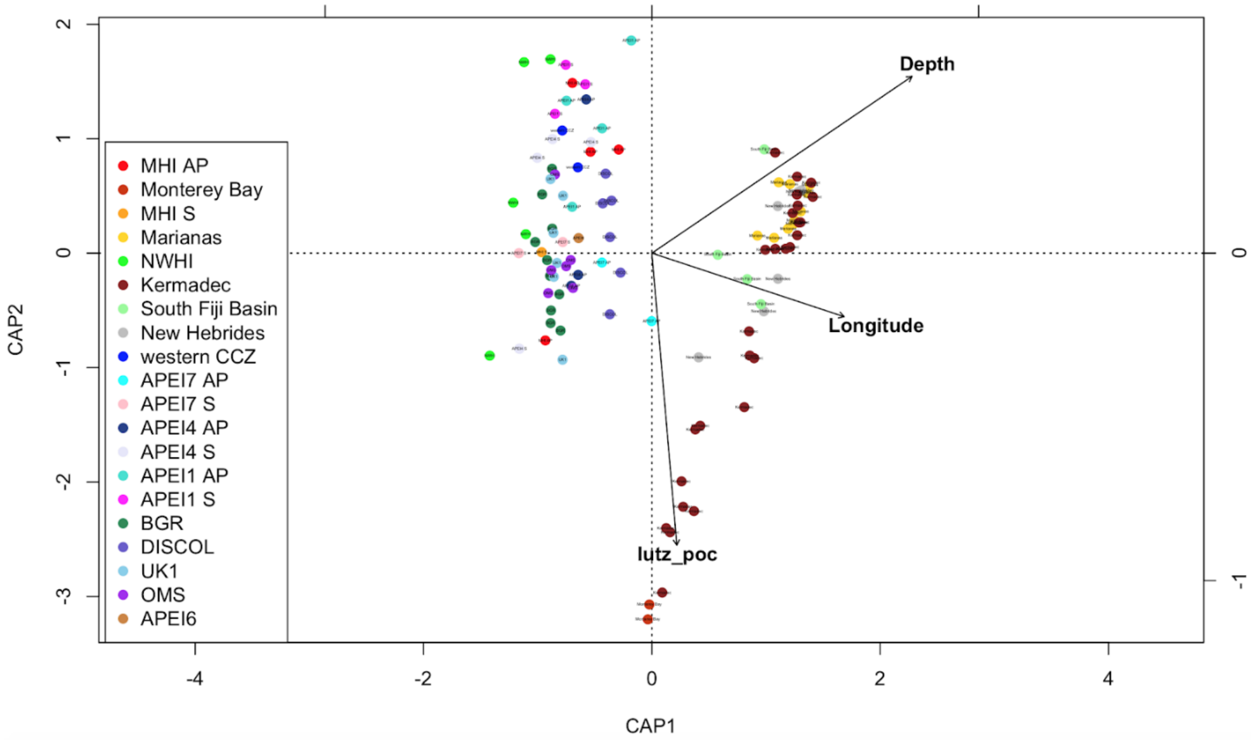

Figure 8. Principal coordinate analysis of the baited camera data (points) across the Pacific evaluated in relation to the environmental drivers of particulate organic carbon flux as estimated by Lutz et al. 2007 (lutz_poc), longitude, and depth (arrows).

Fish ranges and overlap from both transect and baited camera data

An important consideration is the ranges of species in relation to the APEI network to ensure adequate representation of species within them and also the potential for connectivity across the reserves. Many species of mobile fishes and scavengers had wide ranges as expected (Fig. 9). It is well known that a number of abyssal species have worldwide distributions (Gaither *et al.*, 2015; King and Priede, 2008). However, quite a few do not, and taxa such as the Liparidae and Zoarcidae frequently have regional endemics (Anderson and Fedorov, 2004; Chernova *et al.*, 2004). Our data suggests that the number of shared taxa increase with shorter distances between sites (Fig. 9). At the scale of the CCZ (~4000 km across) sites share from 1-6 species again highlighting the changes in the fish community across the CCZ region.

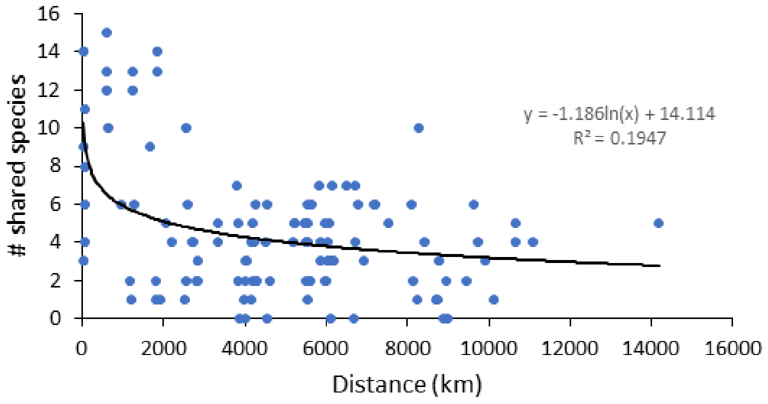

Figure 9. The number of shared species between all possible combinations of station pairs as a function of distance between the pair.

Though not all transect data is quantitative, we used it, in combination with baited camera data, to evaluate species overlap across the Pacific. Overlap between fish species was very high (12 species) for a subset of the areas examined (Fig. 10). This high level of similarity related principally to sampling technique, in that all of them were sampled with baited cameras and a few with both baited cameras and camera transects. The sites with little overlap (OMCO, GSR, Kiribati, TOML, APEI3, 9, and KODOS) were sampled only with camera transects and often lacked adequate spatial coverage to fully characterize diversity. This analysis illustrates clearly that more sampling is required, including with baited cameras, more broadly across the CCZ. Despite the potential sampling limitations which could lead to pseudo-endemism, the majority of fishes were found to have broad overlap between the sites (Fig. 11). Only 8 taxa were found at a single site and most taxa were found at more than 10 sites across the Pacific.

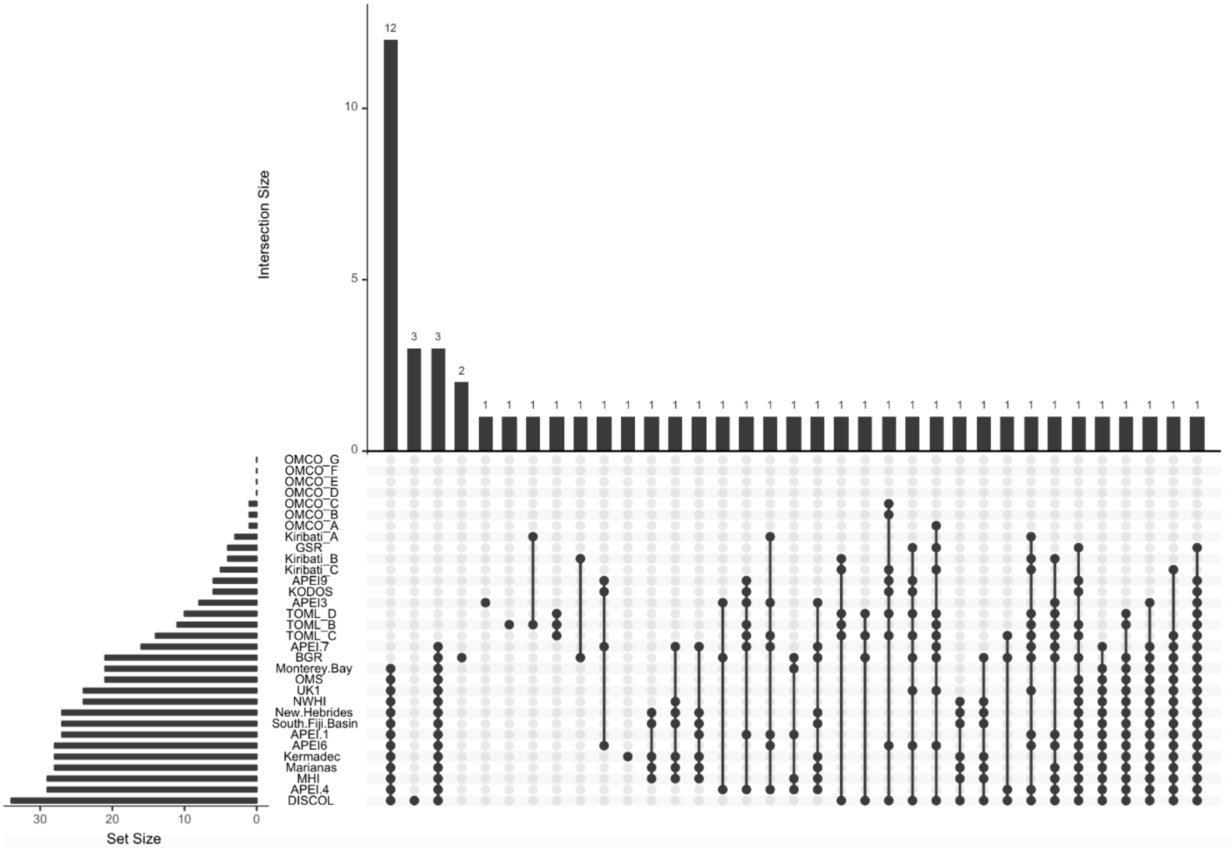

Figure 10. Visualization of intersecting pairs of deployments (upset plot) showing fish species overlap by sampling site from the combination of baited camera and camera transect data. For each site, numbers of taxa are shown on the left. The numbers of shared taxa patterns are given at the top with the stations for which the similarities are found are given as connected dots.

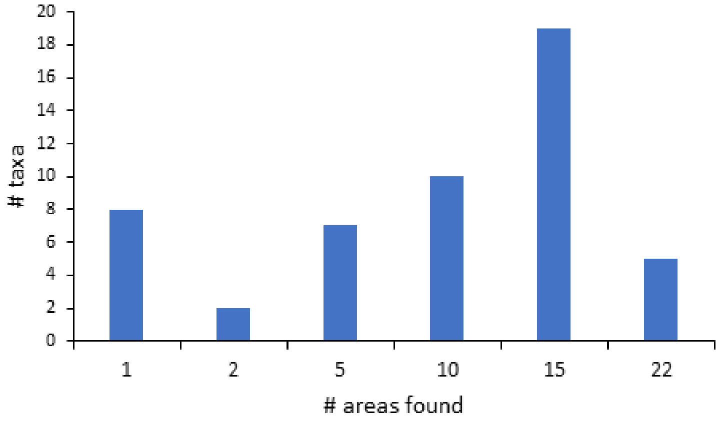

Figure 11. Frequency distribution of the numbers of fish taxa with varying levels of species overlap (# of sites in which each was found) across the Pacific from both camera transect and baited camera data (as in Fig. 9).

It is important to note that the present analysis is largely based on morphological taxonomy, specifically photographic identification. Ideally datasets could be compared to those employing eDNA approaches. However, specimens for fishes and scavengers are limited which makes their coverage in barcoding databases very sparse. Studies are currently underway on scavenging amphipods using genetic methods to examine their population connectivity (Dahlgren, Glover et al; see Connectivity section of this report). eDNA approaches have identified regional variation in the numbers and diversity of fish sequences between seamounts and abyssal plains for instance (Goetze et al; see eDNA section of this report). However, the various datasets are too sparse at this time for concrete comparisons. We need a concentrated fish and scavenger sampling effort using benthic trawls across the CCZ where captured specimens are first morphologically identified and then barcoded with entry into global databases for future reference.

Conclusions

1) Baited camera deployments should be expanded into more CCZ regions using standard methods. Data exist for eastern CCZ contract areas and western CCZ APEIs only. Standard methods (view area of 1-3m^2), duration (18-24 hours at the seafloor), bait (1kg of mackerel or small jack) tied tightly to the camera system in the field of view are critical to data intercomparisons.
2) Taxonomic consistency during annotation is key! Researchers should actively work with others to ensure consistency.
3) There are a number of taxa, particularly ophidiids, where we need significant additional specimens for vouchers and tissue to create DNA barcodes for eDNA work.
4) Fish and scavenger communities and diversity vary significantly across the CCZ and the Pacific suggesting that even for these highly mobile species that not all regions of the CCZ are equivalent, and it cannot be managed as one homogenous region. A network of APEIs that covers the spectrum of available habitats will be key to conserving biological diversity.
5) Seamounts have a significantly different scavenger community than neighboring abyssal plains and thus contribute to regional diversity. Macroscale habitat must be considered in spatial management.

6) At this stage fish and scavenger data is too limited to compare communities between contract areas and neighboring APEIs to evaluate their representativity.
7) Studies elsewhere show seasonal and interannual variation in scavenger abundance (Drazen *et al.*, 2012; Priede *et al.*, 2003), so temporal variation in the CCZ should be investigated.
8) Many fishes and scavengers have very large, even worldwide, ranges. It should be noted that there are a few species that have small ranges too. Despite generally large ranges it is not presently possible to determine if any biogeographic boundaries exist in the CCZ region due to the coarse spatial resolution of the data.
9) Regional variations in community composition are largely the result of varying abundances of species rather than species presence/absence.

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g. GENETIC CONNECTIVITY –

Population connectivity and resilience in the CCZ with respect to the APEI Network

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Introduction

An understanding of the connectivity of populations within marine reserves, between marine reserves and between marine reserves and potentially exploited regions is critical to conservation biology (Palumbi, 2003). Typically, in the marine realm this would involve the study of larval transport but in regions such as the abyssal CCZ, there are no data available on larval transport and as such connectivity must be inferred from population genetic data.

The oligotrophic (food-poor) life at the abyssal seafloor typically sustains species with very low population densities, in contrast to species that inhabit ephemeral habitats such as hydrothermal vents, seeps and large-organic falls, that are often found in large abundances. The effort required to achieve large enough sample sizes for population genetic studies has thus hampered our knowledge of population structure of abyssal fauna (Glover et al. 2016a, Taylor & Roterman 2017).

Among non-scavenging benthic fauna, only four studies have addressed population connectivity in species inhabiting ‘abyssal plains’ i.e., away from seamounts, ephemeral habitats, continental slopes, and other marginal areas (Etter et al. 2011, Gubili et al. 2017, Taboada et al. 2018a, Jansen et al. 2019). These four studies include a macrofaunal bivalve from the Atlantic (Etter et al. 2011), a megafaunal epibenthic holothurian from Atlantic and Pacific samples (Gubili et al. 2017), a macrofaunal, sessile sponge living on polymetallic nodules in the Pacific (Taboada et al. 2018), and five macrofaunal mud-dwelling species each of annelids and isopods from the Pacific (Janssen et al. 2019). Only the latter two studies are actually based on samples from the CCZ, and of these only Taboada et al. (2018a) includes data from any APEIs. No studies have been published on the meiofauna.

Despite the lack of published studies, there has been a significant improvement in our knowledge of connectivity across the CCZ in particular within and between contracted regions. Several new studies are in preparation and are summarized below. Important caveats remain, specifically (1) the lack of samples within APEIs, (2) the reliance on mitochondrial DNA (mtDNA) data only, (3) the absence of any information on rare species (only abundant taxa have been analyzed with mtDNA), (4) the absence of any data on the meiofauna and (5) a strong geographic bias with most samples originating from the eastern CCZ.

Review of existing published studies

In general, the four published studies on abyssal connectivity demonstrate a lack of geographically-driven genetic structure. The first paper to be published suggested that abyssal populations of a small infaunal bivalve, *Ledella ultima,* may well be cosmopolitan at an ocean-basin scale, with large stable populations with high connectivity (Etter et al. 2011). In a study of the large abyssal holothurian *Psychropotes longicauda* (Gubili et al. 2017), high genetic diversity and potential cryptic speciation was discovered, but it was not clearly based on geographic separation. Cryptic species were distributed sympatrically, suggesting that distance was not necessarily a barrier to connectivity. In the recently-described CCZ abyssal sponge *Plenaster craigi* (Lim et al. 2017), significant population structure was observed using microsatellite DNA data, but again it was not linked to geographic distance, suggesting that populations could be broadly connected (Taboada et al. 2018a). The most recent publication (Janssen et al. 2019) examined CCZ macrofaunal taxa and showed only weak or no structuring at the 50-60-km scale and shared haplotypes (suggestive of connectivity) at the 1300-km scale.

Two additional studies on the scavenging fauna of the abyss - a quite separate and mobile faunal component of abyssal systems - (Havermans et al. 2013, Havermans, 2016) demonstrated significant bathymetric structuring but limited geographic structuring, again suggestive that for regions at the same depth, connectivity may be high.

All of these studies are subject to the same caveats outlined above, perhaps the most important being that as only abundant taxa are necessarily pre-selected for connectivity studies, we may be selectively sampling only well-connected species with large populations. Nothing is thus known about connectivity in the vast majority of abyssal species.

Summary of new data on the connectivity of the CCZ

Several new studies are in preparation on the broad-scale connectivity of the CCZ benthos. These were discussed at the DeepCCZ workshop and some preliminary conclusions are presented here to facilitate the review of the CCZ REMP. These conclusions should be treated as preliminary and are based on unpublished data.

The first study discussed is based on a broad sampling effort in the eastern CCZ, with some sampling in APEI-6 (Dahlgren et al. in prep). Eight species representing infauna (one bivalve and four annelids), nodule epifauna (two annelids and one sponge), and motile epifauna (pycnogonid) were studied (Figure 1). This suite of species also represents different life history strategies (Table 1; Dahlgren et al. in prep). One taxon, the bivalve *Nucula profundorum* (Smith, 1885) is common in the CCZ and was first collected on the Challenger expedition. It is currently one of very few infaunal taxa with a published species name (Figure 2). Two taxa are also represented by samples from outside of the CCZ, i.e., from the DISCOL site in the South Pacific and from the Atlantic. The genetic data are collected from two mitochondrial markers, COI and 16S. In addition, allelic-frequency data from 11 microsatellite loci are available for one species (the sponge *P. craigi*). Analyses of these data broadly corroborates the indications from the published studies. There is no evidence for population genetic structure in any of the sampled species. In contrast, shared haplotypes over 5000 km scale is indicated in two of the species. A second connectivity study, of the Ophiuroidea (brittle stars - one of the most abundant megafaunal groups in the CCZ) is in preparation (Martinez Arbizu et al. in prep), detailed below.

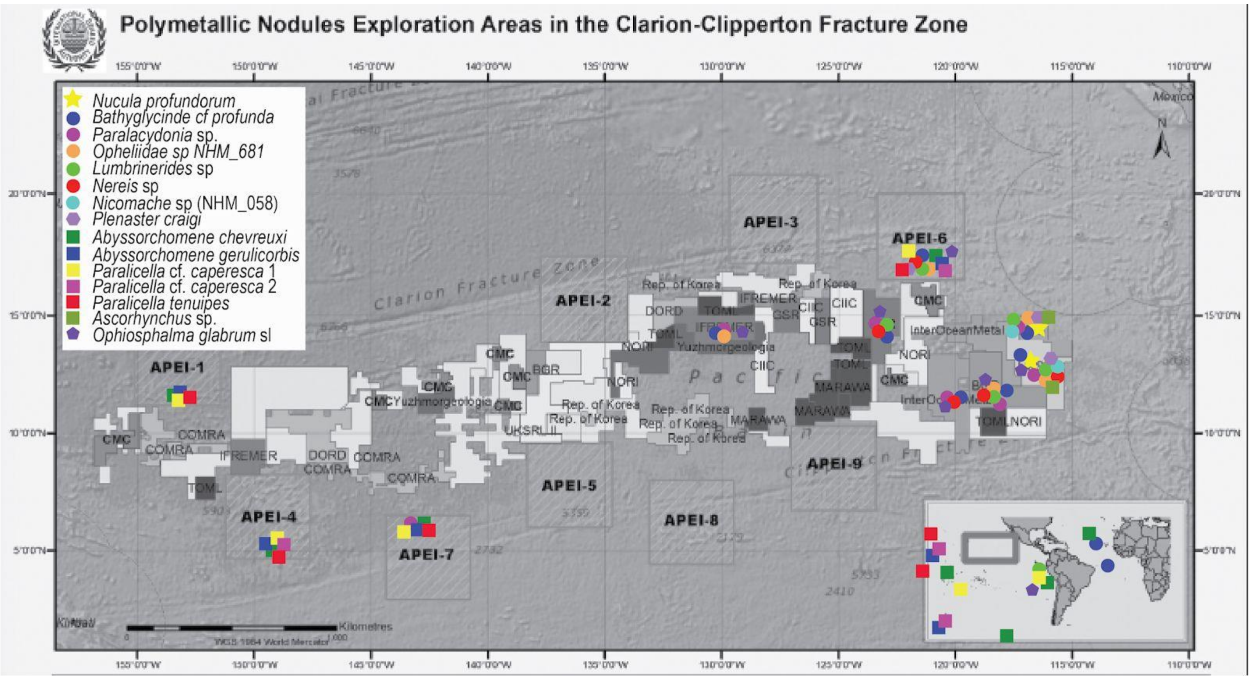

Figure 1. Map indicating sampling sites of species, from five different phyla, included in the connectivity analyses. (star = Mollusca, circle = Annelida, hexagon = Porifera, square = Arthropoda, hexagon = Echinodermata).

Analyses of haplotype data from the infauna and motile epifauna also show generally high levels of genetic diversity with negative Tajima’s D, indicating an excess of rare alleles which could be caused by selective sweeps, bottleneck events or changing population size in general. This pattern is not evident in the sampled nodule epifauna species.

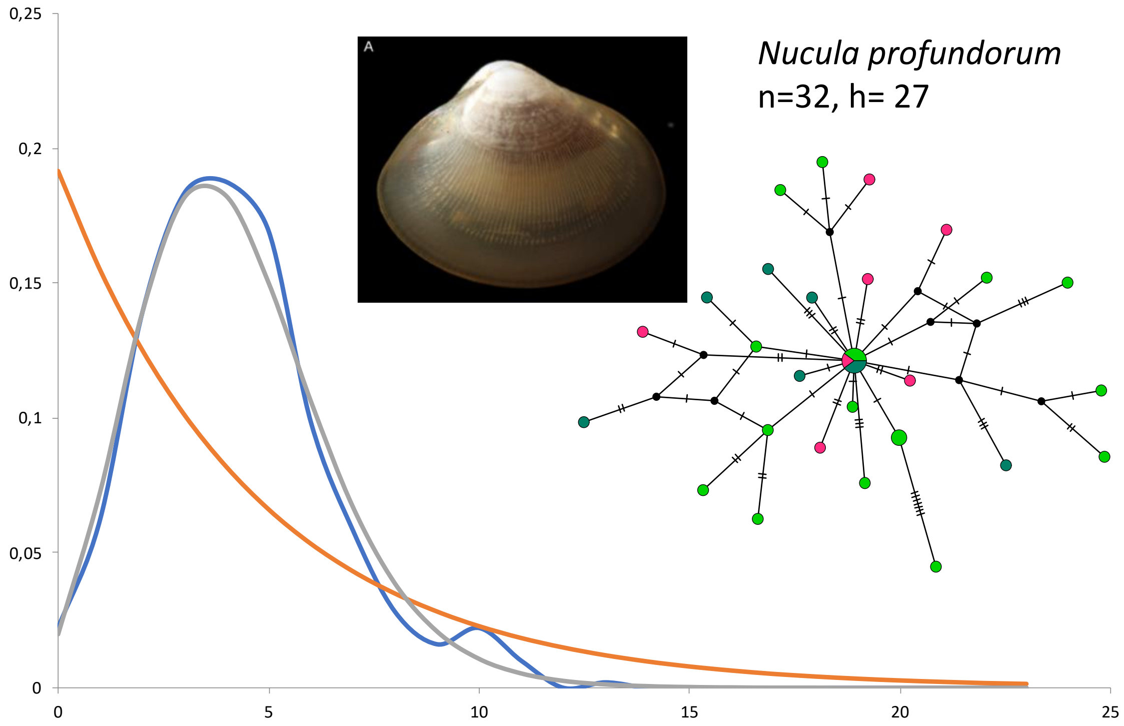

Figure 2. *Nucula profundorum*. Haplotype network and mismatch distribution. 27 haplotypes were found in a sample number of 32 individuals from the eastern CCZ. The Tajima's D was significantly negative (excess of rare alleles) for both COI and 16S markers suggesting an instable population (Dahlgren et al. in prep).

Table 1. Summary of taxa for which population genetics and connectivity data are currently being studied. The life history strategy data are inferred from close relatives or based on preliminary data and is thus only indicative. Areas sampled as in Figure 1.

Species	**Phylum**	**Habitat**	**Indicative life history**	**Areas sampled**	**Reference**
Nucula profundorum E. A. Smith, 1885	Mollusca	Infauna	Broadcast spawner	UK1, OMS	Dahlgren et al. in prep

Bathyglycinde cf. *profunda*	Annelida	Infauna	Brooding	UK1, OMS, GER, FR, IOM, BE, APEI-6, DISCOL, ATL	Dahlgren et al. in prep
Paralacydonia cf. *weberi*	Annelida	Infauna	Broadcast spawner	UK1, OMS, GER, FR, IOM, BE	Dahlgren et al. in prep
Lumbrinerides sp.	Annelida	Infauna	No data	UK1, OMS, GER, BE, DISCOL	Dahlgren et al. in prep
Ophelina martinezarbizui Wiklund et al. 2019	Annelida	Infauna	Brooding	UK1, OMS, GER, FR, APEI-6	Dahlgren et al. in prep
Nereis sp.	Annelida	Nodule epifauna	Broadcast spawner	UK1, OMS, GER, IOM, BE	Dahlgren et al. in prep
Nicomache sp.	Annelida	Nodule epifauna	Broadcast spawner	UK1, OMS	Dahlgren et al. in prep
Plenaster craigi Lim & Wiklund, 2017	Porifera	Nodule epifauna	No data	UK1, OMS, APEI-6,	Dahlgren et al. in prep
Ascorhynchus sp.	Arthropoda	Motile epifauna	Parasitic non-swimming larvae	UK1, OMS	Dahlgren et al. in prep
Ophiosphalma glabrum sl	Echinodermata	Motile epifauna	planktonic larvae (?)	UKSRL, IOM,IFREMER, BGR, GSR, APEI-6, DISCOL	Christodoulou et al. submitted. Wiklund et al. unpublished data

Paralicella cf. *caperesca* 1	Arthropoda	Mobile scavengers	Brooding	APEI-1, APEI-4, APEI-6, APEI-7, South Pacific, East Pacific Basins	Bribiesca-Contreras et al. in prep
Paralicella cf. *caperesca* 2	Arthropoda	Mobile scavengers	Brooding	APEI-6, APEI-7, South Pacific, North Central Pacific	Bribiesca-Contreras et al. in prep
Paralicella tenuipes Chevreux, 1908	Arthropoda	Mobile scavengers	Brooding	APEI-1, APEI-4, APEI-6, APEI-7, Aleutian-Japan Province, North Central Pacific, East Pacific Basins	Bribiesca-Contreras et al. in prep
Abyssorchomene chevreuxi (Stebbing, 1906)	Arthropoda	Mobile scavengers	Brooding	APEI-1, APEI-4, APEI-6, APEI-7, Mid-Atlantic Ridge, East Antarctic Indian, East Pacific Basins, Antarctica	Bribiesca-Contreras et al. in prep

Abyssorchomene gerulicorbis (Shulenberger & Barnard, 1976)	Arthropoda	Mobile scavengers	Brooding	APEI-1, APEI-4, APEI-6, APEI-7, South Pacific, North Central Pacific	Bribiesca-Contreras et al. in prep

A series of cruises since 2012 that took place in five different exploration contract areas (IFREMER, BGR, UK-1, GSR, IOM), two areas protected from mining (APEI-3, APEI-6) and in the DEA (DISCOL Experimental Area) resulted in the collection of numerous ophiuroids (Christodoulou et al. 2019; Christodoulou et al. submitted; Wiklund et al. unpublished data). The brittle star *Ophiosphalma glabrum* (Figure 3) is the second most common echinoderm collected and one of the most abundant megafaunal animals found in the CCZ (Glover et al. 2016b, Christodoulou et al. submitted). So far *O. glabrum* is found to be present in five exploration contract areas and in the APEI-6, while no specimens were found so far in APEI-3 (Christodoulou et al. submitted, Wiklund et al. unpublished data). This species has also been collected from the DEA, in the Peru Basin. Because of the high abundance of *O. glabrum* in the CCZ it was selected as a suitable taxon for connectivity studies. A preliminary COI haplotype network for *Ophiosphalma glabrum* is shown in Figure 4 based on the sampling areas. Two groups of haplotypes (Groups A and B) are discriminated with high genetic divergence indicating the presence of a possible cryptic species or the presence of two highly diverse populations. Within the two groups, no spatial patterns are observed. All the CCZ areas were found to share haplotypes, indicating a high connectivity between them. No barriers seem to prevent gene flow between the populations in different areas. A reason for this broad range might be related to the life history of the ophiuroid which most likely has planktonic larvae that will allow drifting in the water column and reaching distant areas.

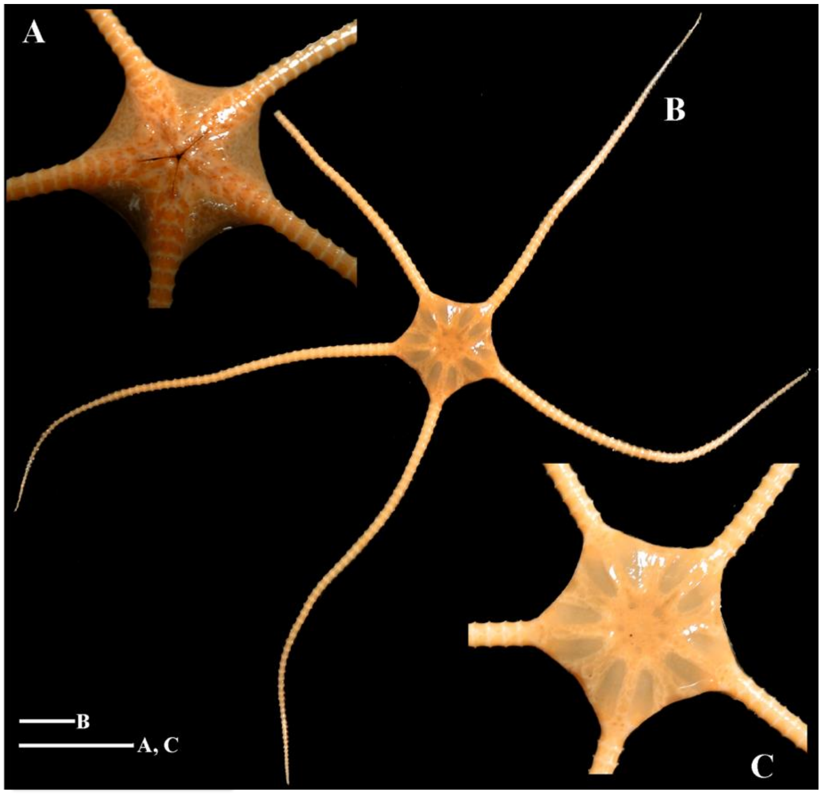

Figure 3. *Ophiosphalma glabrum* (Lütken & Mortensen, 1899). Adult specimen: A, ventral side of central disk; B, whole individual; C, dorsal side of central disk. Scale bars 1 cm.

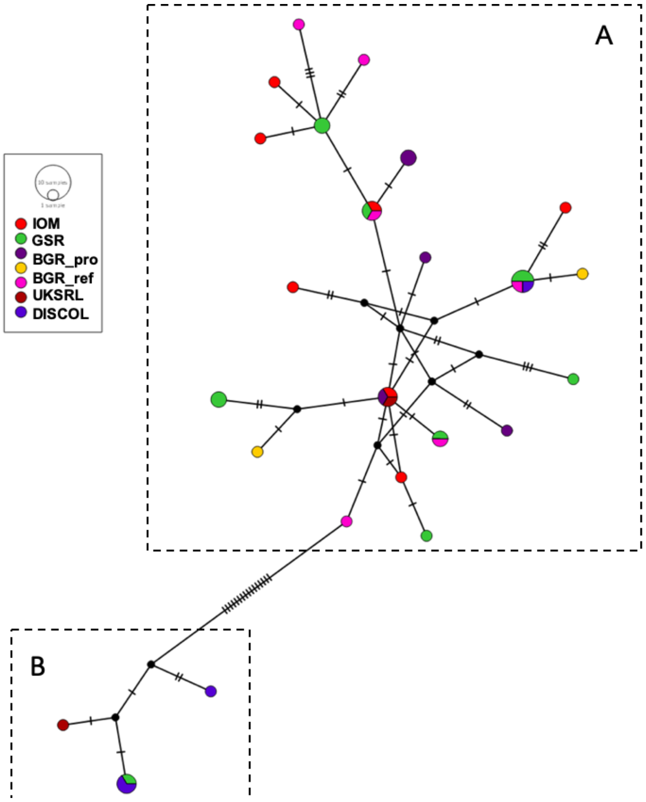

Figure 4: Minimum spanning network showing CO1 haplotype relationships for *Ophiosphalma glabrum* sl. Circle sizes are proportional to the number of individuals with the same haplotype. UKSRL, (UK-1 contract area); BGR, (German contract area); IFREMER, (French contract area); IOM, Interoceanmetal (Consortium of countries), GSR, (Belgian contract area); APEI-3, Area of particular Environmental Interest; DISCOL, Disturbance and recolonization experimental area (Martinez Arbizu et al. in preparation).

Five species of scavenging amphipods (*Paralicella* cf. *caperesca* 1, *P*. cf. *caperesca* 2, *P. tenuipes* Chevreux, 1908, *Abyssorchomene chevreuxi* (Stebbing, 1906), and *A. gerulicorbis* (Shulenberger & Barnard, 1976) have been found to be widespread across the CCZ, and thus are being considered for broad-connectivity studies. In addition, these species are known from outside the CCZ, this includes Pacific regions such as Kermadec, New Hebrides, Marianas, off Japan (Figure 5), but also from the Atlantic Ocean (Ritchie et al. 2017; Figure 6). Genetic data (mtDNA: COI) were generated for samples collected during the DeepCCZ cruise in the western CCZ (APEI-1, APEI-4, and APEI-7). Additional genetic data for APEI-6 in the eastern CCZ (Taboada et al. unpubl. data) and from published studies on scavenging fauna (Havermans et al. 2011, 2013, 2018, Havermans 2016, Corrigan et al. 2014, Ritchie et al. 2015, 2017) have been included. In accordance to previous studies and with other taxa discussed herein, preliminary studies indicate no evidence for geographic structure (e.g. Figures 5 and 6). For the five species, haplotypes are shared across the CCZ, including other Pacific areas and even the Atlantic Ocean.

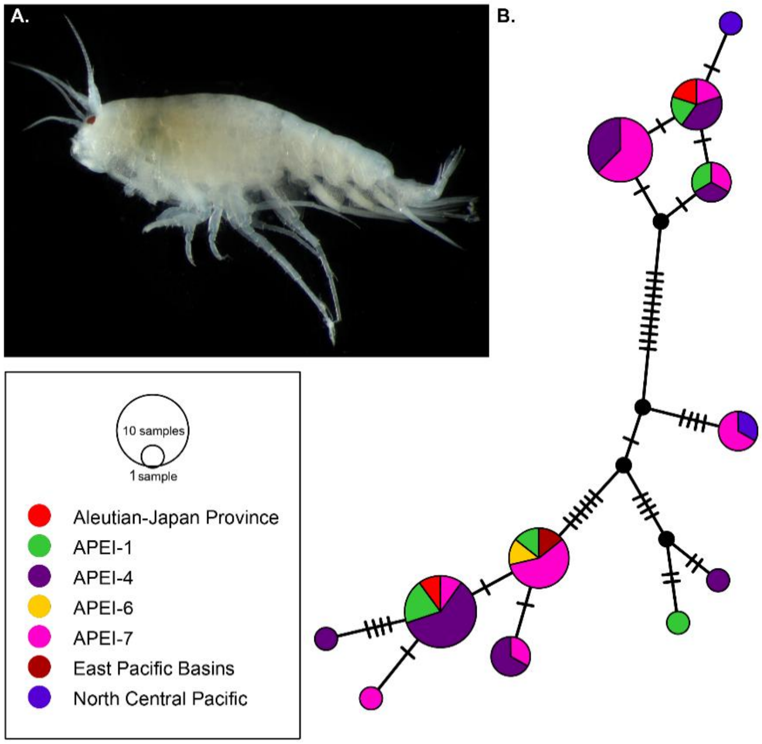

Figure 5. TCS network (B) showing COI haplotypes of *Paralicella tenuipes* Chevreux, 1908 (A) from the CCZ and adjacent areas. Areas outside the CCZ were categorized following the GOODS (Global Open Oceans and Deep Seabed) biogeographic classification (Agostini et al. 2009). APEI, Area of Particular Environmental Interest.

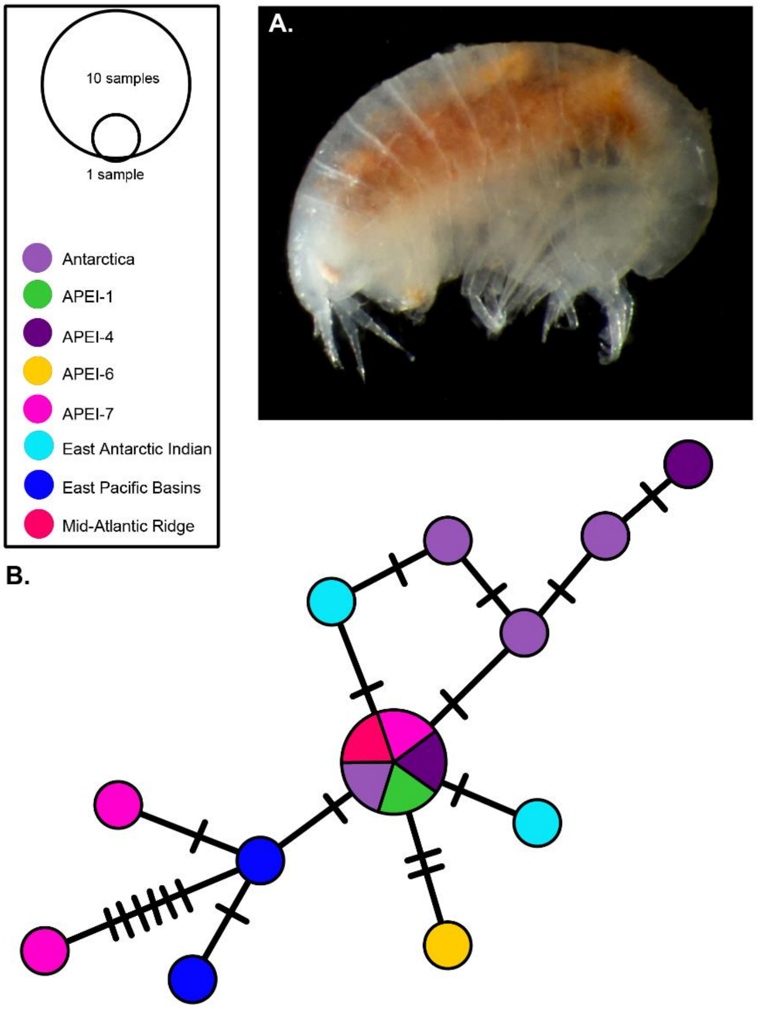

Figure 6. TCS network (B) showing COI haplotypes of *Abyssorchomene chevreuxi* (Stebbing, 1906) (A) from the CCZ and adjacent areas. Areas outside the CCZ were categorized following the GOODS (Global Open Oceans and Deep Seabed) biogeographic classification (Agostini et al. 2009). APEI, Area of Particular Environmental Interest.

General conclusions

For all of the taxa studied to date, two clear general patterns can be drawn. Firstly, that the populations in general show very limited geographic structure. This is suggestive of, but not conclusive evidence for, broad genetic connectivity in these taxa. Secondly, with the exception of the nodule-dwelling sessile fauna, genetic diversity is higher than might be expected based on studies of other marine invertebrates.

With regards to the applicability of these conclusions to the design of the APEI network, some significant caveats must be drawn. Firstly, the majority of the data originate from within contractor zones and there are extremely few studies of connectivity within or between APEIs and other parts of the CCZ. There is only a single published study of connectivity between APEIs and contract areas in the CCZ (Taboada et al. 2018a, 2018b). The remaining conclusions with regards to the available data from APEI 1, 4, 6 and 7 are based on unpublished data. For the western CCZ APEIs 1, 4 and 7, connectivity data are restricted to the mobile scavenger fauna (Bribiesca-Contreras et al. in prep), which are suggestive of limited geographic structure and hence significant connectivity across these sites. For APEI 6 in the north-east CCZ, three taxa show high connectivity with the contractor areas to the south.

Whilst these results could be interpreted as ‘encouraging’ in the sense that there is at least some connectivity between APEIs and between APEIs and contractor zones, it should be noted that this is for just 8 species out of an estimated macrofaunal and megafaunal diversity of well over 1000 species. The taxon sampling is also necessarily biased; only the abundant taxa (which may also happen to be the ones with high connectivity) are able to be sampled for connectivity. It is thus clearly impossible to make any sort of generalisation.

It is not clear what processes are creating the genetic diversity observed at small spatial scales. This should be the subject of further research.

Gaps: Taxonomic impediment to biodiversity syntheses in the CCZ

Background to the problem

Taxonomy provides the basis of all biological science as it allows organisms to be identified based on a common reference database. This links information about organisms and permits the iterative development of biological knowledge. For example, to analyze the biogeographic distribution of species across our planet, we need to use a consistent taxonomy (naming system) to allow different sites to be compared.

The absence of taxonomic data on almost all CCZ fauna (Glover et al, 2018) has thus hindered almost all aspects of our biological knowledge of the region. For example, whilst we might have reasonable data on the abundance of animals from a single contractor region, we do not know what animals those are as they have not been identified using a common reference database (e.g. a field guide or paper with species descriptions using standardized taxonomy).

There are fundamental differences between discriminating species from each other, identifying species and describing species (taxonomy):

1. *Discriminating species* based on DNA or morphology uses an informal naming system (sp. A, B, C etc) to determine if one species is different to another. For example, a researcher in the CCZ distinguishes different polychaetes from one another by their appearance but cannot provide a name from the published literature.
2. *Identifying species* can be done with either DNA or morphology but is confirming what a species is based on comparison to a published reference database and/or published literature. For example, a researcher in the CCZ finds a sponge living on a nodule and identifies it by

sequencing its mitochondrial COI DNA and comparing this to a known reference database (a genetic sequence linked to a name).

3. *Describing species (taxonomy)* is the creation of the database. This would typically be a morphological description of a species with reference specimens (types) deposited in a museum collection, linked to genetic sequences uploaded to global databases (e.g GenBank).

Within a region such as the CCZ, there have been significant efforts at (1) but because of the absence of (3), it has not been possible to do (2). Thus, in most cases, almost none of the species-level data collected by contractors or researcher groups is comparable. This means, for example, that important variables in the development of REMP such as biogeographic comparisons of species ranges to determine if APEIs could act as refuges is impossible. It creates an additional problem in that for comparisons of diversity, although technically possible without a taxonomy, they are likely to be inaccurate as each sample set is identified without any common reference database. It also makes it very hard to carry out assessments over time.

Possible solutions

The taxonomic impediment in the CCZ has been discussed in the past and led the ISA to fund a series of taxonomic workshops on the megafauna, macrofauna and meiofauna. Whilst these workshops were very useful for identifying problems, creating expertise networks and proposing solutions, they did not in themselves provide new taxonomic work.

There are two main approaches for overcoming the taxonomic impediment:

1. *Temporary taxonomic databases.* One immediate solution is the creation of temporary databases of informal species names linked to genetic sequences (e.g. mtCO1 barcodes) and/or morphological data. These databases can be shared and used to confirm species ranges and identify some species by comparison of sequence data. However this solution has some severe limitations:
    a. Absence of quality-control through the peer review system
    b. As specimens and data are not archived in perpetuity at museums or at reliable global databases the data are controlled by individual researchers and are thus likely to be lost
    c. This solution relies on informal networks of researchers, and is thus likely to exclude many research and contractor groups
2. *Fully integrative taxonomy.* The full publication of new species names with morphological descriptions linked to online databases including genetic sequences, coupled with the archiving of specimens and tissue samples in museum collections in perpetuity. This solution has some major advantages over (1):
    a. Archiving of data and specimens at online data systems and museums creates an *iterative growth in knowledge* rather than a ‘snapshot’ that will likely become out of date
    b. Full taxonomic data allows the creation of long-lasting and reliable field-guides, and the connection of DNA barcodes to known names. This enables DNA-based identification either of whole animals or potentially based on metabarcoding (eDNA) approaches.
    c. Full taxonomic data allows future studies, e.g. of temporal change, to use the same taxonomy and thus measure change over time, a critical component of all EIS
    d. New species names and the long-term use of them enables communication of findings as well as enabling an understanding of the natural environment by the general public

One possible way forward to delivering taxonomic data from the CCZ is to emphasize a quality-over-quantity approach and to develop funding streams for small-scale quality taxonomic projects that are achievable in budget and scope. Targets could be set each year for new taxonomic descriptions (e.g 50 new species/year) with costs spread amongst contractors and administered through a competitive fund. Taxonomists could propose small-scale taxonomic projects based on material already collected and linked to DNA sequences. It would also be important to enable this from a bottom-up approach, i.e. allowing the expertise in the community to organize the priority taxonomic goals rather than dictating this from 'the top'.

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h. eDNA –

Environmental DNA surveys of metazoan diversity across abyssal substrates and habitats within the CCZ

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Introduction

Abyssal seafloor assemblages within the CCZ harbor high diversity but low densities of animals, due to low POC flux and food limitation in this broadly oligotrophic to mesotrophic ocean ecosystem. Given the remoteness of the habitat (3800-5300 m water column depths), the area is challenging to comprehensively sample for baseline ecological surveys prior to polymetallic nodule mining using conventional survey techniques. There is need for survey methods that can assess community diversity, composition, and spatial variability of abyssal communities at a range of spatial scales in a timely and cost-effective manner.

Environmental DNA, or eDNA, biodiversity surveys could circumvent some of the challenges of comprehensively sampling these diverse deep ocean habitats. eDNA metabarcoding analyses involve whole-community amplicon sequencing of DNA sampled from seawater, sediments, and nodules with the aim of identifying the suite of taxa present within the target habitat. eDNA survey methods have proven useful for baseline environmental assessments in other applied contexts, for example monitoring fish farming or oil drilling impacts on the benthos (Lejzerowicz et al. 2015, Laroche et al. 2016), due in part to high sensitivity to detect changes in community composition and structure as well as relatively rapid environmental assessments. In the deep sea, an eDNA approach could facilitate detection of rare and/or cryptic taxa within habitats, enabling more comprehensive surveys than can be achieved using conventional methods alone (Bik et al. 2012, Boschen et al. 2016). Because many species in the abyssal CCZ are currently undescribed (e.g., Amon et al. 2016, Bonifácio et al. 2019), whole community sequencing also provides a particularly valuable baseline assessment of the community that has limited dependence on taxonomic species descriptions, and that could readily be repeated following mining disturbance. Regarding the goals of this workshop report, eDNA data could be informative for assessments of diversity, biogeography, and connectivity among habitats across the CCZ, including exploration of mining areas and APEIs.

eDNA research in the CCZ is at an early stage, and no metazoan datasets have yet been published. Several research groups have begun eDNA research surveying metazoan diversity in sediments, seawater and on polymetallic nodules, and several groups are also using metabarcoding methods to assess diversity in whole community biological samples (e.g., meiofauna, meroplanktonic larvae, midwater zooplankton; Kersten et al. 2019, Macheriotou et al. 2019, Martinez-Arbizu et al. in prep).

We provide here an overview of existing unpublished eDNA data, current methods, and preliminary results and syntheses. One primary goal of this report is to facilitate standardization of methods in order to encourage intercomparable datasets that are necessary for large spatial scale syntheses in future work.

Sampling overview – Current and ongoing datasets

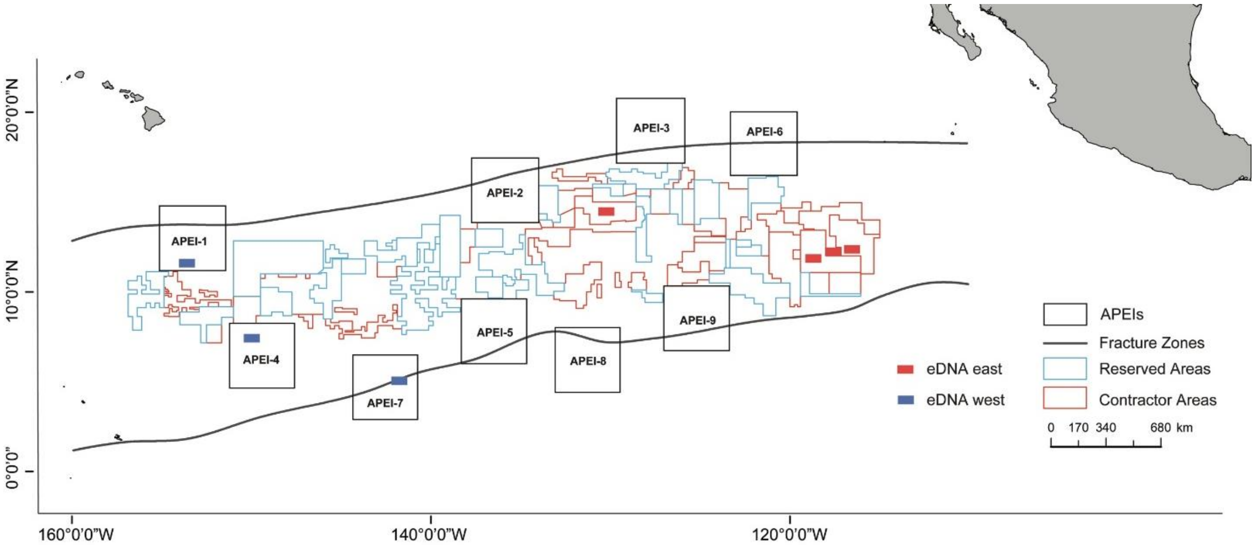

Figure 1. Overview of sampling locations for eDNA biotic surveys within the CCZ. Regions sampled in APEIs 1, 4 and 7 are indicated in blue (DeepCCZ; Goetze/Laroche, eDNA west), regions sampled in UK1, OMS1, BGR and IFREMER claim areas are marked in red (ABYSSLINE and other programs; Lejzerowicz/Gooday, eDNA east). Base map – Laroche.

Western CCZ – Sediments, polymetallic nodules and seawater were sampled within APEIs 1, 4, and 7 (Fig. 1). Sediments and nodules were collected by ROV push cores (7 cm diameter) or with the ROV manipulator arm (nodules, scooped to biobox), with 3 dives in APEI7, 4 dives in APEI4, and 2 dives in APEI1. Sediment cores were collected with 2 biological replicates in close proximity, and 2-5 cores were collected for eDNA on each ROV dive. Sediments were subsampled for eDNA in 0-2 cm and 3-5 cm sediment intervals (sterile 60 ml Falcon tubes), homogenized, and DNA extracted from 10-g of sediment for each sediment interval. Remaining sediment was preserved in DESS (Yoder et al. 2006, Fonseca and Fehlauer-Ale 2012), for meiofaunal extraction and comparative studies (organismal versus eDNA). Seawater samples were collected by conductivity-temperature-depth (CTD) casts with Niskin bottle rosette sampling at 7 depths between the sea surface (5 m) and the seafloor (5 mab, meters above bottom). 5-6 replicates were obtained on each cast at each depth (1-5L filtered per replicate), with seawater filtered onto 0.2 µm sterile Supor filters.

Eastern CCZ – Sediment samples were collected in the eastern CCZ in the UK-1, OMS, BGR and IFREMER claim areas (Fig. 1). A nested sampling design was employed with up to 2 cores sampled per multicorer deployment (station), with up to 3 subsamples within each core. 2 g of sediment were sampled from the surface for each sample/subsample.

Ideal sampling designs combine use of larger sample volumes (>10g sediment, >10 L of seawater filtered), with both technical and biological replication (replicate PCR reactions, replicate samples or subsamples; Lejzerowicz et al. 2014, Laroche et al. in review). Each study also faces necessary trade-offs due to overall cost and the need to sample across habitat heterogeneity and patchiness in the abyss. Results to date indicate under-sampling of diversity at all scales (e.g., Fig. 2), even with large sample volumes (10 g sediment, 10 L of seawater filtered), with the majority of alpha diversity unique within each biological replicate. Increasing the amount of material processed is important to effectively capturing the diversity of these systems (e.g., also see Nascimento et al. 2018)

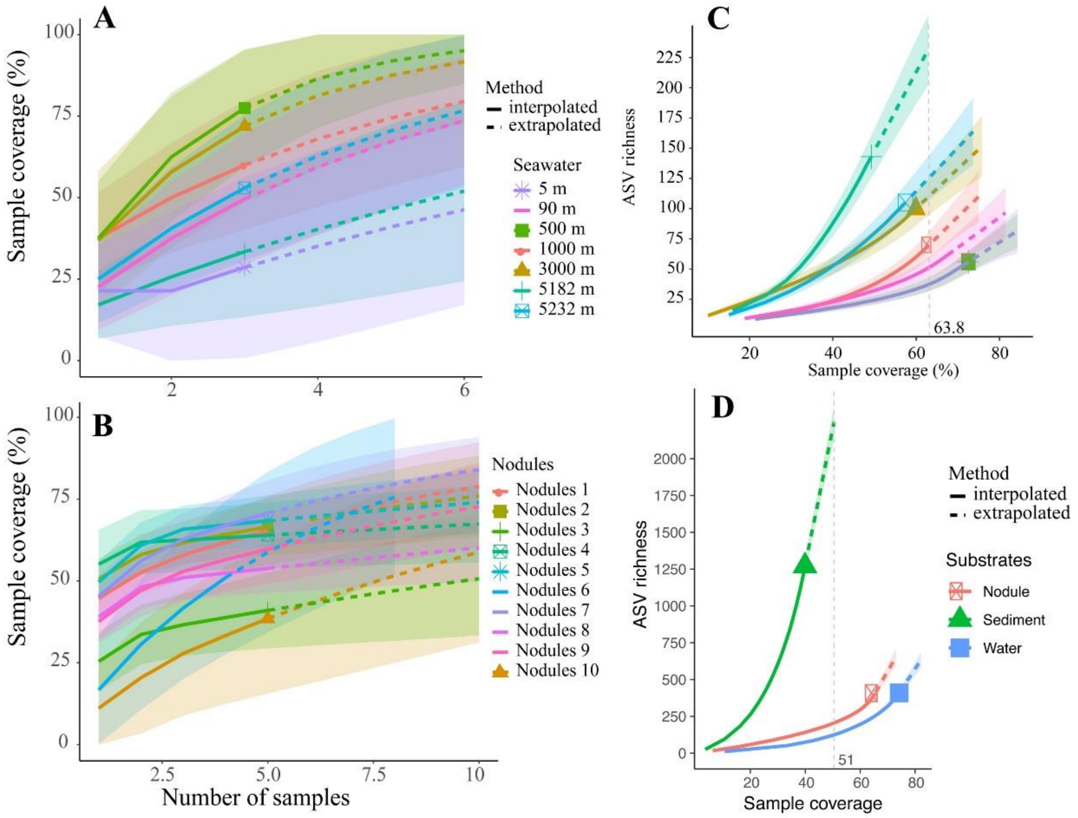

Figure 2. Sampling coverage achieved for alpha and gamma diversity in the western CCZ (APEIs 1, 4, 7), based on 18S V4 amplicon sequencing. Sampling coverage is a measure of sampling completeness as described in Chao and Jost (2012). (A, B) Alpha diversity. Sampling coverage obtained across biological replicates of seawater samples (3 replicates each of 7 water column depths) (A), and polymetallic nodule (B) samples (5 replicates each of 10 nodules). (C, D) Gamma diversity. Amplicon sequence variant (ASV) richness as a function of sampling coverage study-wide for seawater samples (C; 12 CTD casts, symbol and color as in A), and across abyssal substrate types, polymetallic nodules, sediments, and BBL seawater (D). In (A) 5232m is 5 meters above bottom (mab) and 5182m is 50 mab. In C, D, the dotted line marks base coverage, the highest coverage value between minimum extrapolated values and maximum interpolated values (e.g., *see* Chao et al. 2014). Data from DeepCCZ – Laroche, Kersten, Smith, Goetze (in review).

Methodological approaches – Data generation

Standardization of methodological details in data generation is essential to large-scale integration and analysis of data from multiple research groups at the CCZ-wide scale. To facilitate communication across research groups and enable ongoing and future eDNA research to be conducted with inter-comparable methods, we provide methodological information regarding the unpublished eDNA data that has been generated within the CCZ to date.

Goetze, Laroche, et al. – Western CCZ: PCR amplifications targeted (1) the V4 region of 18S using primers Uni18SF: 5′-AGG GCA AKY CTG GTG CCA GC-3′ and Uni18SR: 5′-GRC GGT ATC TRA TCG YCT T-3′, designed to target marine invertebrates (Zhan et al. 2013), and (2) mitochondrial cytochrome *c* oxidase subunit 1 (mtCOI), using universal metazoan primers mlCOIintF: 5′-GGW ACW GGW TGA ACW GTW TAY CCY CC-3′ and jgHCO2198: 5′-TAI ACY TCI GGR TGI CCR AAR AAY CA-3′ (Geller et al. 2013, Leray et al. 2013). Annealing temperatures during polymerase chain reaction (PCR) amplification were 50 °C for COI and 55 °C for 18S V4.

Lejzerowicz et al. – Eastern CCZ: PCR amplifications targeted (1) the V1-V2 region of 18S using primers R22mod [5′- CCT GCT GCC TTC CTT RGA -3′] and F04 [5′- GCT TGT CTC AAA GAT TAA GCC -3′] for deep-sea metazoa (Blaxter et al. 1998, Fonseca et al. 2010, Sinniger et al. 2016), (2) the V4 region of 18S using primers TAReuk454FWD1 [5′- CCA GCA (G ⁄ C)C(C ⁄ T) GCG GTA ATTCC – 3′] and TAReukREV3 [5′- ACT TTC GTT CTT GAT (C ⁄ T)(A ⁄ G) A – 3′] (Stoeck et al. 2010) as eukaryote-universal primers (Pawlowski et al. 2012), and (3) the mitochondrial COI gene (mtCOI) using primers mlCOIintF [5′- GGW ACW GGW TGA ACW GTW TAY CCY CC -3′] and dgHCO2198 [5′- TAA ACT TCA GGG TGA CCA AAR AAY CA -3′] (Leray et al. 2013).

Bioinformatic processing and data archiving – key points:

- This field moves very quickly, with continuous improvements in analytical methods. Best-practice bioinformatic methods will certainly change in the near future. A review of current methods is necessary at the start of every project.
- There is often a long tail of rare ASVs/OTUs (unique genetic variants/clustered operational taxonomic units) known to contain real, biologically rare organisms as well as spurious reads: distinguishing these is difficult. Because the majority of ASVs/OTUs in metabarcoding datasets are rare, decisions regarding how these taxa are handled can have a large impact on data interpretations. The majority of species in abyssal environments also are rare (e.g., Llodra-Ramirez et al. 2010), and entirely excluding or failing to interpret these rare ASVs/OTUs is not advisable.
- Taxonomic assignment or classification of reads remains challenging for abyssal samples, due to the limited reference sequences available from the deep ocean (marker specific). Ongoing research in DNA taxonomy is critically important to improve taxonomic assignment of eDNA reads.
- Given the importance of baseline studies, public release of all data is important. It is certain that re-analysis of these data will be required in the longer-term, and both raw data files and comprehensive metadata need to be appropriately archived so that pre- and post- mining analyses can be conducted on the raw data files with contemporary bioinformatic and data analysis methods. Continuous advances in reference databases due to DNA taxonomy research will also mean that re-analyses of baseline metabarcoding data may yield greater classification and more ecological insights in the future.

Data Available for Analyses

Table 1. Overview of eDNA metabarcoding data that has been generated from material collected within the CCZ. Authors: FL – Franck Lejzerowicz and colleagues, GL – Goetze/Laroche and colleagues. Markers listed are COI = cytochrome *c* oxidase subunit 1, 18S rRNA V1-V2, and 18S rRNA V4 regions. Nod = nodules, Sed = sediments, * = both RNA and DNA sequenced per sample. Regions sampled are as shown in Figure 1. Numbers listed include both samples and replicates.

	Author	***COI Nod***	***COI Sed***	***COI Water***	***18S V1-2 Nod***	***18S V1-2 Sed***	***18S V1-2 Water***	***18S V4 Nod***	***18S V4 Sed***	***18S V4 Water***
BGR	FL	-	45*	-	-	44*	-	-	-	-
IFREMER	FL	-	3*	-	-	3*	-	-	-	-
OMS1	FL	-	65*	-	-	66*	-	-	46*	-
UK1	FL	-	65*	-	-	65*	-	-	47*	-
APEI1	GL	30	18	42	-	-	-	30	18	42
APEI4	GL	20	33	28	-	-	-	20	33	28
APEI7	GL	-	20	28	-	-	-	-	20	28

Results and Discussion

Preliminary results reported here provide insight into broad-scale patterns in biodiversity and biogeography across the CCZ based on eDNA survey results. Because the spatial scale of sampling for eDNA is limited at present, and we do not have published data spanning across APEIs and exploration claim areas, we are not in a position to address the overarching workshop goal of determining whether the APEIs are representative of the exploration contract areas. Nonetheless, the results below are useful to consider in reference to large-scale patterns across the CCZ.

Biodiversity – how does it vary along and across the CCZ?

One primary observation is that assemblages sampled in sediments, on nodules, and in seawater of the benthic boundary layer (BBL) are highly distinct, with little overlap in the ASVs (18S) or OTUs (COI) found in association with these abyssal substrate types (at equivalent sampling coverage, Laroche et al. in prep). This observation is important in the context of using eDNA metabarcoding for biomonitoring surveys, as it confirms that eDNA has sufficient power to detect distinct nodule-attached and sediment communities that have been described in prior work (e.g., Veillette et al. 2007, Vanreusel et al. 2016). The taxonomic resolution obtained in 18S rRNA eDNA surveys is also comparable to or higher than that of image-based survey methods (e.g., ROV video transects for megafauna).

A second primary observation is that ASV (18S) and OTU (COI) metazoan richness is far higher in sediments than in nodules or BBL seawater, at equivalent sampling coverage (Fig. 2D). Much of this diversity is in small soft-bodied taxa that are difficult to identify or sample effectively using conventional methods (e.g. Platyhelminthes, Nematodes).

In terms of large spatial-scale patterns, we find higher ASV/OTU richness in APEIs 4 and 7, which receive higher POC flux in comparison to APEI1 (e.g., see habitat modeling report/Howell & McQuaid). In APEI4, material also was collected in a region with both polymetallic nodules and soft sediment assemblages (Laroche et al. in prep). Although somewhat limited in spatial extent, these results are concordant with other evidence that both POC flux and nodule abundance are key environmental drivers of community diversity, composition, and animal abundance in the CCZ (e.g. Simon-Lledo et al. 2019). (Data in Laroche et al. in prep).

Biogeography – are species ranges large compared to distances between APEIs?

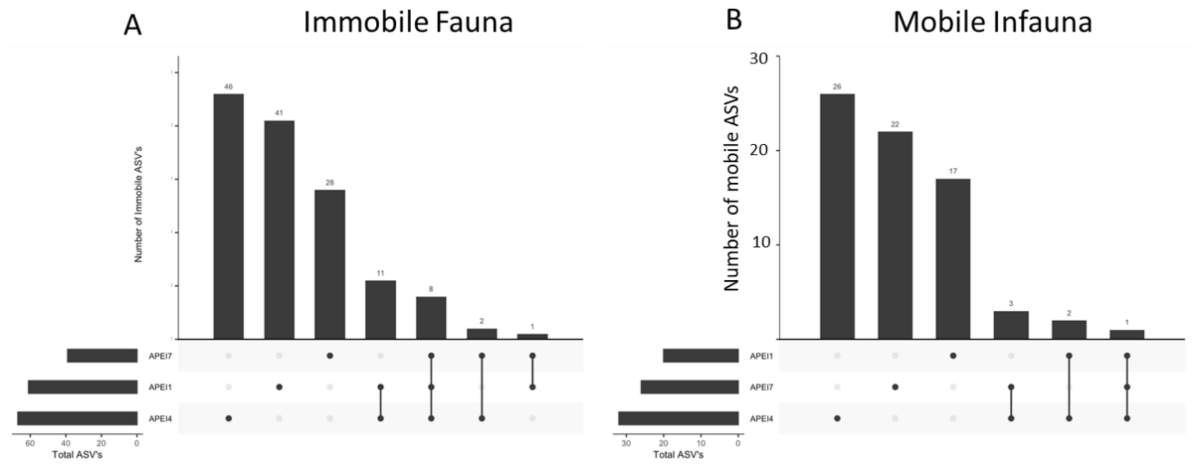

Figure 3. UpSet plots illustrating faunal sharing and range distributions across APEIs 1, 4, and 7 in the western CCZ for (A) immobile fauna, and (B) mobile infauna. Data are from COI OTUs clustered at 97% for putative species-level taxonomic resolution. Immobile fauna includes sponges, crinoids, hard corals, anemones, sea pens, soft corals, and tube-dwelling polychaetes. Mobile infauna includes hemichordates, nemerteans, bivalves (not mussels), gastropods, non-tube-dwelling polychaetes, and meiofaunal groups (rotifers, harpacticoids, gastrotrichs, nematodes, tardigrades, kinorynchs, xenocoelomorpha).

The overwhelming majority of benthic taxa detected by eDNA in our data were found to be unique to one of the APEIs, regardless of body-size, phylum or mobility type. On the order of 7-16% of OTUs were shared across multiple APEIs, providing evidence of range distributions on the scale of 100s to 1000s of km, sufficiently large to span across APEIs and exploration claim areas. For example, in figure 3 above, 84% of immobile fauna, including many nodule-attached organisms that require hard substrate, were unique to one APEI, while 16% were shared across 2 or more APEIs (figure 3A). Although we might expect mobile infauna to have broader range distributions, we find that 92% are unique to an APEI, while 8% are shared across 2 or more APEIs. All mobile epifauna sampled and classified at COI (e.g., echinoderms, not including crinoids, & decapods) were found to be unique to a single APEI (results not shown). Comparable analyses conducted with 18S data find similar patterns, with 87-92% of ASVs unique to a single APEI for these same mobility categories (immobile fauna, mobile infauna, mobile epifauna).

Although eDNA can support range distribution mapping of putative species (COI OTUs, clustered at 97% similarity) in principle, our current material greatly under-samples the diversity present. We interpret our results to mean that the probability of detecting species, even if they are present in any given region, is fairly low. Such results are not unique to eDNA data types. The majority of species in abyssal sediment habitats of the CCZ are rare, often with limited overlap observed in community composition among regions (e.g. see the meiofaunal report section). Inferences of range distributions would be greatly enhanced by a large-scale CCZ synthesis including data from multiple programs and investigators. Application of occupancy modeling in such a synthesis may increase our understanding of species distributions inferred from eDNA data (e.g., Doi et al. 2019).

In sum, while we do find evidence of broad distributions for a small proportion of the community (~ 2-16% of OTUs), the majority of putative species sampled to date are restricted to a single APEI in our material, with very little faunal overlap among APEIs in the western CCZ.

Bathymetry – Seamounts as potential refugia

One primary goal of the DeepCCZ program was to evaluate whether deep seamounts within the CCZ could serve as refugia from mining disturbance on the abyssal plains, with seamount populations serving as source populations for larvae to the adjacent abyssal seafloor. We find evidence that sediment community composition on seamount summits is distinct from adjacent abyssal plains, with limited overlap in community composition (499 of OTUs [16 %] and 379 of OTUs [19 %] for APEIs 4 and 7, respectively; Laroche et al. in prep).

Several lines of evidence suggest that bathymetric habitat, abyssal plains vs seamounts, plays a dominant role structuring biological communities even at abyssal depths, and is therefore important to consider in the context of mining disturbance. Pre- and post-mining impact surveys should target bathymetrically equivalent habitats in order to control for this environmental variable. Our results also imply that preservation of communities on seamounts will not be sufficient for protecting abyssal assemblages more generally, as they are a very distinct habitat type and harbor distinct communities in comparison to areas targeted for nodule mining.

Synthesis Conclusions

1. We recommend adoption of eDNA metabarcoding as a standard assessment tool for monitoring metazoan biodiversity and biogeography in the deep ocean (CCZ). eDNA metabarcoding methods are complementary to conventional methods to assess these properties in the deep sea and have proven very effective for biomonitoring community change pre- and post-impact in other applied settings.
2. The diversity of metazoans in the deep ocean has been poorly studied using eDNA, with no published studies within the Clarion-Clipperton Zone. Unpublished data exists for the western APEIs (APEI1, 4, 7 – Goetze/Laroche) and eastern claim areas (BGR, IFREMER, OMS1, UK1 – Lejzerowicz). There are partially overlapping markers and primer sets for these existing data; broader-scale synthesis for metazoans will be possible (COI, 18S).
3. Standardization of methods is essential to large-scale spatial synthesis and integration of results across research groups. Achieving convergence on several key aspects of methods is an important goal (markers, primers) to support broad-scale analyses in the future.
4. Early results suggest that eDNA methods detect a wide range of diversity for organismal groups that are small, soft-bodied, and not typically included in conventional sampling and assessment methods (e.g., Platyhelminthes). The potential for important new insights into this diversity is high.
5. Biodiversity in sediments is very high in comparison to polymetallic nodules and BBL seawater, with the majority of OTUs/ASVs rare.
6. The majority of sediment ASVs/OTUs are sampled within only a single APEI (~ 90%, western CCZ), but 'cosmopolitan' taxa (present across all sampled sites) can be detected, and ranges can be mapped using eDNA methods. Preliminary results suggest very limited ASV sharing across regions (e.g., ~ 8-16% of ASVs shared among 2 or more of APEIs 1, 4, 7; Figure 3).

7. There are bathymetric and/or topographic influences on community composition as assessed by eDNA, with distinct communities on seamounts and nearby abyssal plains, but with some overlapping ASVs (Laroche et al. in prep). Consideration of bathymetric features will be important when using eDNA methods for biomonitoring pre- and post-mining, and in consideration of eDNA spatial sampling design.

Gaps and Limitations

1. All existing data are unpublished, and analysis is in progress. It is too early for cross-program synthesis of these data, but this could be possible within the next ~ year.
2. We are under-sampling diversity at all spatial scales in existing datasets.
3. Spatial coverage of sampling for individual programs is limited, and insufficient at present to assess representativity of APEIs in comparison to exploration claim areas.
4. Taxonomic classification is problematic for mtCOI reads, the most commonly used marker for putative species-level assessment of metazoans. Alpha-taxonomy combined with DNA barcoding of meiofaunal diversity should be a high priority to enhance classification of reads. More complete classification of reads will greatly enhance the power of eDNA methods.

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i. ECOSYSTEM FUNCTIONS –

DEEPCCZ Biodiversity synthesis workshop report: Ecosystem functioning

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Introduction

Deep-sea ecosystem functioning refers to the interactions that occur between abiotic and biotic elements of deep-sea ecosystems and habitats (Thurber et al. 2014). The concept of deep-sea ecosystem functioning is related to ecosystem services. Essentially, ecosystem functions, such as sediment community oxygen consumption (SCOC or respiration) and nutrient cycling, can be characterized outside a human context and may (but not all do) provide ecosystem services with direct or indirect human benefits (Thurber et al. 2014). The indirect benefits from SCOC and nutrient cycling include calcite dissolution (Berelson et al. 1997, Wenzhofer et al. 2001) that controls changes in ocean pH, and the production and release of nutrients that fuel surface ocean primary production and fisheries.

With the exception of deep-water chemosynthetic habitats, deep sea biological communities greatly depend on the transfer of carbon (C) and nitrogen (N) from surface waters (Smith et al 2008, Smith et al. 2009). The rate of transfer of C and N from the surface structures many characteristics of the deep seafloor, including, but not limited to benthic community structure (Smith et al. 2008, Corliss et al 2009, Wei et al. 2010, Durden et al. 2017, Sweetman et al. 2017) and benthic ecosystem functions, such as bioturbation (Smith et al. 1997, Smith and Rabouille 2002, Sweetman et al. 2017), C-cycling processes (Sweetman and Witte 2008, Sweetman et al. 2019), nutrient cycling (Hammond et al. 1996), and respiration (Berelson et al. 1997, C.R. Smith et al. 2008, K.L. Smith et al. 2009, Sweetman et al. 2017). The food supply to the seafloor, defined as the particulate organic carbon (POC) flux, often corresponds to only 0.5-2% of the total surface net primary production (Dunne et al. 2007, Smith et al 2008). As such, the deep sea is considered an extremely food limited environment with C and N limitation increasing with increasing water depth down to 6000 m depth.

In the Clarion-Clipperton Zone (CCZ), a north-south and east-west gradient in surface ocean primary production and POC flux exists (Lutz et al. 2007), with production and POC flux declining northwards from the equator as well as from east to west. The north-south gradient in POC flux in the central Pacific along the 140°W transect has been shown to strongly influence benthic processes including SCOC, opal dissolution, and nutrient fluxes (Hammond et al. 1996, Berelson et al. 1997).

The abyssal seafloor underlying the eastern equatorial Pacific receives an annual POC flux equivalent to ~ 20% of the flux at the equator (Berelson et al. 1997, Smith et al. 1997; Smith and Demopoulos 2003). The low POC flux to the seabed here allows the precipitation of manganese and iron oxides from pore waters forming polymetallic nodules at the seafloor that are rich in nickel and copper, as well as other commercially important metals (e.g., cobalt, molybdenum, and lithium) (International Seabed Authority 2010). Because of the sheer density of nodules in the Clarion-Clipperton Zone and the increasing difficulty of mining on land, the CCZ has become a prime area of interest for future resource extraction. If mining proceeds in the CCZ, nodule extraction will significantly disturb the seafloor environment (e.g., 600–800 km^2 of seabed per mining operation per year affected, Levin et al. 2016). This therefore represents a serious environmental issue, which is heightened by limited knowledge of the area. In particular, we know very little about how benthic ecosystem function processes change as a function of POC flux across the central Pacific and the Clarion-Clipperton Zone, and how representative benthic ecosystem functioning in the 9 Areas of Particular Environmental Interest (APEIs) are relative to similar processes in the contract areas.

The following report describes a meta-analysis that was carried out as part of the DEEPCCZ biodiversity synthesis workshop at Friday Harbor Laboratory (Univ. Washington) from the 1st – 4th of October 2019. We assessed how benthic ecosystem functioning changed as a function of POC flux across the central Pacific Ocean and the Clarion-Clipperton Zone to determine if benthic ecosystem functioning measurements made within the APEIs were representative to similar measurements made in other regions of the CCZ/ central Pacific.

Methods

Between November 2018 and August 2019, we undertook a thorough literature search gathering published literature data, and unpublished data on benthic ecosystem functioning measurements made in the central Pacific as well as the Clarion-Clipperton Zone. The data-set comprised benthic ecosystem functioning measurements from numerous sites (Fig. 1), which were made ex situ using recovered mega-cores and gravity cores, as well as measurements made in situ using benthic lander systems. Due to known experimental artefacts associated with ex situ measurements (e.g., decompression and warming effects, Glud et al. 1994, 1999), we focused our meta-analysis on benthic ecosystem function measurements made in situ.

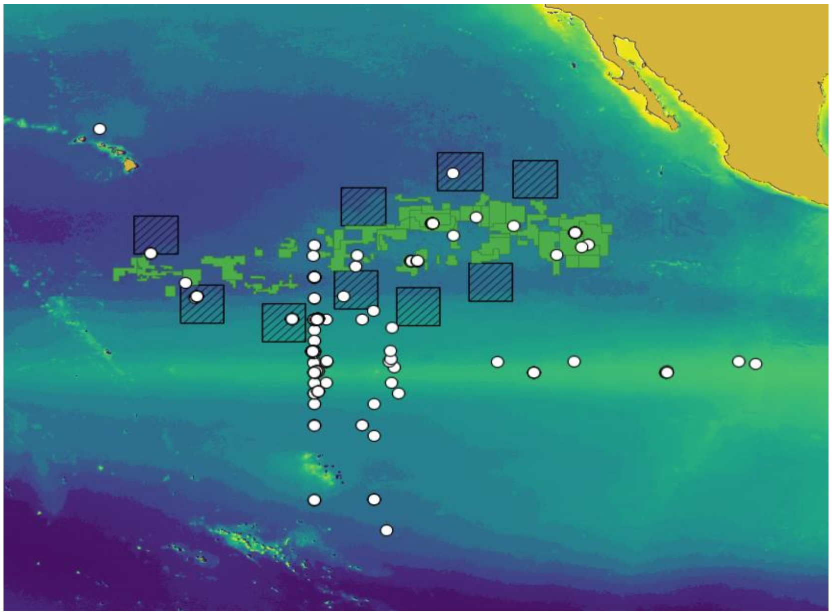

Figure 1. Locations (white circles) in the central Pacific and CCZ where benthic ecosystem function data was generated during our extensive literature search. The contract areas and APEIs are shown in green and grey striped boxes, respectively

We specifically focused on sediment community oxygen consumption (SCOC) and nutrient flux rates in our meta-analysis since more data-sets on these specific ecosystem functions were available (Fig. 2). SCOC is a commonly used state-of-the-art method to measure the overall benthic activity and biogeochemical process and thus seafloor ecosystem function. In situ measurements carried out directly at the seafloor are the optimal technique to quantify fluxes across sediment-water interface, since in situ hydrodynamics can hardly be reproduced in laboratories. Moreover, samples can be altered chemically, physically and biologically, while being sampled at the seafloor, brought to the surface and processed on board; core recovery will change oxygen penetration and availability (through core heating, pressure release and lysis of labile organic matter).

In situ exchange rates across the water-sediment interface are commonly measured by two approaches: 1) enclosed benthic chamber systems and 2) oxygen concentration profiles in the benthic boundary layer determined by microsensors. Fluxes measured by benthic chamber incubations following the decrease in water oxygen concentration over time represent the total oxygen consumption (= total flux; TF) including diffusion as well as advective oxygen transport across the sediment-water interface due to benthic organisms (micro-, meio-, macrofaunal) activity flushing their burrows with overlying water (bioirrigation). In contrast to total fluxes measured by chamber incubations, fluxes measured by microprofiling represent the diffusive oxygen consumption. The diffusive flux (DF) is calculated from the linear oxygen gradient in the diffusive boundary layer based on Fick's first law of diffusion, which states that the diffusive flux (J) is directly proportional to the concentration gradient ($\partial C/\partial x$) under steady state conditions; the factor of proportionality is the temperature- and substance-dependent molecular diffusion coefficient (D_0): $J = -D_0\ \partial C/\partial x$.

The ratio between the total and diffusive oxygen flux can be used as a measure of the benthic fauna-mediated oxygen consumption (TF – DF = fauna-mediated flux; e.g. Wenzhöfer and Glud, 2002). Thus, benthic chamber incubations represent the better flux estimation, but microsensor measurements allow an insight into the sediment providing information on the vertical zonation of biogeochemical processes. Comparing TF and DF measured at the same site during one deployment at a nodule site in the Peru basin (DISCOL) revealed similar fluxes using benthic chambers and microprofiles; thus we conclude that both methods can be used to estimate SCOC. This similarity in TF and DF is further confirmed from other deep-sea studies (e.g. Wenzhöfer & Glud, 2002; Glud et al., 2008), and suggests that benthic oxygen consumption in deep-sea sediments is dominated by microorganisms.

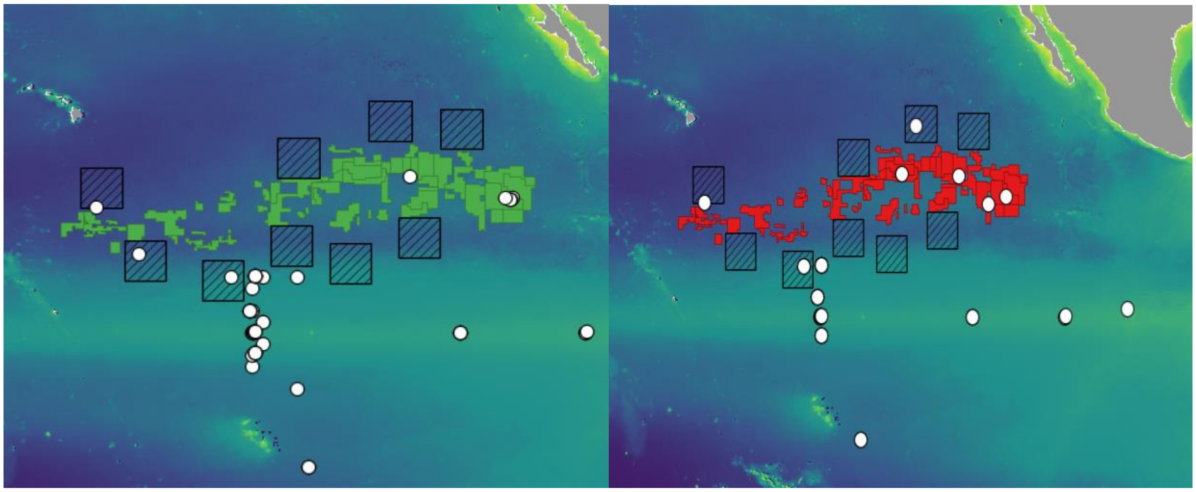

Figure 2. Locations for which SCOC (left) and nutrient flux (right) data were available in the central Pacific and CCZ.

Benthic nutrient fluxes (nitrate, phosphate, silicate) can be measured in situ by chamber incubations by isolating a specific volume of water and sediment, and measuring nutrient concentration changes over-time of target-compounds within the enclosed waterbody. Nutrient depletion or enrichment of the water inside the chamber is measured by collecting water samples with syringes at pre-programmed times and analyzing on board. Fluxes can be estimated by calculating the mean rate of change in concentration during the incubation period by regression analysis. This provides the total solute exchange across the sediment water interface.

Sedimentary POC flux can be estimated from modeled and extrapolated surface productivity maps (Lutz et al. 2007) and provides information on the food available for benthic organisms. Alternatively, organic carbon supply to the seafloor can also be derived from in situ measured SCOC data using a respiratory quotient (RQ) to convert oxygen fluxes into carbon equivalents.

We compared modeled sedimentary POC with SCOC-derived carbon equivalents (using a RQ = 0.85) and found a high uncertainty within the data set (Fig. 3). Therefore, we recommend that in situ POC flux information is generated during baseline surveys so that particle and oxygen fluxes can be compared directly. Additionally, information on POC quality is lacking. Therefore, an additional method could be to study sediment-bound chlorophyll a (Chl-a) and its degradation products, or phaeopigments. The ratio of Chl-a/ (Chl-a + phaeopigments) can be used as an indicator for the freshness of the settling material and may show a much better relationship to SCOC.

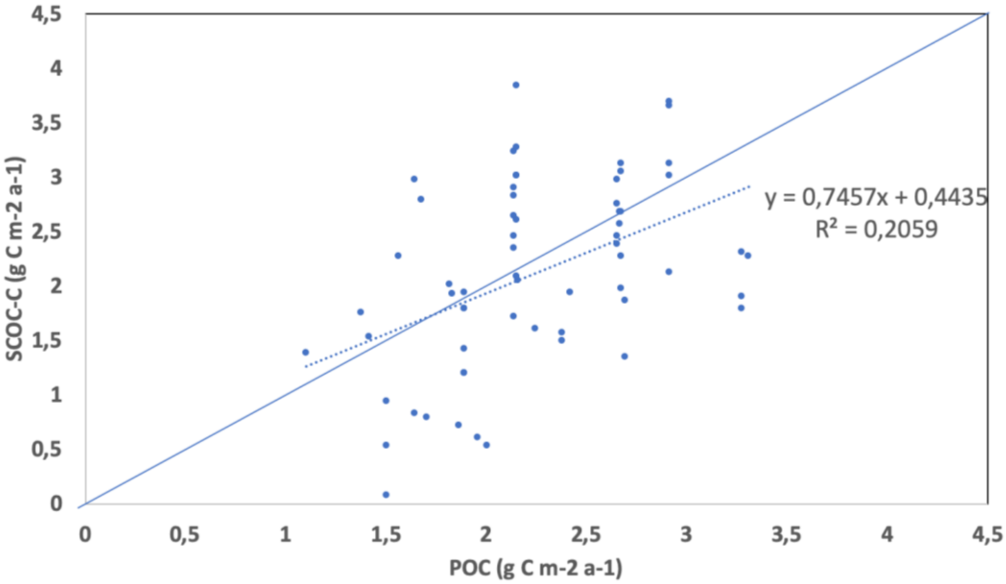

Fig. 3. Relationship between annual estimated POC fluxes (based on Lutz et al. 2007) and annual SCOC rates. The solid line is the 1:1 relationship between SCOC and POC flux

We compared SCOC and nutrient flux data from the central Pacific and CCZ together with modeled POC flux data by regression analysis to quantify how POC flux modified SCOC and nutrient cycling at the abyssal seafloor. We further explored if there were any relationships between SCOC and nutrient flux and nodule abundance. To do this, we first carried out Aikake Information Criterion analysis to determine which polynomial models best fit the data-sets of 1) POC vs. SCOC or nutrient fluxes and 2) nodule abundance vs. SCOC or nutrient fluxes. Once the best model was selected, we analyzed the data to test for significant relationships between the independent (POC or nodule abundance) and dependent variables (SCOC or nutrient fluxes). Multiple regression analysis was used to assess which independent variable had the largest influence on the benthic ecosystem function data-sets. All data sets were analyzed for normality and heteroscedasticity and transformed when data-sets failed to meet parametric assumptions. All data was analyzed using the statistical programming language platform "R".

Results

(1) Ecosystem functions across CCZ

SCOC in the central equatorial Pacific was positively correlated with POC ($F_{1,\,57}$=9.123, p=0.004), and explained 21% of the variability in the SCOC data (Fig. 4). However, when only measurements from the CCZ were considered, the relationship between SCOC and POC was still visible, but no longer significant (p=0.21; Fig. 4). SCOC was not significantly related to nodule abundance (p=0.39; Fig. 4, 5), though we lacked SCOC data from areas with moderate to high nodule coverage, so caution must be taken when interpreting this result.

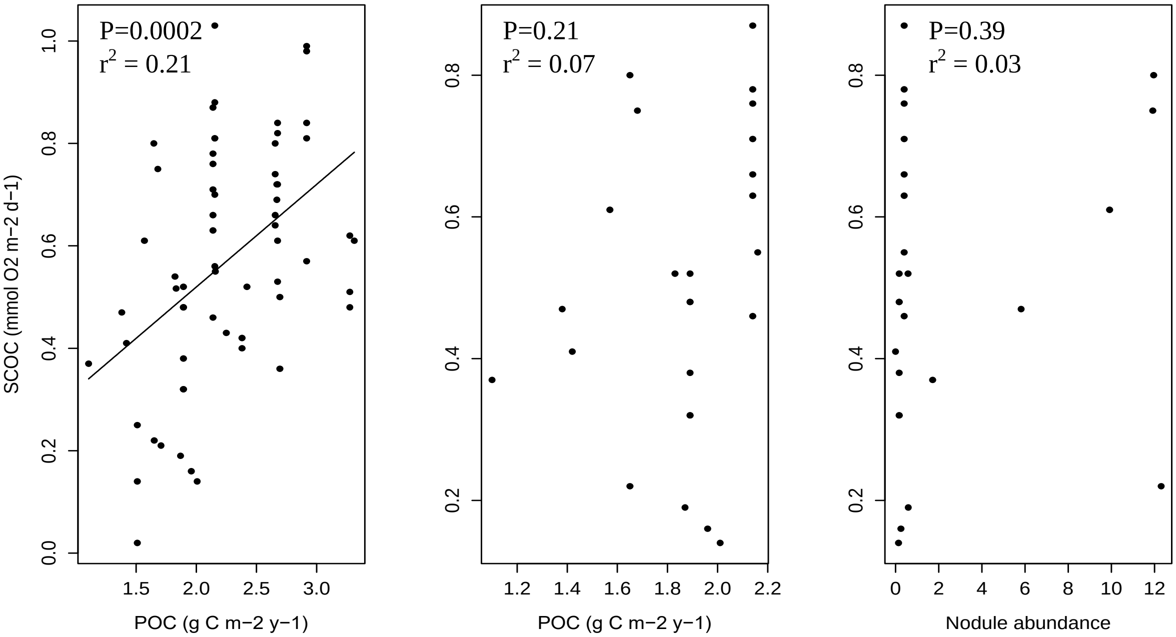

Fig. 4. Relationship between POC (in g C m^{-2} yr^{-1}) and SCOC (in mmol O_2 m^{-2} d^{-1}) in the central equatorial Pacific (left) and the CCZ (middle), and relationship between nodule abundance (kg nodules m^{-2}) and SCOC (in mmol O_2 m^{-2} d^{-1}).

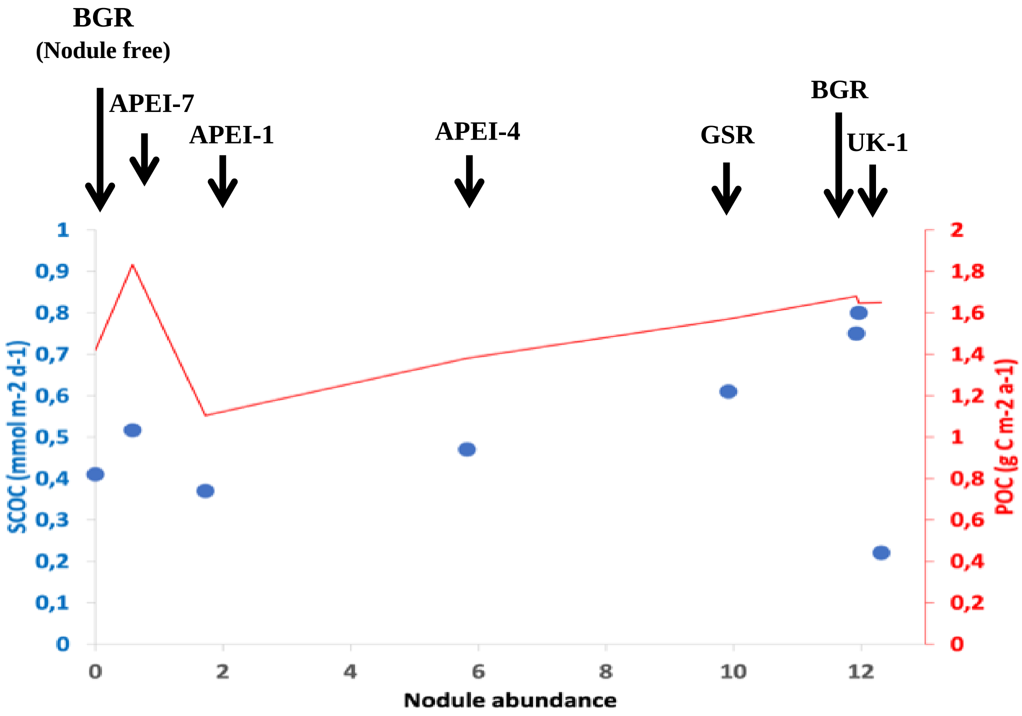

Fig. 5. Impact of nodule abundance on SCOC (left y-axis) in different contractor areas and APEIs of the CCZ. The right line shows the POC flux at each specific sampling point and shows that SCOC at higher nodule densities is related to higher POC fluxes, which was also confirmed by multiple regression analysis.

Phosphate and silica fluxes were significantly and positively correlated with POC flux in the central equatorial Pacific (phosphate: $F_{1,\ 47}$=9.434, p=0.004; silica: $F_{1,\ 47}$=19.06, p<0.001; Figs. 6 & 8). However, there was no significant relationship between nitrate and POC flux in the central equatorial Pacific (p=0.28; Fig. 7). In the CCZ, the positive relationship between phosphate and POC was still significant ($F_{1,\ 17}$=23.75, p<0.001), but the relationship between silica and POC and between nitrate and POC were not significant when only datasets from the CCZ were analyzed ($p_{nitrate}$=0.28, p_{silica}=0.34). In the CCZ, nodule abundance only had a significant influence on phosphate fluxes ($F_{1,\ 17}$=15.62, p=0.001, Fig. 6). However, as with the SCOC data we lacked phosphate and silicate flux data from areas with moderate to high nodule coverage so caution must again be taken when interpreting these results.

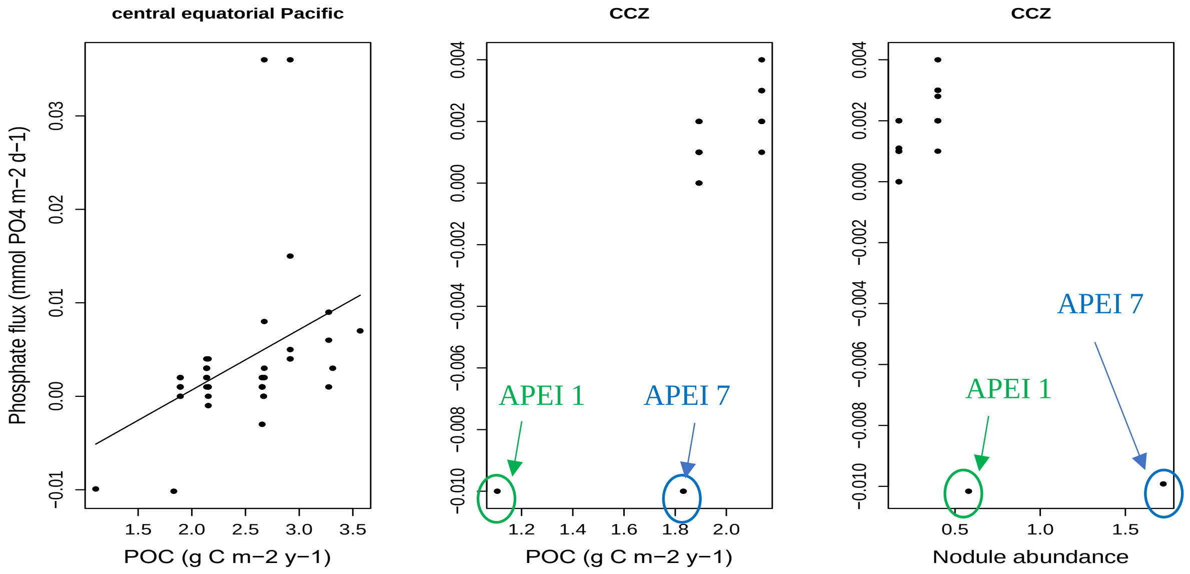

Fig. 6. Relationship between POC (in g C m^{-2} yr^{-1}) and phosphate flux (in mmol PO_3^{4-} m^{-2} d^{-1}) in the central equatorial Pacific (left) and the CCZ (middle), and the relationship between nodule abundance (kg nodules m^{-2}) and phosphate flux.

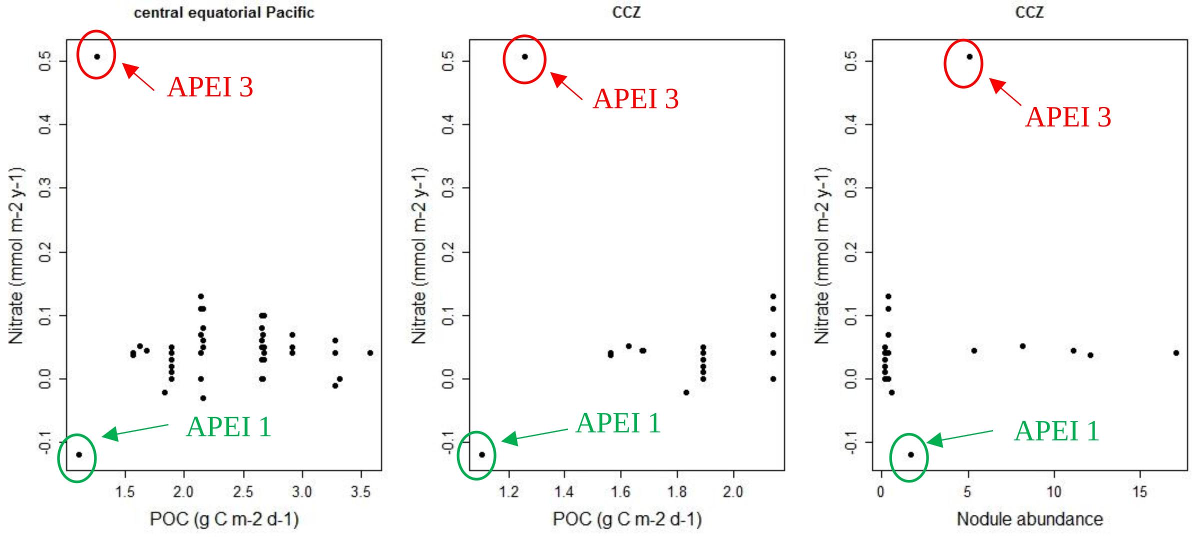

Fig. 7. Relationship between POC (in g C m^{-2} yr^{-1}) and nitrate flux (in mmol NO_3^- m^{-2} d^{-1}) in the central equatorial Pacific (left) and the CCZ (middle), and the relationship between nodule abundance (kg nodules m^{-2}) and nitrate flux.

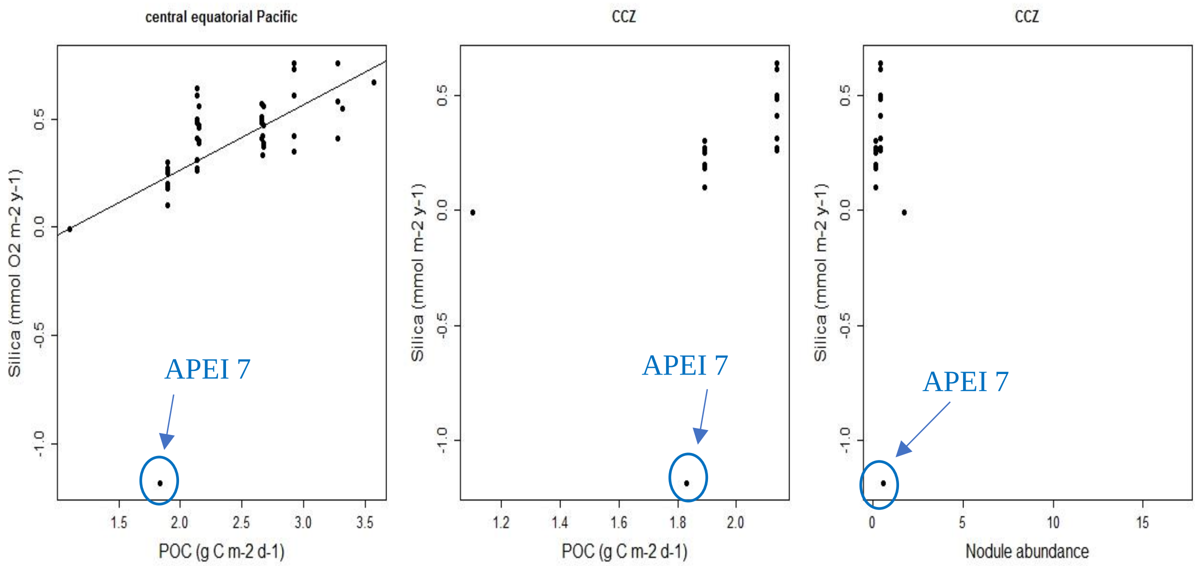

Fig. 8. Relationship between POC (in g C m^{-2} yr^{-1}) and silica flux (in mmol Si m^{-2} d^{-1}) in the central equatorial Pacific (left) and the CCZ (middle), and the relationship between nodule abundance (kg nodules m^{-2}) and silica flux.

(2) Ecosystem functions comparison APEIs vs contractor areas

When comparing the APEIs vs. contractor areas, the limited data showed higher SCOC rates in the contractor areas than in the APEIs (Fig. 9 left), but this trend was related to the west-east gradient in POC flux with higher POC fluxes in the eastern CCZ compared to the western CCZ (Fig. 9 right).

Nutrient flux analysis showed that the regression model of phosphate fluxes vs. POC in the CCZ was strongly influenced by phosphate fluxes measured in APEI 1 and APEI 7 (Fig. 6). Silica fluxes in APEI 7 had the largest influence on the relationship between silica fluxes and POC in the CCZ (Fig. 8) and nitrate fluxes in the APEI 1 and APEI 7 also influenced the (non-significant) relationship between nitrate and POC (Fig. 7).

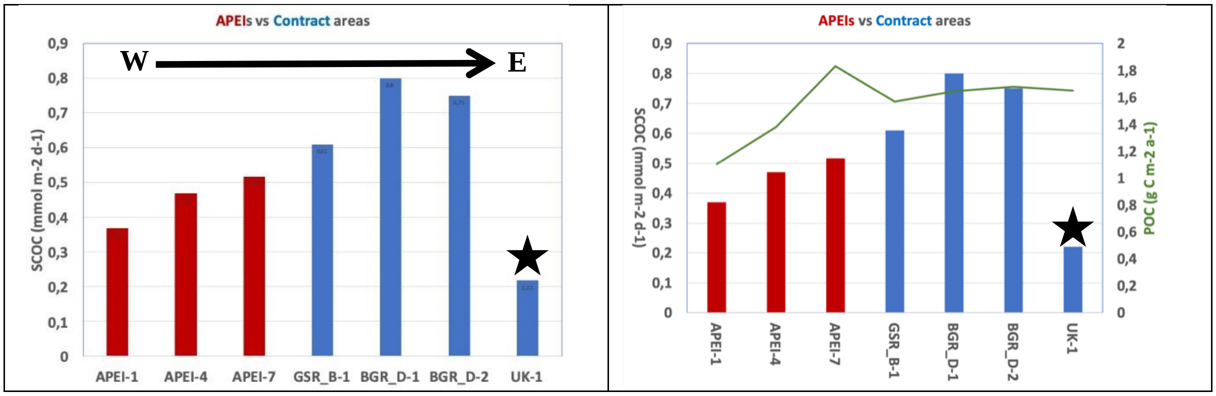

Fig. 8. Individual SCOC measurements in APEIs (red bars) and contractor areas (blue bars) along a west-east gradient in the CCZ. The blue bar marked with a star might be an artefact.

Discussion, conclusions and knowledge gaps

The primary aim of the meta-analysis was to assess how benthic ecosystem functioning varied as a function of POC flux and nodule abundance in the central Pacific Ocean, as well as contract areas and APEIs in the Clarion-Clipperton Zone. However, due to limitations in the amount of data that were available, we were only able to examine how sediment community oxygen consumption (SCOC) and nutrient fluxes across the sediment-water interface varied as a function of POC flux and nodule abundance.

The SCOC data-sets used comprised in situ data collected by benthic chamber landers as well as micro-profiling landers. SCOC rates quantified using the different approaches did not reveal any major differences suggesting that the quality of the SCOC rate data gathered from benthic chambers and microprofiles was of the same quality. SCOC rates quantified across the central equatorial Pacific showed a highly significant relationship with modeled POC flux data, which has been shown in numerous deep-sea studies from other regions (C.R. Smith et al. 2008, K.L. Smith et al. 2009), though the amount of variance explained by POC flux was quite low ($r^2 = 0.21$). This suggests that other factors were also influencing benthic respiration and could have included differences in POC quality as well as using modeled as opposed to measured POC fluxes. We therefore recommend that both POC fluxes to the seafloor and POC quality are quantified during baseline investigations of contract areas. Multiple regression analysis on SCOC rates measured across the central Pacific revealed that POC flux was the only significant factor ($p=0.006$) affecting benthic respiration rather than nodule abundance. However, caution must be taken when interpreting the relationships between functions and nodule abundance since our in situ ecosystem function data largely came from areas with little nodule cover (<10-15 kg m^{-2}). Therefore, relationships across a range of nodule coverage were not possible to assess. Also, we cannot exclude the possibility that nodules may modify SCOC rates through their effect on bottom water current flow and particle and organic C deposition dynamics, though this needs to be assessed in follow-up studies.

When only the CCZ data sets were considered, a positive relationship between SCOC and POC flux was still apparent, but the relationship was no longer significant and less variance in the SCOC rates could be explained by modeled POC flux. This highlights that more in situ data on benthic respiration is needed for the CCZ. Nevertheless, SCOC rates seem to increase from the western CCZ to the eastern CCZ albeit with the exception of a single SCOC measurement from the UK1 contract area. SCOC rates measured in situ in APEI 1,4, and 7 were within the same order of magnitude as SCOC rates measured in the other areas of the CCZ, albeit they were lower than SCOC rates documented in the more productive central and eastern CCZ. Nevertheless, more SCOC rate information will be required from contract areas situated closer to APEI 1, 4, and 7 to be able to conclusively state whether SCOC in these particular APEIs is similar to SCOC rates in nearby contract areas.

Opal fluxes to the seafloor have been shown to be higher in high POC flux areas situated along the 140°W EQPAC transect (Berelson et al. 2007). Statistically significant relationships were detected between benthic phosphate and silica fluxes and modeled POC flux when the central equatorial Pacific data-set was interrogated (Figs. 6,8). The higher silica flux across the sediment water interface in regions of high POC flux is most likely related to higher fluxes of siliceous POC settling to the seafloor and its subsequent remineralization in regions of high POC flux. While statistically significant relationships between silica and phosphate flux and POC supply were seen in the larger data-set, neither silicate, phosphate or nitrate flux was significantly related to POC flux nor nodule abundance when only the CCZ data-set was analyzed. This highlights both the need for more nutrient flux data in this region, and the possibility that benthic nutrient cycling may possibly not be related to seafloor nodule cover. Although data on silicate, phosphate and nitrate fluxes in the APEIs is minimal and limited to only APEI 1, 3, and 7, phosphate fluxes in APEI 1 and 7, nitrate flux in APEI 1 and 3 and silicate fluxes in APEI 7 were distinct (based on Cook's Distance's) relative to fluxes in other contract and non-contract areas of the

CCZ. While this appears to suggest that benthic nutrient cycling processes may be different in these particular APEIs, the limited amount of data available on benthic nutrient fluxes in these areas and other contract areas makes this presently difficult to conclude.

Summary of conclusions

1) SCOC was positively and significantly related to POC flux across the region, while multiple regression analysis showed that nodule abundance did not seem to exert much of an effect on SCOC. However, caution needs to be taken when interpreting this result for the reasons outlined above. The abundance of nodules may have an effect on sediment accumulation around the nodules and the availability of POC at the seafloor and lead to a secondary effect on benthic ecosystem function. This requires further study.

2) Only 20% of the variance in SCOC is explained by modeled POC flux, suggesting other factors (e.g., organic matter quality) also need to be measured in baseline studies.

3) The significant relationship observed between SCOC and POC flux does not hold when only data-sets from the CCZ are used. Although a positive relation is still observed it highlights the need for more data on benthic ecosystem function in the region.

4) The different in situ methodologies used to measure SCOC (benthic chambers, micro-profilers) do not appear to have an effect on SCOC rates, and we recommend that nodule volume data is consistently collected when benthic ecosystem functioning studies are carried out using benthic chambers.

5) Available in situ SCOC rates from the western APEIs are within the range of flux measurements from the central equatorial Pacific and CCZ, but are at the low end of the scale. There appears to be an increase in SCOC from the western to eastern CCZ (with the exception of SCOC rates from UK1). Currently, it is not possible to assess if the SCOC rates measured in the APEIs 1, 4, and 7 are similar to SCOC rates in contract areas situated nearby.

6) In terms of nutrient fluxes, we see a significant and positive effect of POC flux on silica and phosphate fluxes across the central equatorial Pacific, but not nitrate. These significant relationships are not seen when data from the CCZ are plotted against POC flux and nodule abundance, which highlights the need for more benthic ecosystem functioning data in the region.

7) In terms of the silicate, phosphate and nitrate fluxes, flux estimates from APEIs 1, 3, and 7 appear to be outliers (confirmed from Cook's Distance analysis) when compared to the available flux data from other areas. However, due to the limited number of APEI data-sets available, it is not possible to robustly conclude whether benthic function (nutrient fluxes) within these and other APEIs are unique/ similar with respect to the greater CCZ/ nearby contract areas.

Key Gaps

1) Information on POC quality is lacking in the data-sets so far identified, as well as other parameters that may influence functioning (e.g., faunal abundance and microbial biomass data from within chambers). In particular, 80% of the variance in the SCOC is still unexplained.

2) We need in situ POC flux information from each APEI and contract area in order to compare particle and oxygen flux within APEIs and contract areas.

3) We need a better understanding of temporal changes in benthic ecosystem functioning.

4) There is still a large knowledge gap on benthic ecosystem functioning (e.g., bioturbation, calcite dissolution) as little to no data are available on these and other benthic ecosystem functions from the APEIs/ contract areas.

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j. FOSSILIZED FAUNA –

DeepCCZ Synthesis Workshop - Fossilized Fauna

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Introduction

For decades, there has been evidence that cetaceans actively use the Clarion-Clipperton Zone (CCZ) in the Pacific Ocean (Ballance et al., 2006). This includes sightings at the sea surface, as well as seafloor observations of Cetacea falls (Smith et al., 2015; Amon et al., 2016), and possible seafloor traces from deep-diving beaked whales (Marsh et al., 2018). There is also growing evidence that this use is not new, with fossilized Cetacea bones trawled from the abyssal CCZ seafloor during the Challenger expedition (Murray & Renard, 1891). These collections have also extended to fossilized shark teeth.

Data Available for Analyses

The data used stem from megafaunal image analyses (see Invertebrate Megafauna Section for further details) and can also be seen in Table 1. Additional image datasets from the DISCOL site in the southeast Pacific and the Kiribati EEZ were included. These datasets were collected during a variety of research cruises using different tools and survey designs, resulting in some which can be treated quantitatively, but others only qualitatively (Table 1). Fossilized teeth could only be reliably counted in imagery that was collected at an altitude of 4 meters or below.

Table 1. Summary of CCZ image datasets, from west to east, to be analyzed for fossilized fauna. Asterisk indicates that the dataset is based on video transect data so can only be used qualitatively. Data from APEIs are in bold.

Area	**Equipment**	**Observed Area (m^2)**	**Source**	**DOI/Contact Person**
Kiribati	Towed Camera	14,666	Nautilus Minerals / NOC	Simon-Lledó et al. 2019b, 10.3389/fmars.2019.00605
APEI 1	**ROV**	**6,539**	**Univ. Hawaii**	**Durden, In prep**
APEI 4	**ROV**	**9,139**	**Univ. Hawaii**	**Durden, In prep**
APEI 7	**ROV**	**7,021**	**Univ. Hawaii**	**Durden, In prep**
OMCO B	Towed	1,599	UK Seabed Res. / Univ. Plymouth	McQuaid, In prep

	Camera			
OMCO A	Towed Camera	800	UK Seabed Res. / Univ. Plymouth	McQuaid, In prep
OMCO C	Towed Camera	802	UK Seabed Res. / Univ. Plymouth	McQuaid, In prep
OMCO D	Towed Camera	800	UK Seabed Res. / Univ. Plymouth	McQuaid, In prep
OMCO E	Towed Camera	801	UK Seabed Res. / Univ. Plymouth	McQuaid, In prep
OMCO F	Towed Camera	1,600	UK Seabed Res. / Univ. Plymouth	McQuaid, In prep
APEI 3	**ROV**	***3,300 m**	**MARE/IMAR/Okeanos-Univ. Azores**	**Cuvelier, in review, 10.5194/bg-2019-304 initial evaluation in Vanreusel et al. 2015**
OMCO G	1	799	UK Seabed Res. / Univ. Plymouth	McQuaid, In prep
GSR	ROV	*1,200 m	MARE/IMAR/Okeanos-Univ. Azores	Cuvelier, in review, 10.5194/bg-2019-304 initial evaluation in Vanreusel et al. 2015
APEI 6 SW	**AUV**	**18,582**	**NOC**	**Simon-Lledó et al. 2019** **10.1002/lno.11157**
APEI 6 SW	**Towed camera**	***32,900 m**	**NOC**	**unpublished**
APEI 6 NE	**AUV**	**TBD**	**NHM/Univ. Hawaii**	**Amon et al., In prep**
BGR	ROV	*1,600 m	MARE/IMAR/Okeanos-Univ. Azores	Cuvelier, in review, 10.5194/bg-2019-304 initial evaluation in Vanreusel et al. 2015
UK1 A	ROV	4,204	NHM/Univ. Hawaii	Amon et al. 2016, 10.1038/srep30492

OMS	AUV	TBD	NHM/Univ. Hawaii	Amon et al., In prep
UK1 B	AUV	TBD	NHM/Univ. Hawaii	Amon et al., In prep
DISCOL	AUV	110,000	NOC	Simon-Lledó et al. 2019, 10.1038/s41598-019-44492

Results

Fossils were observed in imagery from all of the above areas within the eastern Pacific, excluding the Exclusive Economic Zone of Kiribati, APEI 7 and the DISCOL area, indicating that they are widespread across the CCZ (Figures 1, 2 and 3). Fossils were observed in both the APEIs and contract areas. There appeared to be an increase in the number of fossils observed from west to east across the Pacific, with the highest densities observed in APEI 6 (>30,000 fossils per km^2) (Figures 2 and 3). Fossilized bone densities in APEI 6 were more than double that found in any of the other areas surveyed (Figure 2). It should be noted that although densities for UK1 and OMS were not included here, preliminary inspections suggest that the abundance of fossils in these areas may be of similar magnitudes to those found at APEI 6, indicating that the extreme east of the CCZ may be a fossil hotspot.

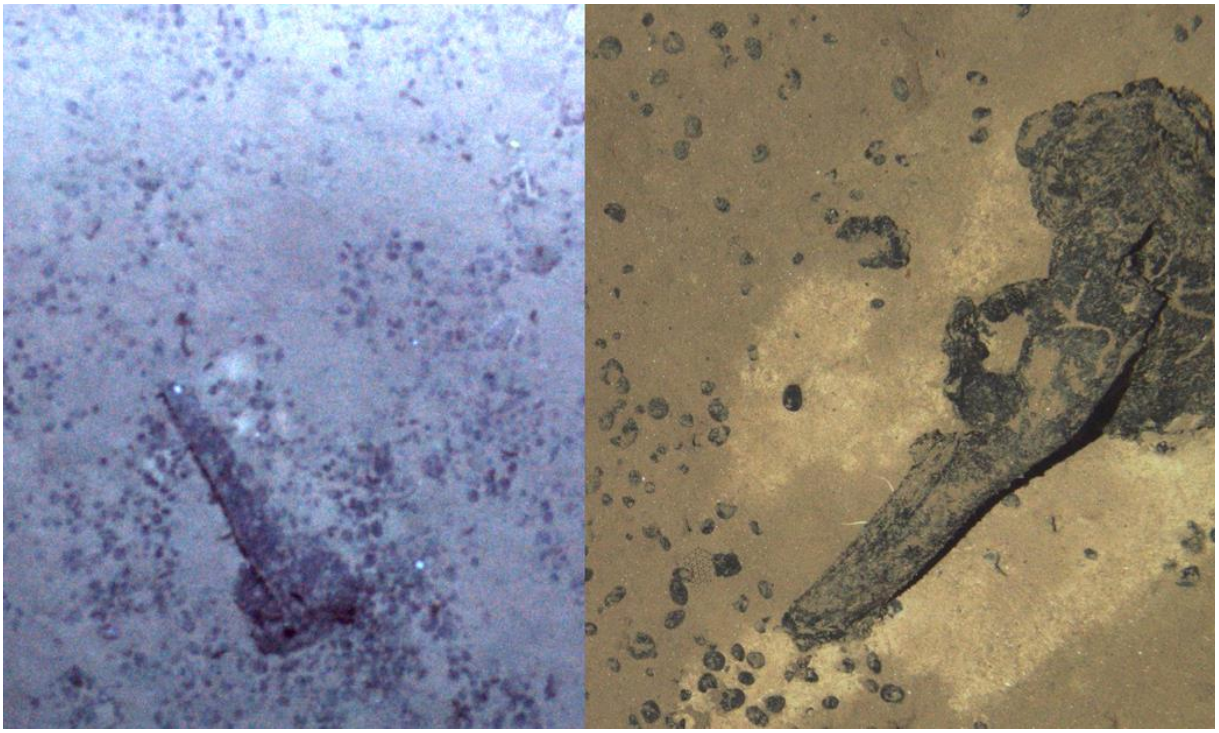

Figure 1. Fossilized whale skulls observed in the UK1 contract area (left) and APEI 6 SW (right).

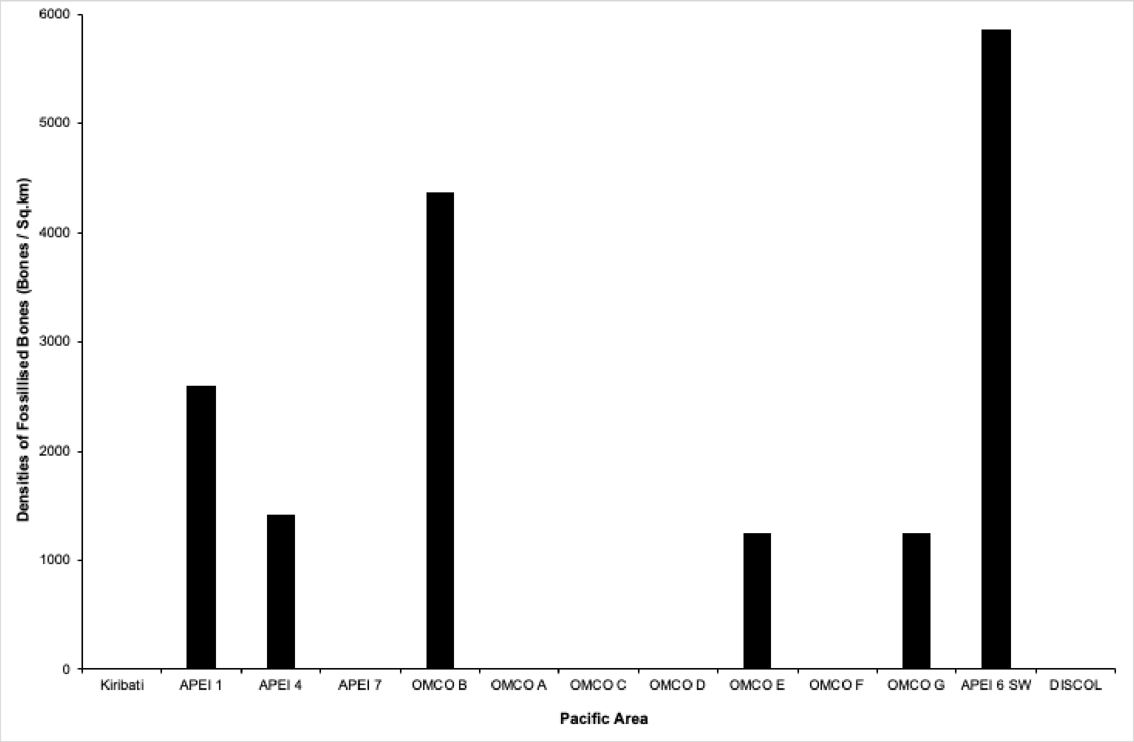

Figure 2. Mean densities of fossilized bones observed from west to east across the East Pacific Ocean. Fossilized bones were very abundant in UK1 A and B, OMS, and APEI 6 NE and will be included in subsequent quantitative analyses. Additionally, fossilized bones were observed in APEI 3, GSR, and BGR but it was not possible to quantitatively assess those image datasets.

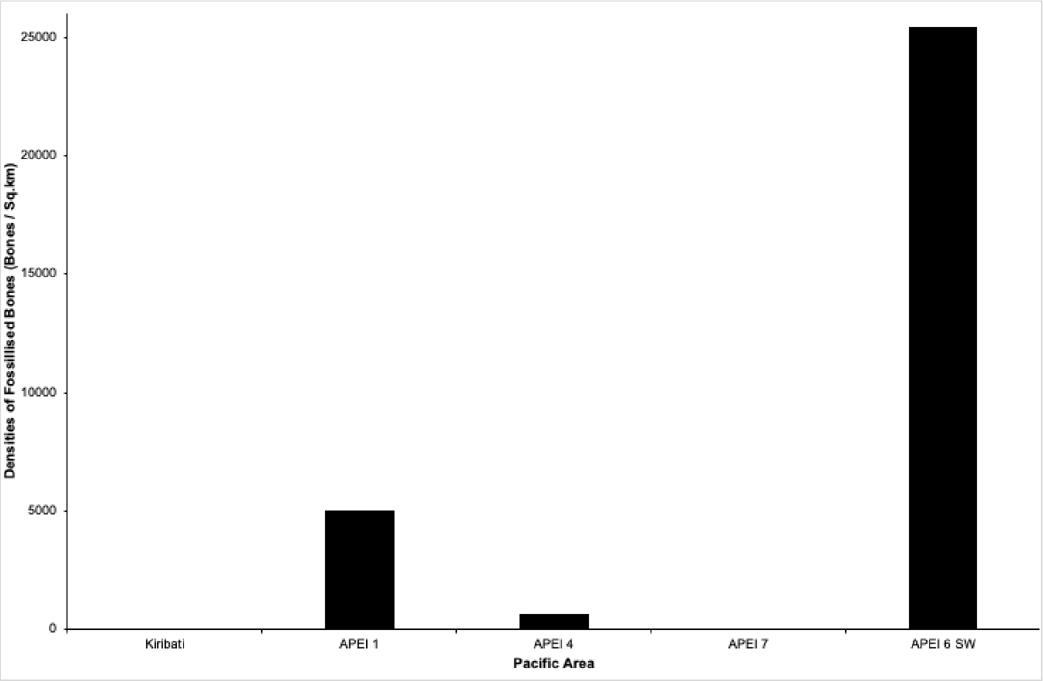

Figure 3. Mean densities of fossilized teeth observed from west to east across the East Pacific Ocean. Fossilized teeth were very abundant in UK1 A and will be included in subsequent quantitative analyses. Additionally, fossilized teeth were observed in OMCO F, APEI 3, GSR, APEI 6 NE, BGR, OMS, UK1 B, and DISCOL, but those datasets could not be reliably quantitatively assessed for teeth.

Fossils found in the CCZ are from a variety of extinct and extant species, and also likely include new species. Extinct species include *Messapicetus longirostris*, *Choneziphius* sp., *Globicetus* sp., *Pterocetus* sp., and many other ziphiid morphotypes. Extant species include *Mesoplodon densirostris* (Blainville beaked whale), *Ziphius cavirostris* (Cuvier's beaked whale) and other odontocete and mysticete whales. The cumulative periods over which these species were known to exist span 16 MYA to present day. The majority of fossils observed exhibited a polymetallic coating, with similar surface texture to that found on polymetallic nodules. These structures add to the available hard substrate on the seabed and are often found colonized by epibenthic megafauna, including anemones, annelid worms, sponges, ophiuroids and corals.

Over the coming months, these datasets will be fully analyzed to describe the types of fossils observed, their densities, and their potential ages. Associations with fauna, as well as the spatial variability of fossils across the CCZ, will also be explored.

Qualifications and Caveats

There are a number of important qualifications and caveats associated with the data used in this work:

- These are preliminary conclusions as data have not been extensively error-checked.
- Fossils can be difficult to detect in imagery. Bone fragments can be hard to differentiate from basalt rock fragments, particularly in imagery collected at high altitudes above the seabed. Additionally, many shark teeth are usually similar in size to polymetallic nodules, and thus hard to detect in imagery collected at altitudes above 4 meters.
- The number of transects and images were low in some of the areas surveyed.
- APEI 6 SW, where we found the highest densities of fossils, was surveyed using images collected at the lowest altitude (range: 2 to 4 meters) above the seabed. Nodules within APEI 6 were of flat-discoidal shape and considerably smaller than in other nodule-bearing areas, like the GSR or UK1 contract areas. These factors could have facilitated the detection of proportionally more fossilized bones (and especially teeth) in seabed imagery from the APEI 6 SW.
- More detailed paleontological inspection is planned ahead of scientific publication of these results to provide deeper insight on the taxonomic range (and hence potential cultural value) of the bone fragments encountered.

Gaps

- Despite an extensive amount of data, a number of regional gaps exist. There is no baseline information available for this analysis across the contract areas west of GSR 6. APEIs 2, 5, 8, and 9 also lack data.
- While imagery data does exist for five contract areas, five APEIs and two areas outside of the CCZ, it is important to note that only relatively small areas of seafloor have been surveyed in these areas.

- As introduced previously, image data varies widely in quality, limiting the ability to resolve smaller fossils such as sharks' teeth, or features needed to accurately assign species identifications.

Conclusions

- Diverse fossils of extinct and extant Cetacea and sharks are found across the CCZ. These appear to be spatially variable, with highest densities in the east CCZ. However, further exploration of the available data will be needed to unveil potential patterns in the distribution of fossil occurrences and densities.
- The presence of high densities of fossils suggests they may provide additional substrate for fauna to occupy/colonize and that special attention and perhaps additional conservation and management measures should be considered in the Regional Environmental Management Plan for the CCZ, as well as in the Exploitation Regulations and other areas of the Mining Code.

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k. HABITAT MAPPING AND ENVIRONMENTAL DATA –

Assessing representativity of the CCZ APEI network using habitat modelling and classification approaches

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Introduction

The principles of habitat modelling rely on the fundamental relationship between species and environment. Species inhabit distinct ecological niches and those niches are defined by a set of environmental conditions. Variation in ecologically relevant environmental conditions can therefore be used as proxies to represent variation in biological communities. The use of biophysical proxies as indicators of benthic habitats has become an established method of habitat modelling and mapping that is widely used in spatial management (Roff and Taylor 2000; Harris and Whiteway, 2009; Howell, 2010). An important first step in the habitat modelling approach is to determine the important environmental variables in the region of study.

In the Clarion-Clipperton Fracture Zone (CCZ), there is a strong gradient in Particulate Organic Carbon (POC) flux, decreasing from east to west, and from south to north (Lutz et al., 2007; Pennington et al., 2006; Smith et al., 1997). This gradient in POC flux across the CCZ has been linked to differences in faunal communities (e.g. Smith et al., 1997; Smith et al., 2008; Vanreusel et al., 2016; Veillette et al., 2007, and new evidence presented in this report), and flux of POC to the seafloor is therefore a key driver of faunal community composition and distribution in the CCZ.

In addition, nodules play an important role in determining the distribution of benthic biota in the CCZ. Nodules provide habitat for encrusting and epifaunal species that depend on hard surface for attachment (e.g. Amon et al., 2016; Dugolinsky, Margolis & Dudley 1977; Gooday, Goineau & Voltski, 2015 and new evidence presented in this report), support a range of fauna in the crevices of individual nodules (Bussau, Schriever & Thiel, 1995; Thiel et al., 1993), and support microbial assemblages distinct from the surrounding sediments and overlying water column (Shulse et al., 2016). A recent study provided the first quantified evidence that variation in nodule cover in the CCZ influences megafaunal standing stock, composition, functional group composition, the distribution of individual species, and some biodiversity attributes (Simon-Lledó et al., 2019a), and other working-group reports from this workshop outline further examples. Nodules are therefore also a key driver of faunal community composition and distribution in the CCZ.

Finally, topographic variation has also been shown to influence biological communities in the CCZ and other abyssal regions (Durden et al., 2015; Stefanoudis et al., 2016; Leitner et al., 2017; Simon-Lledó et al., 2019b; Meiofaunal Report from this workshop). The CCZ features seamounts, troughs and ridges, and community composition is likely to vary in response to these features. Bathymetry and derived variables, including slope and bathymetric position index (BPI), can be used to discriminate these different topographic conditions (e.g., Leitner et al., 2017) and therefore also form a key variable in habitat mapping for the CCZ.

While there are many other variables that may influence faunal communities in the CCZ, at present there is little direct evidence to support the application of further environmental variables to the habitat classification approach. In addition, some potentially important variables are not available as data layers at the scale of the whole CCZ, and therefore cannot be considered here.

We used two approaches to assess the representativity of the APEI network. The first approach follows established habitat-mapping methods of classifying the environment into distinct ecological-niche based classes (habitats) (e.g. Howell, 2010; Evans et al., 2015) and assessing the percentage of each habitat class contained within the APEI network versus mining exploration and reserved areas, and unmanaged areas (i.e. areas outside both APEIs and mining areas) (McQuaid et al., in prep). The second approach uses a simple comparison of the distribution of values of key environmental variables contained within the APEI network versus mining exploration and reserved areas and unmanaged areas, similar to methods used in Dunn et al. (2019).

Methods

Prior to the workshop, the habitat modelling team collated available relevant GIS layers of environmental data for the CCZ (Table 1) and carried out a habitat classification of the CCZ (McQuaid et al., in prep). Boundaries of the study region were defined by the CCZ Environmental Management Plan (EMP) (ISA, 2011) (shifted slightly east to encompass all contract areas comfortably): 0°-23°30’ N x 114° W-159° W.

The habitat classification was developed using key variables that are known to influence faunal community composition and distribution in the CCZ and wider deep-sea, and for which environmental data layers were available. Topographic variables were derived from the General Bathymetric Chart of the Oceans (GEBCO) bathymetry layer at 1 km^2 resolution. All variables were generated in ArcMap 10.4 using the Benthic Terrain Modeler extension (Wright et al., 2005). Slope is determined as the largest change in elevation between a cell and its 8 nearest neighbors. BPI gives the relative elevation of a point in relation to the overall landscape and was derived at both broad and fine scale (BBPI and FBPI, respectively), to capture topographic features at different scales across the region. BBPI was derived with an inner radius of 1 cell and an outer radius of 100 cells (1 cell = 1 km), giving a scale factor of 100 km. This broad scale layer identified large geomorphological units, such as abyssal plains, steps and troughs. This choice of scale draws from the US Federal Geographic Data Committee’s Coastal and Marine Ecological Classification Standard (CMECS) (FGDC, 2012). FBPI was derived with an inner radius of 1 cell and an outer radius of 10 cells, with a scale factor of 10 km. This finer-scale layer identified smaller megahabitats or features on the scale of kilometers to tens of kilometers, as defined in Greene et al. (1999). These features include seamounts, abyssal hills, canyons, plateaus, large banks and terraces.

Estimates for POC flux in the CCZ were obtained from a global model produced by Lutz et al. (2007). Lutz et al. (2007) modelled flux of POC to the seafloor based on water depth and seasonal variability in remote-sensed net primary productivity between 19 August 1997 and 24 June 2004. These estimates were interpolated to a 1 km^2 resolution in the CCZ using kriging.

Modelled estimates of nodule abundance across the CCZ region were obtained from ISA ‘Technical Study No. 6: A Geological Model of Polymetallic Nodule Deposits in the Clarion Clipperton Fracture Zone’ (ISA, 2010). In addition, Charles Morgan provided new nodule density data for the eastern CCZ as modelled estimates. These data were interpolated to 1 km^2 gridded data layer using inverse distance weighting.

All of the above variables were projected in WGS 1984 PDC Mercator projection, an equal-area projection suitable for use in the Pacific Ocean. An equal-area projection was used so that estimates of the area of each habitat identified through the classification could be calculated.

Table 1: *List of environmental variables used prior to and during workshop.*

Variable	Units	Manipulation	Original cell size	Source
Topographic variables				
Depth	meters	None	0.016°	GEBCO2008
Slope	°	Created using ArcGIS Spatial Analyst Extension	0.016°	GEBCO2008
Bathymetric position index – broad scale	-	Created using ArcGIS Benthic Terrain Modeler extension (Wright et al., 2005). Inner radius 1, outer radius 100, scale factor is 100km	0.016°	GEBCO2008
Bathymetric position index – finescale	-	Created using ArcGIS Benthic Terrain Modeler extension (Wright et al., 2005). Inner radius 1, outer radius 10, scale factor is 10km	0.016°	GEBCO2008
Other variables				
Particulate organic carbon flux to seabed	$mg/m^2/year$	Interpolated to 1kmx1km	7x7km	Derived from Lutz et al. (2007)
Nodule abundance	kg/m^2	Interpolated to 1kmx1km	0.25°	ISA, 2010
Nodule abundance	kg/m^2	Interpolated to 1kmx1km	0.5°	Charles Morgan

Cluster analysis was used to carry out unsupervised clustering on groups of variables: (1) topographic variables (FBPI, BBPI and slope), (2) modelled POC flux, and (3) modelled nodule abundance. This provided an overview of topographic features within the region, and areas of high to low POC and nodule abundance. Average silhouette width (ASW) was used to objectively determine the number of clusters each group of variables should be classified into. Expert judgment was also used to further refine the final choice of number of clusters. The three classified layers were combined in ArcGIS 10.4 using the "Combine" tool to produce the final habitat classification.

In order to test the representativity of the current APEI network, the proportion of each habitat type (from the classification) in the study region, APEI network, exploration and reserved areas and unmanaged areas of the CCZ management area were calculated and used in analyses. In addition, to assess representativity of continuous environmental variables we used ArcGIS 10.4 to crop environmental layers to the APEI network, exploration and reserved areas and unmanaged areas of the CCZ. Cropped layers were loaded in R and histograms produced to compare the distribution of environmental conditions within the APEIs and the exploration and reserved areas and unmanaged areas.

Finally, using new data on nodule abundance presented at the workshop, the CCZ was reclassified with boundaries extended to the east of the CCZ management area to explore potential areas of habitat with little protection identified in the previous steps.

No biological validation of the classification was carried out using point sample data for several reasons. The underlying environmental models used to build the classification contain known, but unquantified, error. The modelled nodule abundance layer becomes less reliable with distance from the central CCZ and is at a native resolution of 0.25 degrees (approximately 22 x 25 km at this latitude). The Lutz et al., (2007) POC layer is a modelled, averaged annual layer derived from several years of data and at a native resolution of 7 x 7 km. The GEBCO bathymetry layer is modelled from gravimetric distortions of the sea's surface, and this method is unable to detect small features. All layers were produced to reflect broad-scale variability only, and thus the constructed habitat classification is also only able to reflect broad-scale variability. It would be unproductive to use limited point sample data to validate a model of this scale.

Results

Habitat classification based assessment

The final habitat map consisted of 24 classes, each representing a different ecological niche (Figure 1).

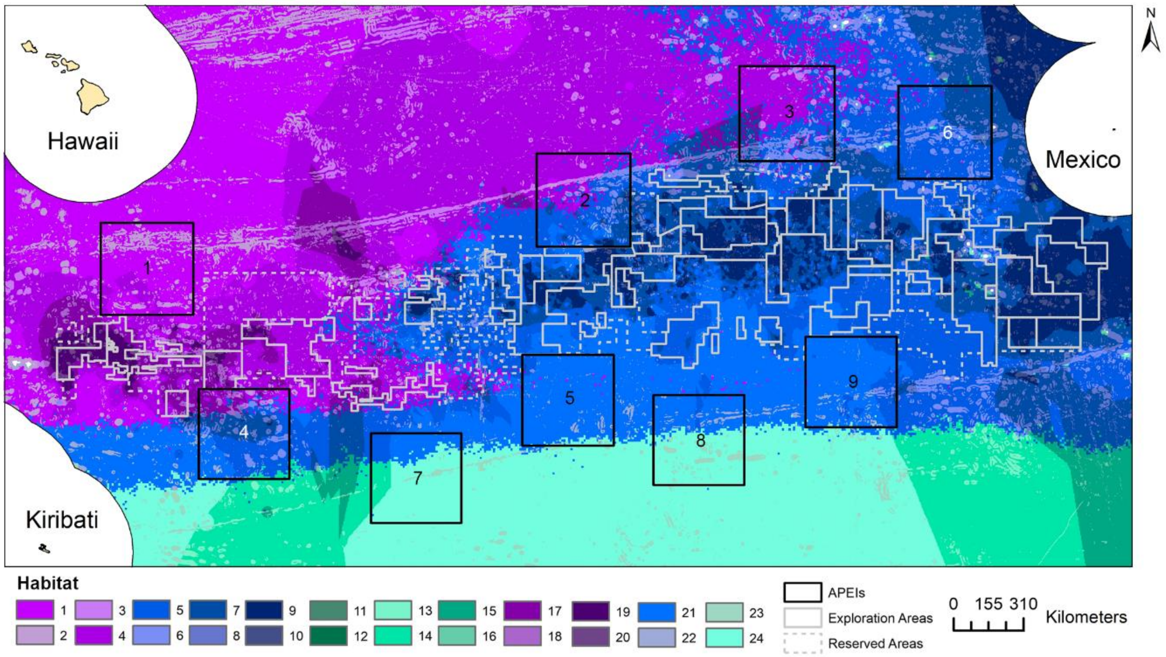

Figure 1: *Final habitat classification produced by combining layers of clustered environmental variables. Areas of low POC are shown in purple, areas of medium POC in blue, and high POC in green. Within a color block (purple, blue or green), pale to dark colors represent a gradient of very low to high*

nodule abundance, and within these divisions light colors indicate sloped areas, while dark colors indicate flat or constant slope.

Some of the habitats were large in extent, while others were much smaller in extent, within the modelled domain (Figure 2, Table 2).

Table 2: *Total area of each habitat within the CCZ, in mining areas (exploration and/or reserved areas), APEIs and outside of these managed areas.*

Habitat	**Total area (km^2)**	**Mining Areas (km^2)**	**APEIs (km^2)**	**Unmanaged (km^2)**
1	1 142 505	43 451	120 597	978 457
2	240 740	7 946	35 353	197 441
3	241 329	24 863	25 955	190 511
4	1 928 272	243 443	162 711	1 522 118
5	1 557 203	477 846	287 916	791 441
6	264 740	26 274	73 550	164 916
7	1 019 185	413 550	96 483	509 152
8	165 080	35 231	26 420	103 429
9	593 231	366 931	2 669	223 631
10	57 997	30 456	639	26 902
11	1 125	236	0	889
12	714	347	0	367
13	56 480	21	793	55 666
14	636 650	369	18 764	617 517
15	166 478	1 155	0	165 323
16	9 228	572	90	8 566
17	280 487	98 127	22 219	160 141
18	66 355	20 526	6 148	39 681
19	70 899	49 105	238	21 556
20	17 923	10 128	44	7 751
21	1 086 811	134 785	387 614	564 412
22	63 133	8 391	23 149	31 593
23	72 864	0	16 667	56 197
24	1 443 516	0	190 948	1 252 568

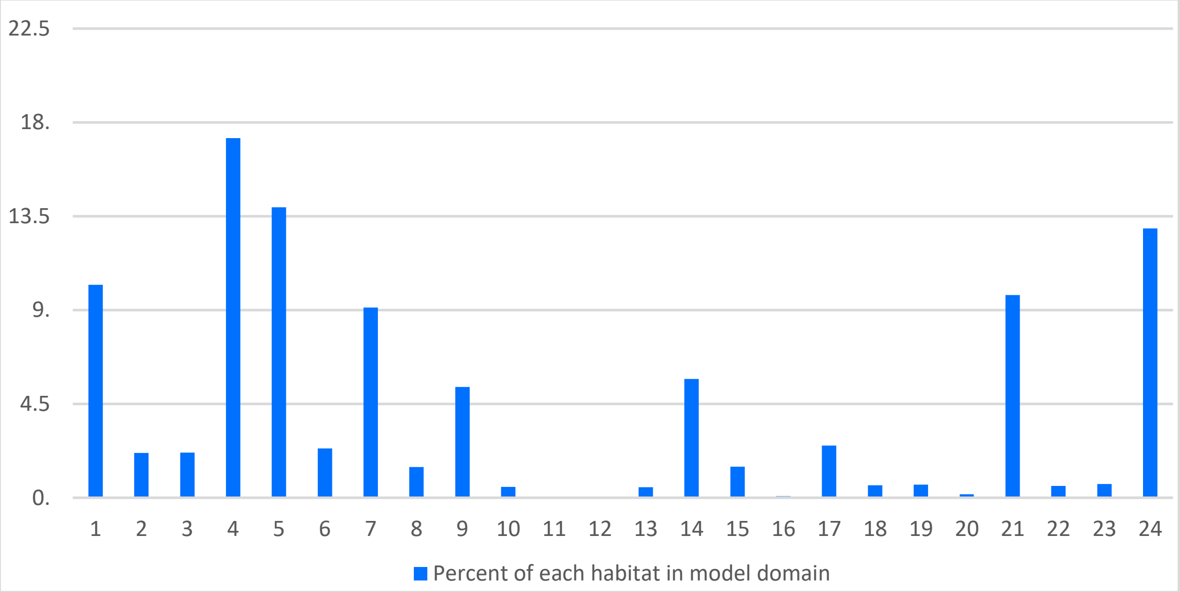

Figure 2: *Percentage of model domain within each habitat.*

Out of the 24 habitat classes present in APEIs and/or mining areas (exploration and/or reserved areas), there were some clear gaps in terms of representation and some clear areas of concern (Figure 3). For example, habitats 9, 10, 19, and 20 had over 50% of their total area of habitat inside mining areas, and little to no area inside APEIs. All of these habitats are areas of high nodule abundance (Table 3). These habitats also had a fairly high proportion of their area in currently unmanaged areas, which could be considered for spatial management. Habitats 11 and 12 also had high nodule abundance and were not represented inside APEIs, although these two habitats are small in total extent (Table 2, Figure 2).

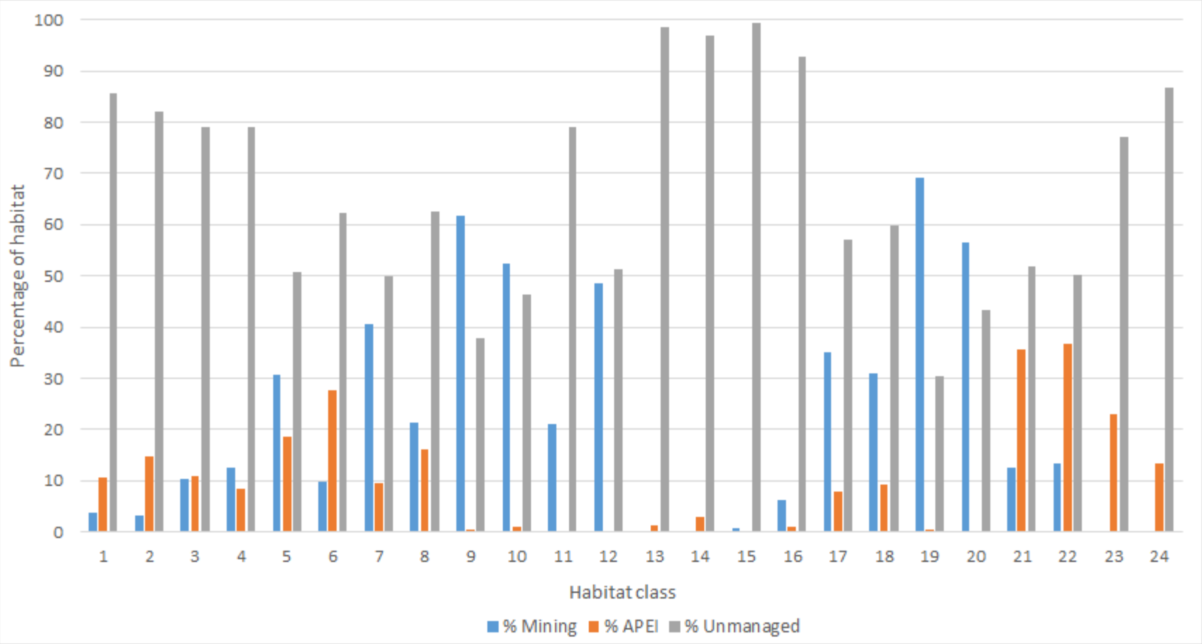

Figure 3: *Area of each habitat class in APEI and mining areas as a percentage of total area of each habitat within the model domain.*

When considering just the area of habitat within some current management areas (i.e., areas within APEIs, exploration or reserved areas), these trends become more apparent. Habitats 9, 10, 11, 12, 15, 19 and 20 have between 90-100% of their managed area inside exploration and reserved areas (Figure 4). It should be noted that only a small percentage of habitat 15 is within a managed area, i.e. it is predominantly found outside of APEIs and exploration and reserved areas (Figure 3).

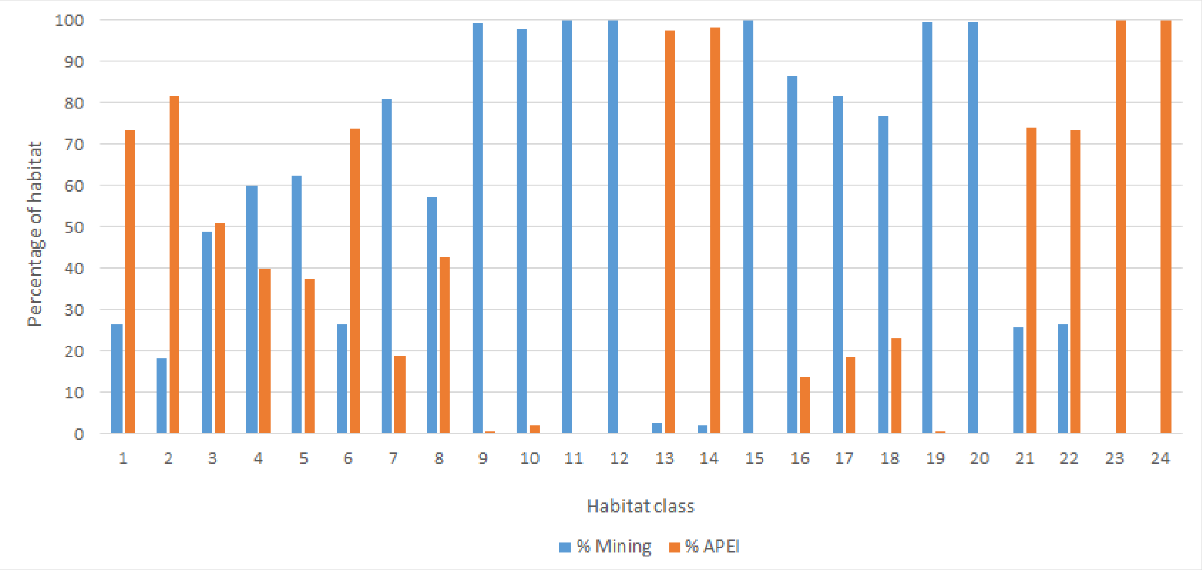

Figure 4: *Area of each habitat class in APEI and mining areas as a percentage of total area either protected or contractd.*

Habitats 9, 10, 11, 12, 19 and 20 are all areas of high nodule abundance (Table 3). Thus, areas of high nodule abundance are not currently well represented within the APEI network.

Table 3: *Environmental conditions of each habitat class (expressed as a mean). Habitats of concern are shaded in grey.*

Habitat	**Nodule Abund (kg m^{-2})**	**POC (g C_{org} m^{-2} y^{-1})**	**Slope (^{0})**	**BBPI**	**FBPI**
1	1.18	1.18	0.78	-17.21	-4.1
2	1.18	1.18	3.64	135.48	37.77
3	3.98	1.18	3.64	135.48	37.77
4	3.98	1.18	0.78	-17.21	-4.1
5	3.98	1.53	0.78	-17.21	-4.1
6	3.98	1.53	3.64	135.48	37.77
7	6.56	1.53	0.78	-17.21	-4.1
8	6.56	1.53	3.64	135.48	37.77
9	11.1	1.53	0.78	-17.21	-4.1
10	11.1	1.53	3.64	135.48	37.77
11	11.1	2.09	3.64	135.48	37.77
12	11.1	2.09	0.78	-17.21	-4.1
13	3.98	2.09	3.64	135.48	37.77
14	3.98	2.09	0.78	-17.21	-4.1
15	6.56	2.09	0.78	-17.21	-4.1
16	6.56	2.09	3.64	135.48	37.77
17	6.56	1.18	0.78	-17.21	-4.1
18	6.56	1.18	3.64	135.48	37.77
19	11.1	1.18	0.78	-17.21	-4.1
20	11.1	1.18	3.64	135.48	37.77
21	1.18	1.53	0.78	-17.21	-4.1
22	1.18	1.53	3.64	135.48	37.77
23	1.18	2.09	3.64	135.48	37.77
24	1.18	2.09	0.78	-17.21	-4.1

How do the APEIs differ?

When considering individual APEIs and their contribution to the overall network, APEI 4 is the most habitat diverse, while APEI 6 and 4 each contain habitats found in only one APEI (i.e. not replicated within the network) (Figure 5). APEI 6 contains habitat 16, which has a very small spatial extent, while APEI 4 contains the only examples of habitats 19 and 20.

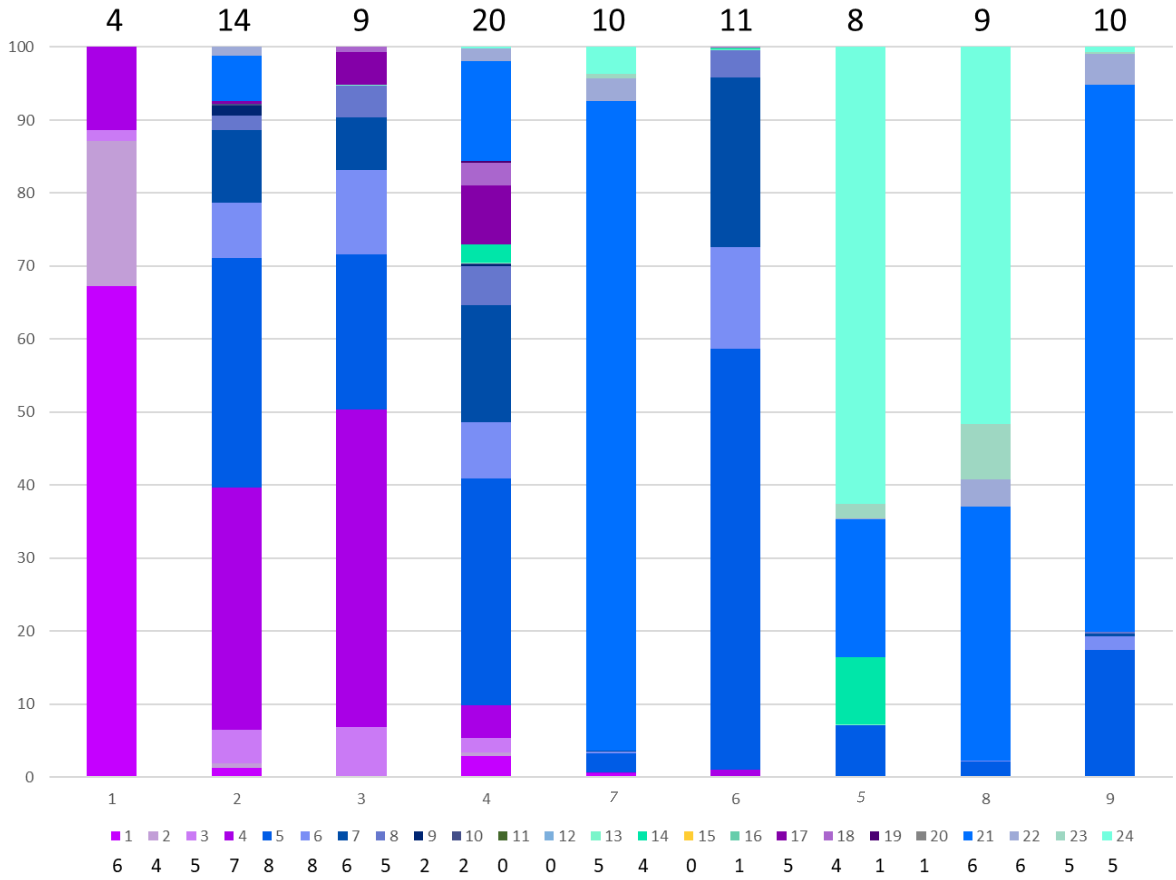

Figure 5*: Percentage of each APEI belonging to each habitat type. Numbers above bars show the number of habitat types within each APEI, while numbers below show number of APEIs within which each habitat is represented.*

<u>Continuous variable assessment</u>

Assessment of continuous variables suggests differences in the frequency distribution of values for nodule abundance and POC inside APEIs vs. inside exploration and reserved areas and unmanaged areas (Figure 6). APEIs have a slight over-representation of areas of medium to high POC flux compared to mining areas. Mining areas, on the other hand, have greater representation of areas of medium to high nodule abundance compared to APEIs, which have over-representation of areas of low nodule abundance. Areas of high nodule abundance are very poorly represented in, or wholly lacking from, APEIs. Areas of very high nodule abundance (>15 kg m^{2}) are not well represented in unmanaged areas.

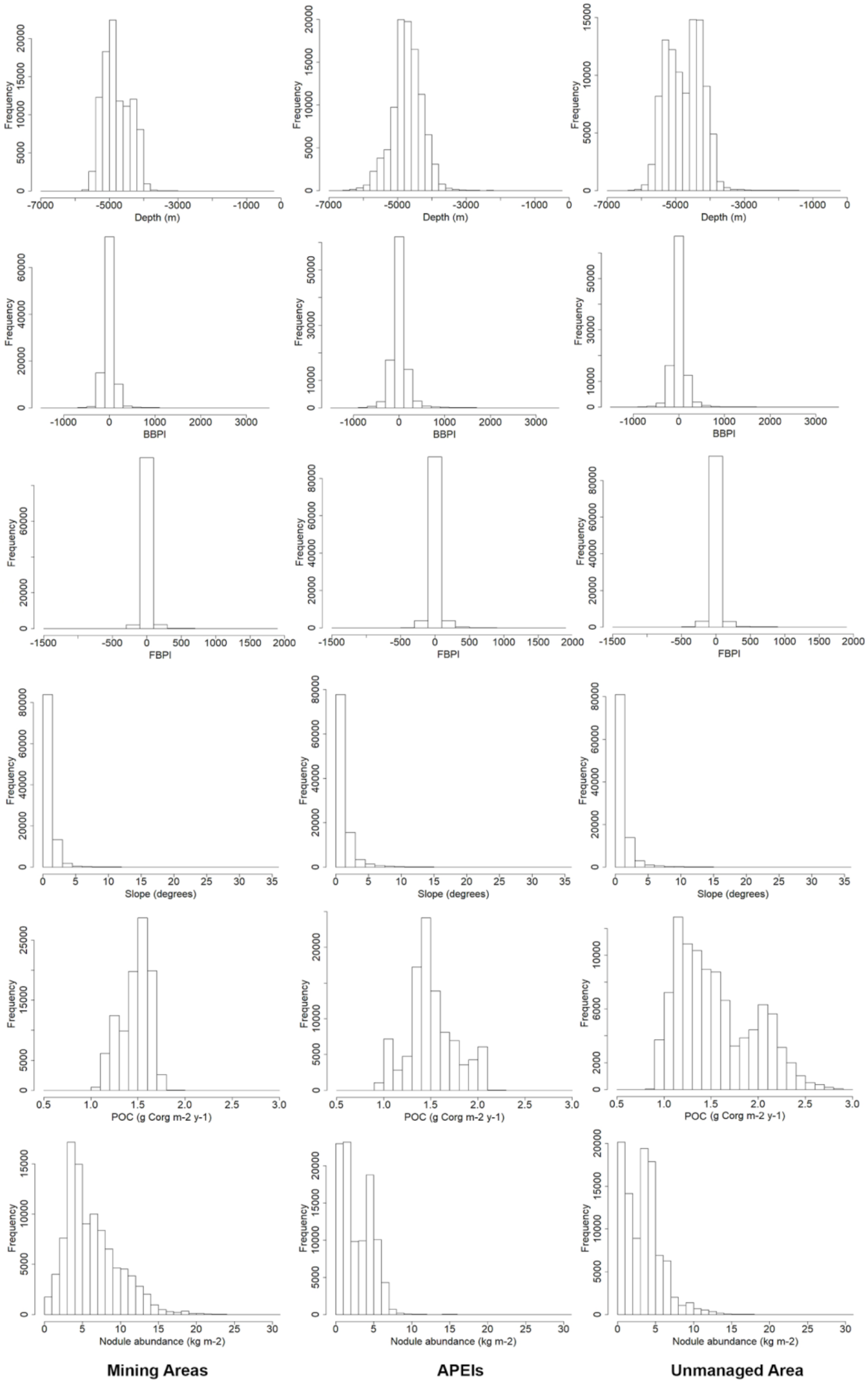

Figure 6: *Histograms comparing environmental conditions in mining areas, APEIs and unmanaged areas.*

Reclassification of CCZ including eastern areas

Both methods of assessing the APEI network indicated that areas of high nodule abundance are not well represented within the current network. Considering the distribution of habitats 9, 10, 11, 12, 19 and 20, it is clear that they are predominantly distributed within exploration and reserved areas. Habitats 11 and 12 are very small in spatial extent and may be focused around particular seafloor features (Table 2, Figure 2, Figure 7 inset). Habitats 9, 10, 19 and 20 represent a challenge in terms of achieving representation. However, reclassification of the CCZ using new data in the east shows that there are areas of habitats 19 and 20 in the north west of the region and areas of habitats 9 and 10 in the east that could be considered for spatial management measures (Figure 7).

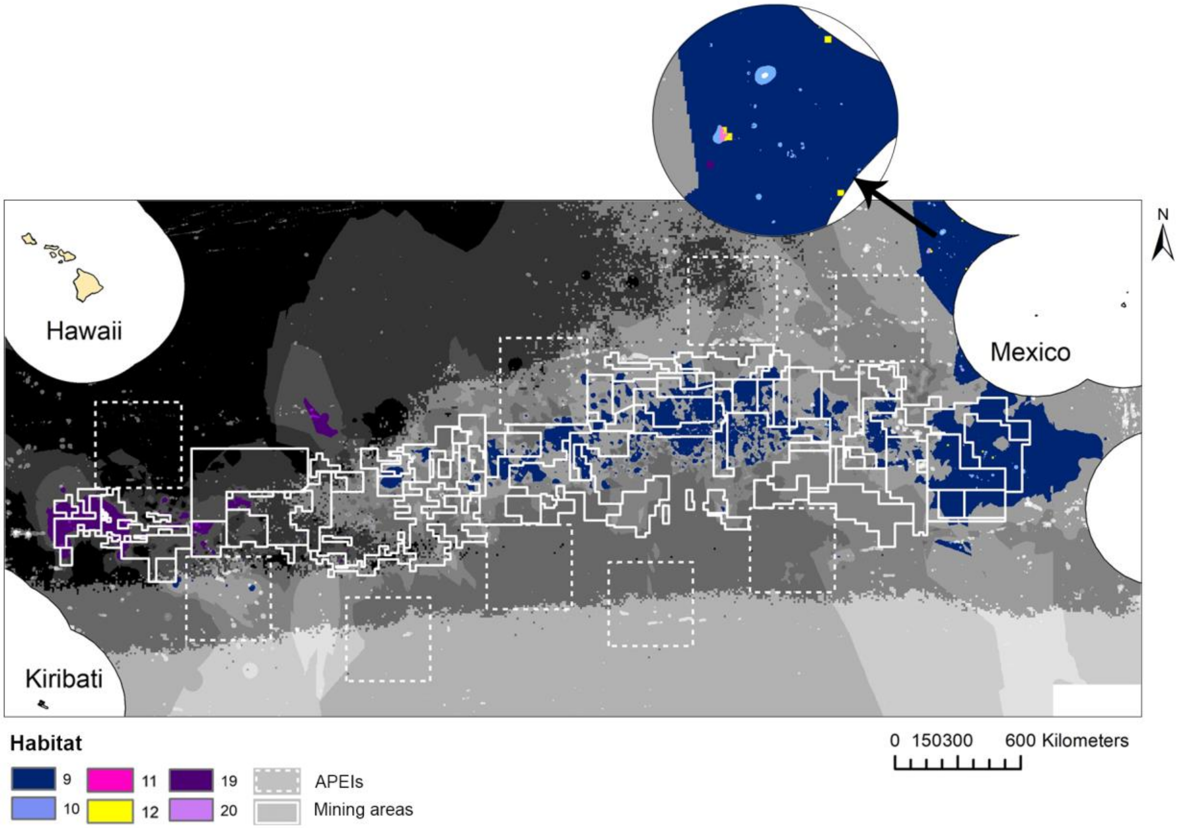

Figure 7: *Reclassification of the CCZ including new data to the east of the CCZ management area, showing the distribution of habitats 9 ,10, 11, 12, 19 and 20 lying outside of exploration and reserved areas.*

Conclusions

- Habitats characterized by high nodule abundance are not well represented (<5% of total area of habitat), and in some cases have no representation, within the current APEI network.
- There are regions to the north-west, east and within the central CCZ not contained within exploration or reserved areas, that could be considered for spatial management, i.e., inclusion in APEIs, in order to more fully represent the range of habitats present.
- Further examples of poorly represented habitats could be considered as part of a process to establish Preservation Reference Zones with a conservation function inside of exploration and reserved areas, and in the establishment of new APEIs in relinquished contract areas.

- APEIs 4 and 6 are of particular importance to the network in terms of representation and/or diversity of habitats contained within them.
- Some habitats are only contained within one or no APEI and therefore lack APEI replication.

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