Deep-ocean macrofauna assemblages on ferromanganese and phosphorite-rich substrates in the Southern California Borderland (#101134)

First submission

Guidance from your Editor

Please submit by **4 Jul 2024** for the benefit of the authors (and your token reward) .

Structure and Criteria
Please read the 'Structure and Criteria' page for guidance.

Author notes
Have you read the author notes on the [guidance page](#)?

Raw data check
Review the raw data.

Image check
Check that figures and images have not been inappropriately manipulated.

If this article is published your review will be made public. You can choose whether to sign your review. If uploading a PDF please remove any identifiable information (if you want to remain anonymous).

Files

Download and review all files from the [materials page](#).

2 Latex file(s)
1 Table file(s)
1 Raw data file(s)

Structure and Criteria

Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

📄 You can also annotate this PDF and upload it as part of your review

When ready submit online.

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your guidance page.

BASIC REPORTING

- Clear, unambiguous, professional English language used throughout.
- Intro & background to show context. Literature well referenced & relevant.
- Structure conforms to PeerJ standards, discipline norm, or improved for clarity.
- Figures are relevant, high quality, well labelled & described.
- Raw data supplied (see PeerJ policy).

EXPERIMENTAL DESIGN

- Original primary research within Scope of the journal.
- Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
- Rigorous investigation performed to a high technical & ethical standard.
- Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

- ℹ️ **Impact and novelty is not assessed.** Meaningful replication encouraged where rationale & benefit to literature is clearly stated.
- All underlying data have been provided; they are robust, statistically sound, & controlled.

- Conclusions are well stated, linked to original research question & limited to supporting results.

Standout reviewing tips

The best reviewers use these techniques

Tip	*Example*
Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
Give specific suggestions on how to improve the manuscript	*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*
Comment on language and grammar issues	*The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult. I suggest you have a colleague who is proficient in English and familiar with the subject matter review your manuscript, or contact a professional editing service.*
Organize by importance of the issues, and number your points	*1. Your most important issue* *2. The next most important item* *3. ...* *4. The least important points*
Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

Deep-ocean macrofauna assemblages on ferromanganese and phosphorite-rich substrates in the Southern California Borderland

Michelle Guraieb [Corresp., 1] , Guillermo F Mendoza [1] , Kira Mizell [2] , Gregory W Rouse [1] , Lisa A Levin [1]

[1] Integrative Oceanography Division and Center for Marine Biodiversity and Conservation, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California, United States

[2] USGS Pacific Coastal and Marine Science Center Santa Cruz, Santa Cruz, CA, USA, Santa Cruz, California, United States

Corresponding Author: Michelle Guraieb
Email address: mguraieb@ucsd.edu

Mineral-rich hardgrounds, such as ferromanganese (FeMn) crusts and phosphorites, occur on seamounts and continental margins, gaining attention for their resource potential due to their enrichment in valuable metals in some regions. This study focuses on the Southern California Borderland (SCB), an area characterized by uneven and heterogeneous topography featuring FeMn crusts, phosphorites, basalt and sedimentary rocks that occur at varying depths and are exposed to a range of temperatures, and oxygen concentrations. Due to its heterogeneity, this region serves as an optimal setting for investigating the relationship between mineral-rich hardgrounds and benthic fauna. This study aims to characterize the density, diversity, and community composition of macrofauna (> 300 μm) on hardgrounds as a function of substrate type and environment (depth and oxygen ranges). Rocks and their macrofauna were sampled quantitatively using remotely operated vehicles (ROVs) during expeditions in 2020 and 2021 at depths above, within and below the oxygen minimum zone (OMZ). A total of 3,555 macrofauna individuals were counted and 417 different morphospecies were identified from 82 rocks at depths between 231 m and 2,688 m. Average density for SCB macrofauna was 11.08 ± 0.87 ind. 200 cm^{-2} and mean Shannon-Wiener diversity per rock ($H'_{[log_e]}$) was 2.22 ± 0.07. A relationship was found between substrate type and macrofaunal communities. Phosphorite rocks had the highest H' of the 4 substrates compared on a per-rock basis. However, when samples were pooled by substrate, FeMn crusts had the highest H' and rarefaction diversity. Of all the environmental variables examined, depth explained the largest variance in macrofaunal community composition. Macrofaunal density and diversity had similar values at sites within and outside the OMZ. This study is the first to analyze the macrofaunal communities of mineral-rich hardgrounds in the SCB, which support deep-ocean biodiversity by acting as specialized substrates for macrofaunal communities.

Understanding the intricate relationships between macrofaunal assemblages and mineral-rich substrates may inform effects from environmental disruptions associated with deep-seabed mining or climate change. The findings contribute baseline information useful for effective conservation and management of the SCB and will support scientists in monitoring changes in these communities due to environmental disturbance or human impact in the future.

Deep-ocean macrofauna assemblages on ferromanganese and phosphorite-rich substrates in the Southern California Borderland

Michelle Guraieb[1], **Guillermo F. Mendoza**[1], **Kira Mizell**[2], **Gregory W. Rouse**[1], **and Lisa A. Levin**[1]

[1]**Integrative Oceanography Division and Center for Marine Biodiversity and Conservation, Scripps Institution of Oceanography, University of California San Diego, La Jolla, California, USA**

[2]**USGS Pacific Coastal and Marine Science Center Santa Cruz, Santa Cruz, California, USA**

Corresponding author:
Michelle Guraieb[1]

Email address: mguraieb@ucsd.edu

ABSTRACT

Mineral-rich hardgrounds, such as ferromanganese (FeMn) crusts and phosphorites, occur on seamounts and continental margins, gaining attention for their resource potential due to their enrichment in valuable metals in some regions. This study focuses on the Southern California Borderland (SCB), an area characterized by uneven and heterogeneous topography featuring FeMn crusts, phosphorites, basalt and sedimentary rocks that occur at varying depths and are exposed to a range of temperatures, and oxygen concentrations. Due to its heterogeneity, this region serves as an optimal setting for investigating the relationship between mineral-rich hardgrounds and benthic fauna. This study aims to characterize the density, diversity, and community composition of macrofauna ($> 300 \ \mu m$) on hardgrounds as a function of substrate type and environment (depth and oxygen ranges). Rocks and their macrofauna were sampled quantitatively using remotely operated vehicles (ROVs) during expeditions in 2020 and 2021 at depths above, within and below the oxygen minimum zone (OMZ). A total of 3,555 macrofauna individuals were counted and 417 different morphospecies were identified from 82 rocks at depths between 231 m and 2,688 m. Average density for SCB macrofauna was 11.08 ± 0.87 ind. 200 cm^{-2} and mean Shannon-Wiener diversity per rock (H'$_{[loge]}$) was 2.22 ± 0.07. A relationship was found between substrate type and macrofaunal communities. Phosphorite rocks had the highest H' of the 4 substrates compared on a per-rock basis. However, when samples were pooled by substrate, FeMn crusts had the highest H' and rarefaction diversity. Of all the environmental variables examined, depth explained the largest variance in macrofaunal community composition. Macrofaunal density and diversity had similar values at sites within and outside the OMZ. This study is the first to analyze the macrofaunal communities of mineral-rich hardgrounds in the SCB, which support deep-ocean biodiversity by acting as specialized substrates for macrofaunal communities. Understanding the intricate relationships between macrofaunal assemblages and mineral-rich substrates may inform effects from environmental disruptions associated with deep-seabed mining or climate change. The findings contribute baseline information useful for effective conservation and management of the SCB and will support scientists in monitoring changes in these communities due to environmental disturbance or human impact in the future.

Key words: biodiversity, deep-ocean, ferromanganese crust, macrofauna, phosphorite, oxygen minimum zones

INTRODUCTION

The deep ocean (> 200 m deep) is the largest habitable space on Earth and it remains the least explored and understood area of the ocean. With an average depth of 3,800 m, the ocean consists mostly of deep

water, which represents over 95% of the volume on earth that is available for living organisms to thrive (Danovaro et al., 2020). The ocean plays a vital role in regulating our climate and providing essential services and resources to humanity (Thurber et al., 2014; Baker et al., 2020). However, the deep-ocean ecosystem services provided to humankind are under pressure from human activities that increasingly impact the natural functions that occur in the ocean (Baker et al., 2020). Advancing our knowledge of the deep ocean through baseline studies supports the development of conservation initiatives and effective marine ecosystem management strategies. This is especially urgent as cumulative impacts from climate change to deep-seabed mining pose challenges for the proper management of the deep ocean (Levin et al., 2016; Baker et al., 2020).

Two deep-ocean mineral types being considered for their resource potential are ferromanganese (FeMn) crusts and phosphorite rocks (Hein et al., 2013, 2016). FeMn crusts, which were first considered as a potential resource for cobalt in the early 1980s, are also enriched with metals, such as copper, nickel and manganese, which are used in electric car batteries and other technologies (Halbach et al., 1982; Hein et al., 2013). FeMn crusts precipitate from seawater and are typically found in open ocean areas with low organic carbon content and low sedimentation rates (Hein et al., 2013; Usui et al., 2017). These crusts are found across a broad range of depths (400-7,000 m) on seamounts, ridges, and plateaus and form within a variety of seawater oxygen concentrations (Hein et al., 2013; Mizell et al., 2020).

Marine phosphorites occur in the Pacific and Atlantic oceans along the western continental margins at depths above 2,500 m in upwelling areas, on seamounts, and in lagoon deposits (Hein et al., 2016). Some shallower, nearshore occurrences of these phosphorous-rich rocks are primarily of interest to the mining industry as a source of macronutrients for fertilizers used in agriculture (PCMSC, 2022); rare earth elements have been studied as a potential secondary ore (Hein et al., 2016).

Extraction of these mineral-rich geological features will affect deep-ocean biodiversity, and research regarding potential impacts from deep-seabed mining to the health of the global ocean is needed (Levin et al., 2016). FeMn crusts and phosphorite rocks are inherently interwoven with the life of deep-ocean fauna as they cover miles of the seafloor where animals live and biogeochemical processes fundamental to the overall balance of ocean ecosystems occur (Jones et al., 2018). Currently, the relationship between macrofaunal communities and mineral-rich hard substrates is not well understood beyond the provision of attachment sites or physical habitat (Schlacher et al., 2014).

Biodiversity and species abundance in the deep ocean are responsible for key ecological functions, such as nutrient cycling, bioturbation, connectivity, primary and secondary production, respiration, habitat, and food supply (Le et al., 2017). These ecological functions translate into provisioning services (e.g., fisheries, pharmaceuticals, industrial agents and biomaterials); regulating services (e.g., climate regulation, biological control and waste absorption); and cultural services (e.g., educational, aesthetic, existence and stewardship) (Le et al., 2017). The loss of biodiversity leads to a decline in these important functions and services on which we rely (Danovaro et al., 2008). Furthermore, the biodiversity in the deep ocean is a crucial component of the resilience of these ecosystems, contributing to their ability to withstand the effects of anthropogenic disturbance (Oliver et al., 2015).

Deep-water fishing, climate change, oil and gas extraction, pollution, and deep-seabed mining are some of the main potential stressors to deep-ocean ecosystems (Ramirez-Llodra et al., 2011). According to Levin et al. (2016), mining of FeMn crusts and phosphorite rocks will remove currently living structure-forming organisms that provide habitat and food for other smaller fauna (Buhl-Mortensen et al., 2010), resulting in the loss of heterogeneity and therefore driving a decline in biodiversity. Moreover, the consequences of climate change, including oxygen loss, have the potential to amplify the adverse impacts stemming from mining operations (Le et al., 2017). The emerging consensus is that more scientific information is needed to inform regulations and decision making regarding these deep-sea ecosystems and their disturbance (Levin et al., 2016; Jones et al., 2018; Montserrat et al., 2019).

0.1 Oxygen Minimum Zone of the Southern California Borderland

The Southern California Borderland (SCB) offers a unique environment to study the relationship between mineral-rich hardgrounds and the benthic fauna that live on them. A variety of geological features (e.g., banks, ridges, knolls, escarpments and seamounts) and environmental conditions (low oxygen, various depths, varying food supply, temperature ranges) add to the heterogeneity of the region and make it a suitable habitat for many marine species that inhabit hard substrates. The SCB exhibits characteristics that allow for a well-formed oxygen minimum zone (OMZ) at bathyal depths (400-1,100 m in the case

of this study) because it is located on the eastern boundary of the Pacific Ocean Basin, where upwelling acts as one of the drivers of oxygen depletion (Gooday et al., 2010). Equatorward winds blowing along the coast in the SCB underpin the upwelling of nutrients from depths of 200 m, supporting high primary productivity (Checkley and Barth, 2009). High productivity in shallow waters leads to large amounts of organic matter sinking to deeper depths where bacteria use oxygen to decompose organic particles, further driving oxygen depletion (Levin, 2003).

Ocean deoxygenation is a phenomenon characterized by the reduction of dissolved oxygen content in the ocean due to human activities, primarily the addition of nutrients and global warming (Breitburg et al., 2018; Oschlies et al., 2018). Warming has contributed to the expansion of OMZs, areas where oxygen concentrations are <0.5 ml L^{-1} or <22 μM kg^{-1} (Levin, 2003; Stramma et al., 2010). Significant loss of oxygen off Southern California is also attributed to the strengthening of the California Undercurrent which brings warm, salty, low oxygen water up from the equator (Bograd et al., 2015). The expansion of OMZs and oxygen reduction can compress the habitat of marine species, which may trigger a variety of biological responses (Stramma et al., 2010). In sediments, macrofaunal densities are lowest in the core of the OMZ (Levin, 2003), and diversity decreases with declining oxygen concentrations (Levin et al., 1991, 2002; Gooday et al., 2010), which we expected to see for hard-ground communities in the OMZ of the SCB.

0.2 Deep-Ocean Faunal Studies and Their Relevance

During the past three decades, technological advancements have allowed scientists to study the diversity, ecology and surrounding environment of deep-ocean macrofauna using multicores (De Smet et al., 2017), submersibles (Li, 2017; Dong et al., 2021), and ROVs (Schlacher et al., 2014). Various studies have explored the relationship between benthic faunal communities and the substrate on which they live (Gage and Tyler, 1991; Gooday et al., 2010; Vanreusel et al., 2010; Schlacher et al., 2014; Simon-Lledó et al., 2019; Pereira et al., 2022); however, most studies that examine this relationship have focused on chemosynthetic ecosystems (Baco and Smith, 2003; Levin et al., 2015, 2017; Bourque et al., 2017; Pereira et al., 2021, 2022), and deep-ocean sediments (Wei et al., 2012; Baldrighi et al., 2014; Leduc et al., 2015; De Smet et al., 2017; Dong et al., 2021). Those studies undertaken on non-reducing, hard substrates have mainly examined the characteristics of the megafauna community (Clark, 2011; Grigg et al., 2013; Amon et al., 2016; De Smet et al., 2021; Vlach, 2022). Studies of macrofaunal assemblages on mineral-rich substrates are limited and often examine the fauna of associated sediments (e.g., Leduc et al., 2015; Chuar et al., 2020). Research on abyssal plains with polymetallic nodules has focused largely on foraminifera (Mullineaux, 1987, 1989; Veillette et al., 2007), meiofauna (Pape et al., 2021), megafauna (Tilot, 2006a,b; Kersken et al., 2018; De Smet et al., 2021; Yan et al., 2024), or macrofauna in the sediments surrounding the nodules (De Smet et al., 2017; Chuar et al., 2020; Lins et al., 2021). Grischenko et al. (2018), adressed macrofauna on nodules in the Clarion Clipperton Zone focusing exclusively on Byrozoa and the one study of macrofauna on FeMn crust was only qualitative (Toscano and Raspini, 2005). Our analysis is the first to examine the macrofaunal relationship with mineral-rich hard substrates in a quantitative analysis of density, diversity, and community composition in the SCB region.

The objective of this study is to understand the relationship of macrofaunal (> 300 μm) assemblages to mineral-rich substrates in the SCB off the Pacific coast of the United States, and to other environmental factors. For substrates collected during two oceanographic expeditions (NA124 cruise in 2020 and FK210726 cruise in 2021), we characterize the macrofaunal (> 300 μm) density, diversity and community composition (hereafter community structure) of the mineral-rich and other hard substrates of the SCB. Specifically, we examine faunal association with a) various substrate types (FeMn crust, phosphorite, basalt and sedimentary rocks); and b) different environmental variables (oxygen and water depth). These data could provide baseline information that can inform decision-making processes and support management strategies for biodiversity in Southern California and in the deep ocean. Studying healthy ecosystems such as the SCB offers opportunities for comparative studies of areas currently under consideration for deep-seabed mining activities. Regions such as the West-Pacific seamounts hosting FeMn crust, as well as the continental margins of Mexico, Namibia, South Africa, and New Zealand with phosphorites are currently or have recently been considered for their economic potential, causing a need to understand the biodiversity of these mineral systems (Levin et al., 2016). Safeguarding and studying analogous ecosystems in regions not currently targeted by mining enterprises, such as the SCB, can contribute to the overarching objective of maintaining the integrity of ecosystems, their services, and functions. These data
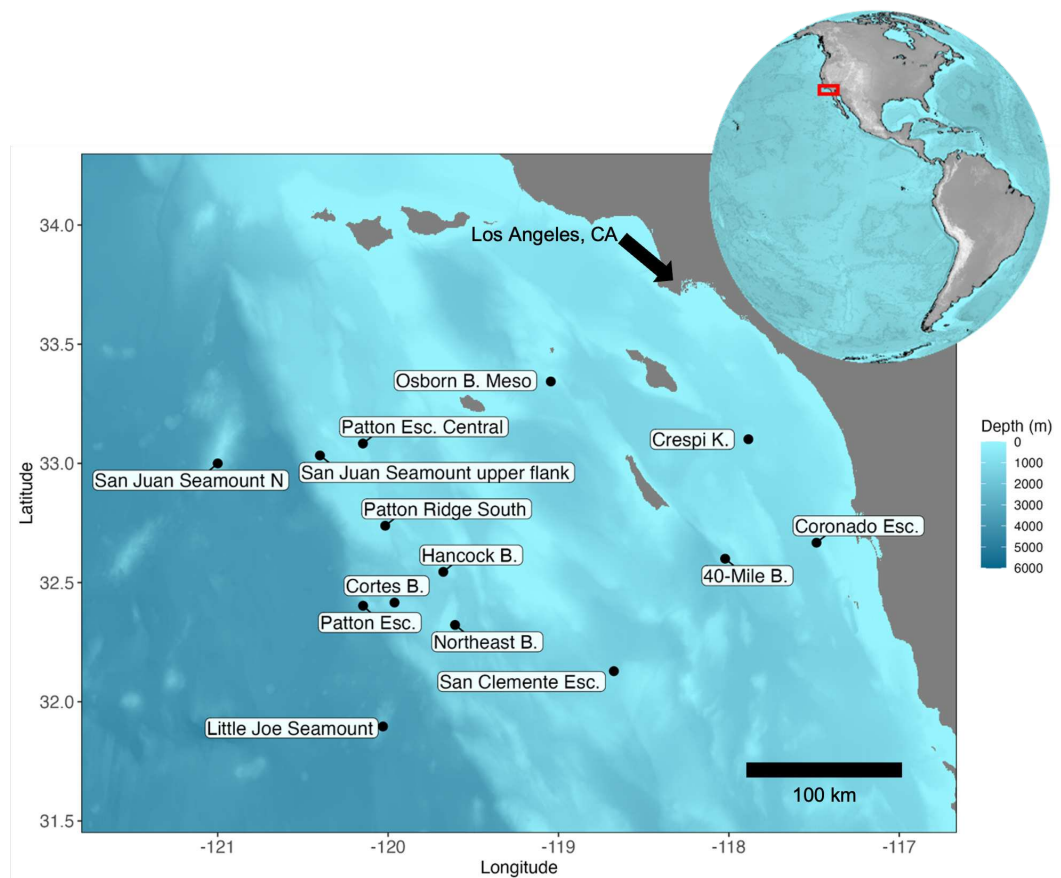
Figure 1. Map of the Southern California Borderland and the locations visited during the expedition aboard E/V *Nautilus* in 2020 (NA 124) and R/V *Falkor* in 2021 (FK210726). Eight sites (Patton Esc. = Patton Escarpment, S.J. Seamount Upper Flank = San Juan Seamount Upper Flank, Northeast B. = Northeast Bank, Cortes B. = Cortes Bank, Patton Ridge South, 40-Mile B. = 40-Mile Bank, San Clemente Esc. = San Clemente Escarpment, Osborn B. Meso = Osborn Bank Mesophotic Zone) were visited on NA 124 and seven sites (Hancock B. = Hancock Bank, S.J. Seamount North = San Juan Seamount North, Patton Esc. = Patton Escarpment, L.J. Seamount = Little Joe Seamount, Crespi K. = Crespi Knoll, Coronado Esc. = Coronado Escarpment) visited on FK210726.

will inform ocean stakeholders, including the people of California, who are spiritually, culturally, and economically connected to the deep ocean (AB1832, 2022).

1 MATERIALS AND METHODS

1.1 Study Area and Data Collection

Rocks of different mineral types (Table 1) along with their biological community were collected in the SCB during two research expeditions aboard the E/V *Nautilus* (NA124 - October 28 to November 6 of 2020) and R/V *Falkor* (FK210726 - July 26 to August 6 of 2021) at the locations shown in Figure 1. Rocks were collected using the remotely operated vehicles (ROVs), *Hercules* (onboard E/V *Nautilus*) and *SuBastian* (onboard R/V *Falkor*). *Hercules* was equipped with two manipulator arms, one high-definition video camera, LED lights, and a CTD (Conductivity-Temperature-Depth) sensor. *SuBastian* was equipped with two manipulators, two high-resolution video cameras, LED lights, and a CTD sensor.

Table 1. Sites visited aboard E/V *Nautilus* (NA124) and R/V *Falkor* (FK210726) with the dive number, date sampled, site name, depth range and physical coordinates at the start of the dive. Substrate type and number of rocks collected are shown for each dive.

| Cruise Number	Site/Location (coordinates)	Depth range of rocks collected (m)	Average temperature (^{o}C)	Oxygen range (μmol L^{-1})	Basalt rocks	Phospho-rite rocks	FeMn crusts	Sedimentary rocks
NA124	Patton Escarpment Central (33.06, -120.12)	587–820	5.34	2.61-5.14	1	1		3
	San Juan Seamount upper flank (33.03, -121)	691–1129	4.39	2.79-14.04			5	
	Northeast Bank (32.31, -119.59)	553–1132	5.15	2.68-15.95	2		3	
	Cortes Bank (32.41, -119.29)	437–529	6.41	4.14-8.31		5		
	Patton Ridge South (32.73, -120.01)	562–726	5.40	2.55-3.96		5		
	40-Mile Bank (32.60, -118.02)	658 –1036	4.76	2.11-14.6			2	3
	San Clemente Escarpment (32.67, -118.13)	1189 –1718	3.03	15.27-37.46	1		4	
	Osborn Bank Mesophotic Zone (33.34, -119.04)	231–396	7.83	19.71-54.79	4			
FK210726	Hancock Bank (32.54, -119.67)	319–594	6.54	9-36.48	6	1	1	
	San Juan Seamount North (33.03, -120.99)	1138 –1442	3.29	26.7–36.48			8	
	Patton Escarpment (32.40, -120.14)	1453 –1797	2.60	46.89–70.9			6	
	Little Joe Seamount (31.89, -120.03)	2366 -2688	1.81	100.17-108.14			8	
	Crespi Knoll (33.10, -117.88)	443 - 525	7.08	12.99-33.11	5			1
	Coronado Escarpment (32.66, -117.48)	443 - 467	7.57	24.16-28.28		7		
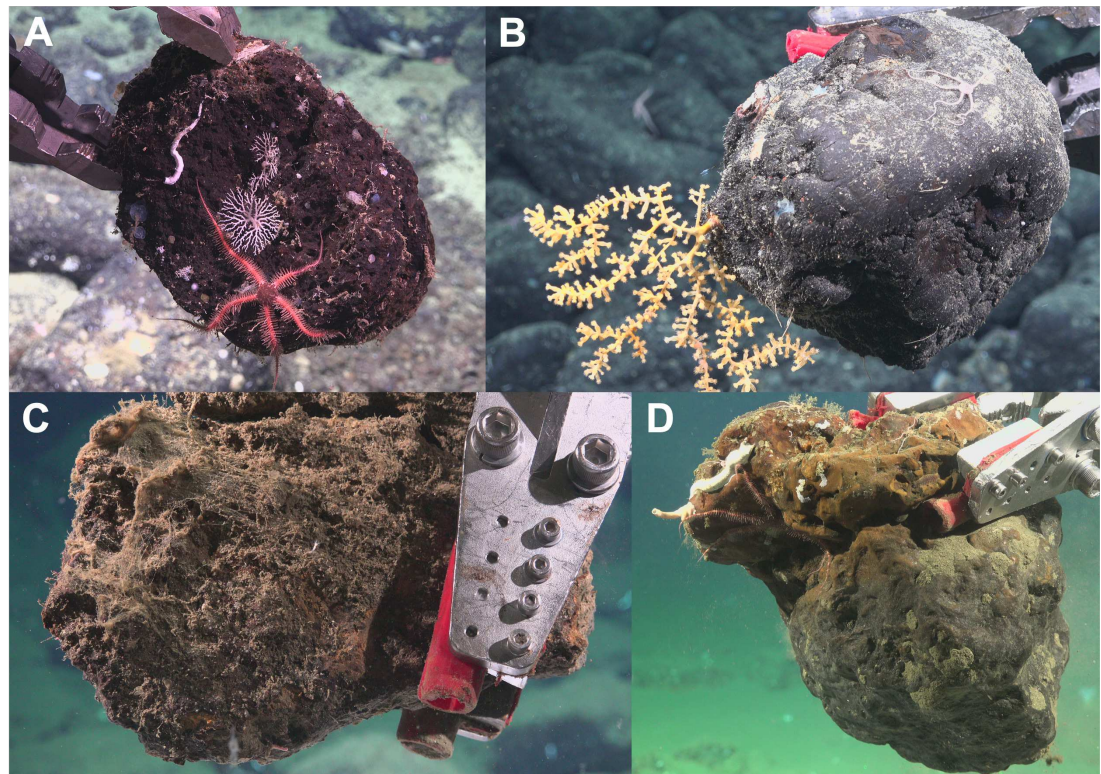
Figure 2. Rock photos of each substrate type upon collection. A) Basalt (Hancock Bank); B) FeMn crust (San Juan Seamount North); C) sedimentary (Crespi Knoll); and D) phosphorite rock (Coronado Escarpment).

1.2 Sampling and At-Sea Processing

The ROVs collected rocks of four different types (Figure 2) with the manipulator arms, maintaining each rock in its in-situ orientation and jostling it as little as possible to preserve fauna settled on the substrate. Each rock was placed into its own isolated biobox compartment on the ROV to avoid cross-contamination or loss of fauna during transport. At each rock collection location, the CTD attached to the ROV obtained measurements of temperature, pressure (depth) and oxygen concentrations. The rock substrates were processed quantitatively for their associated biological community. Every rock was photographed on six sides with a scale and label. All the visible biology was removed using forceps and kept in crystalizing dishes with cool seawater. The residual water contained in each biobox compartment holding individual rocks was washed through a 0.3 mm mesh to collect the macrofauna, and the retained biota were preserved in ethanol. Then, the rocks were wrapped in a monolayer of aluminum foil, which was later weighed to obtain surface area (as in Levin et al. 2015). Each rock was left overnight in a seawater bucket at room temperature to allow the remaining fauna to crawl away or fall out of the rock's crevices. Then, the water in each bucket was sieved again to recover the fauna, and these were combined with the previous day's collections from the same rock.

1.3 Substrate Identification

Rock samples were cut along their longest axis using a diamond blade, and the cut face was described in detail regarding apparent mineral type, stratigraphy, texture, and size. FeMn crusts are identifiable by color and morphology; they are black precipitates occurring on hard substrate of a different rock type (often basalt or other volcanic rock). Phosphorites are typically smooth, shiny, and dense; however, in some cases, it was difficult to tell if a sample was carbonate or phosphorite. To confirm mineral type when ambiguous, representative slabs were cut from each sample for crushing and powdering. The powdered sample was then analyzed by x-ray diffraction (XRD) to determine the presence of carbonate fluorapatite (phosphorite), calcite (carbonate or limestone), clay minerals (mudstone), or volcanic minerals (e.g.,

feldspar). XRD data were produced by a Panalytical X'Pert3 x-ray diffractometer with CuKα radiation and graphite monochromator. The first and primary measurement for all samples was collected every 0.02 ^{o}2 theta between 4 ^{o} and 70 ^{o}2 theta at 40 kV and 45 mA. Diffraction peaks from the digital scan data were identified using Phillips X'Pert High Score software, and mineral patterns were matched to patterns from the ICDD PDF4+ database.

1.4 Lab Processing and Data Synthesis

The surface area of collected rocks was obtained by weighing the aluminum foil wrap pieces using a top-loading balance. The foil weight was divided by the weight of 1 cm^2 of foil to determine the rock surface area in cm^2. Densities below are expressed as the number of organisms per 200 cm^2.

The macrofauna preserved in ethanol at sea, was re-sieved in the lab using a 0.3 mm mesh and sorted under a dissecting microscope at 12x magnification. Taxonomic identification was done to the lowest taxonomic level of identification possible using morphological characteristics. A total of 143 individuals were assigned a specific genus and, of those, 61 were given a species name; the remainder were identified to their lowest taxonomic level possible and designated as morphospecies. Encrusting Bryozoa were quantified by analyzing photographs capturing each profile of every rock at the time of collection aboard the ship. Voucher specimens of some macrofaunal morphospecies have been deposited to the Scripps Institution of Oceanography Benthic Invertebrate Collection (SIO-BIC).

1.5 Univariate Analysis

The dataset used to calculate densities included encrusting Bryozoa and Hydrozoa specimens, which were not identified at the species level. Total densities per sample were tested for normality using Shapiro-Wilk test. A square-root transformation was applied to generate a normal distribution. Then, Bartlett's test was used to assess if the variances were homogeneous. A Kruskal-Wallis test followed by a Dunn's test using Benjamini-Hochberg adjustment (Benjamini and Hochberg, 1995) was performed when comparing densities across substrate types because the assumption of homogeneity was not met. We utilized regression analysis to explore how variations in oxygen concentration and depth correlate with changes in density.

The diversity metrics used in this study to analyze the differences in macrofaunal community structure across various substrate types and environmental variables were: Shannon-Weiner diversity index (H') evenness (J'), rarefaction diversity $ES_{(20)}$ and species richness. The Shannon-Weiner diversity index ($H'_{[log e]}, H'_{[log 10]}$), Pielou's evenness (J'), and species richness (S) were calculated using functions "diversity", "evenness", and "Estimate", respectively, from the *vegan* package in R. $ES_{(20)}$ rarefaction values and Shannon-Weiner diversity index (H') were tested for normality using Shapiro-Wilk test and then a Cochran test was used to assess if the variances were homogeneous. To determine if there were any statistically significant differences within $ES_{(20)}$ values and Shannon-Weiner diversity index (H') across substrate, a Kruskal-Wallis test followed by Dunn's tests using Benjamini-Hochberg adjustment was performed due to the variance of homoscedasticity. We utilized regression analysis to explore how variations in oxygen concentration and depth correlate with changes in diversity. All statistical analyses were performed in R software using the packages *vegan* and *car*.

1.6 Multivariate Analysis

A multivariate analysis was performed to provide a measure of the dissimilarity of macrofaunal community composition between rock samples across different substrates and environmental variables. Total macrofaunal counts excluding encrusting Bryozoa and Hydrozoa (not identified to species level) were standardized to densities per 200 cm^2 and 4th root transformed before performing a multi-dimensional scaling analysis of Bray-Curtis dissimilarities using Primer v7 software. In addition, permutational multivariate analysis of variance (PERMANOVA) and analysis of similarities (ANOSIM) were used to test for the differences in community composition across substrate types; a similarity percentage (SIMPER) test was used to examine which taxa were creating those dissimilarities.

2 RESULTS

2.1 Ecology of the SCB Hardground Macrofauna Community

In this study, a total of 3,555 macrofauna individuals were counted and identified from 82 rocks collected from 231 m to 2688 m deep. Average temperatures across dives ranged from 1.8 to 7.8 and oxygen
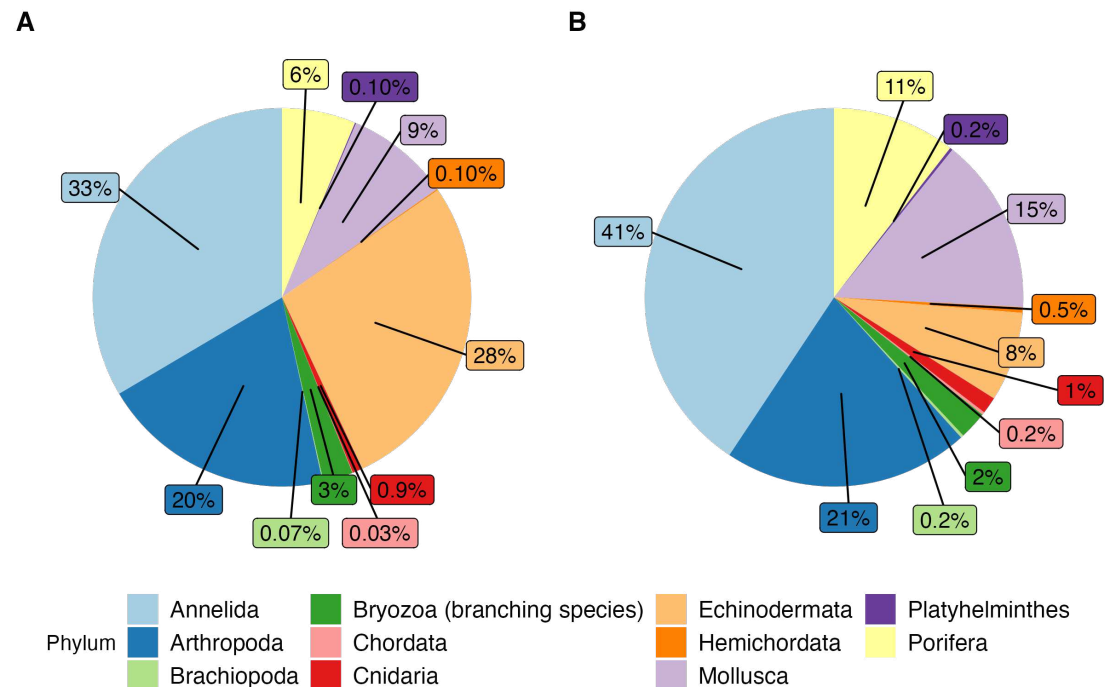
A

B

Phylum		
Annelida	Bryozoa (branching species)	Echinodermata	Platyhelminthes
Arthropoda	Chordata	Hemichordata	Porifera
Brachiopoda	Cnidaria	Mollusca	

Figure 3. A) Phyletic composition of the macrofaunal community in the SCB based on the number of individuals. (B) Phyletic composition of the macrofaunal community in the SCB based on the number of species. These figures do not include encrusting bryozoan colonies (455 individuals) and hydrozoans (88 individuals) since they were not identified to species level and therefore a proper comparison of individuals to species is not possible.

concentrations ranged from 2▦o 108.14 μmol L^{-1} (Table 1). Average densities (± 1 S.E) were 11.08 ± 0.87 ind. 200 cm^{-2} and ranged from 0.5 to 39 ind. 200 cm^{-2} (Table A1). Overall, the community was mainly dominated by the phyla Annelida (∼33%) and Echinodermata (∼27%). The other 2/5 of the individuals consisted of Arthropoda (∼19%), Mollusca (∼8%), and Porifera (∼6%). Less abundant phyla (4% of the total) were Bryozoa (considering only branching colonies), Cnidaria, Hemichordata, Platyhelminthes, Brachiopoda, and Chordata (Figure 3A).

In terms of morphospecies representation, these animals cover a total of 417 different taxa excluding encrusting Bryozoa and Hydrozoa. The phylum Annelida had 170 morphospecies (∼ 40% of morphospecies), dominating more so than for density. There were 88 morphospecies of Arthropoda (∼ 21% of morphospecies), 62 morphospecies of Mollusca (15% of morphospecies), 44 morphospecies of Porifera (∼ 10% of morphospecies), and 32 morphospecies of Echinodermata (∼ 7% of morphospecies). Less dominant phyla (5% of the total morphospecies) included Bryozoa (considering only branching colonies); Cnidaria; Hemichordata; Chordata; Brachiopoda; and Platyhelminthes (Figure 3B). Macrofaunal diversity measured as H'$_{[loge]}$ on SCB rocks averaged 2.22 ± 0.07. H' increased with increasing density (R = 0.36, p = 0.0008); at around 10 ind. 200 cm^{-2}, diversity stops increasing and remains relatively constant or shows little variation (Figure 4). Notably, 3 of 4 rocks with the highest densities (> 25 ind. 200 cm^{-2}) displayed a lower diversity than the mean (H'$_{[loge]}$ = 2.22 ± 0.07) (Figure 4). Diversity metrics for each rock, including species richness, Shann▦einer index (H'), evenness (J'), and rarefaction ES$_{(20)}$, are provided in Table A2.

Overall, the five most abundant taxa (∼ 21% of the total individuals) in the entire study were: Ophiuroidea sp. 5 (postlarvae) (304 individuals, ∼ 10%), *Ophiocten* cf. *centobi* (160 individuals, ∼ 5%), *Protocirrineris* nr. *socialis* (137 individuals, ∼ 4%), *Astrophiura marionae* (105 individuals, ∼ 3%), and Porifera sp. 5 (71 individuals, ∼ 2%). The following five taxa occurred on the most number of rocks: Ophiuroidea sp. 5 (postlarvae), Ophiuroidea sp. 7 (postlarvae), *Sphaerosyllis* nr. *ranunculus*, *Ophiocten* cf. *centobi* and *Munnopsurus* sp. 1. Twenty-five species (6%) account for over half (52.4%) of the animals collected. However, 235 of the 417 morphospecies collected (56.35%) were represented by only 1 or 2
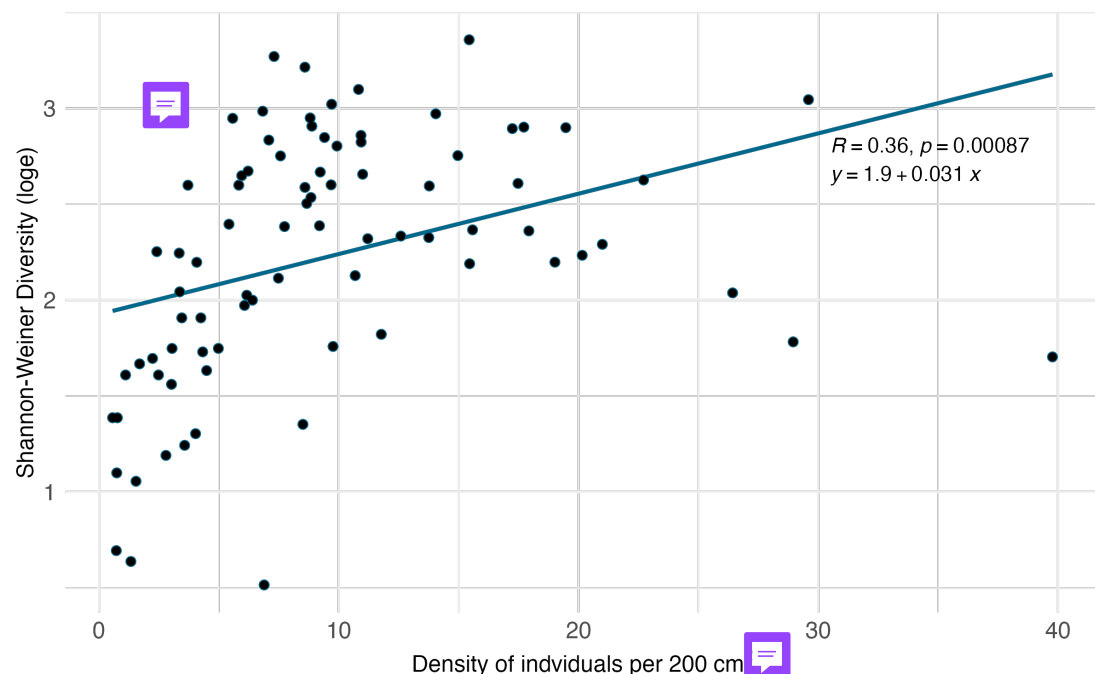
Figure 4. Regression of density (no. ind. 200 cm^{-2}) vs. diversity (H'$_{[loge]}$) of the macrofaunal communities on each rock.

individuals (38.6% were singletons), thus most of the macrofaunal diversity in the hardgrounds lies with rare species. Over half of the morphospecies were found on only 1 rock (213 morphospecies) suggesting that much of the diversity may remain undiscovered. All 137 individuals of the third most abundant taxon, *Protocirrineris* nr. *socialis* were found on only one rock. The top ten taxa included five Ophiuroidea morphospecies, which together accounted for 21% of all the fauna. The most frequently occurring taxa included five Ophiuroidea morphospecies, three Polychaeta morphospecies, one Isopoda, one Tanaidacea, and a branching Bryozoa. Only 4 morphospecies occurred on 30% of the rocks or more (>25 rocks).

2.2 Faunal Relationship with Substrate Type

The highest average macrofaunal densities were found on basalt (15.62 ± 1.83 ind. 200 cm^{-2}) and phosphorite (14.72 ± 1.28 ind. 200 cm^{-2}) rocks (Chi-squared = 30.116, df = 3, p = 1.305e-06). These two substrates had approximately 50% more animals than ferromanganese crusts (7.13 ± 1.25 ind. 200 cm^{-2}) and sedimentary rocks (9.78 ± 0.64 ind. 200 cm^{-2}) (FeMn crusts vs basalt: z = 4.48, p = 0.00001; phosphorite vs FeMn crusts: z = -4.49, p = 0.00001) (Figure 5A). Macrofauna did not exhibit significantly different densities on phosphorite compared to basalt and sedimentary rocks, and on FeMn crusts compared to sedimentary rocks (Table A3).

Macrofaunal diversity was highest on phosphorite (avg H'$_{[loge]}$ =2.75; avg ES$_{(20)}$ = 12.74) and lowest on FeMn crust (avg H'$_{[loge]}$ =1.80; avg ES$_{(20)}$ = 7.65) as calculated per rock (Table 2 and Figure 5B). Macrofaunal diversity on FeMn crust was significantly lower than on phosphorite, basalt and sedimentary rocks; and significantly higher on phosphorite compared to basalt rocks (H': FeMn crusts vs basalt : z = 2.82, p = 0.004; FeMn crusts vs phosphorite: z = -5.31, p = 0.0001; FeMn crusts vs sedimentary rocks: z = -3.06, p = 0.003; phosphorite vs basalt: z = -2.16, p = 0.02) (Table A4). All substrates exhibited similar, relatively high evenness (0.88 - 0.94) (Table 2).

FeMn crust macrofaunal assemblages were most similar to basalt rocks, followed by phosphorite (25 and 24 taxa in common, respectively) and least similar to sedimentary rocks (5 taxa in common). When rock assemblages were pooled by substrate type, FeMn crusts and sedimentary rocks exhibit the highest rarefaction diversity (Figure 5C). FeMn crusts had the greatest number of unique taxa (95/200), followed by basalt (78/178), phosphorite (72/177) and sedimentary rocks (22/89) (Figure 6).

In terms of phyletic composition, the macrofaunal communities on FeMn crust, phosphorite and
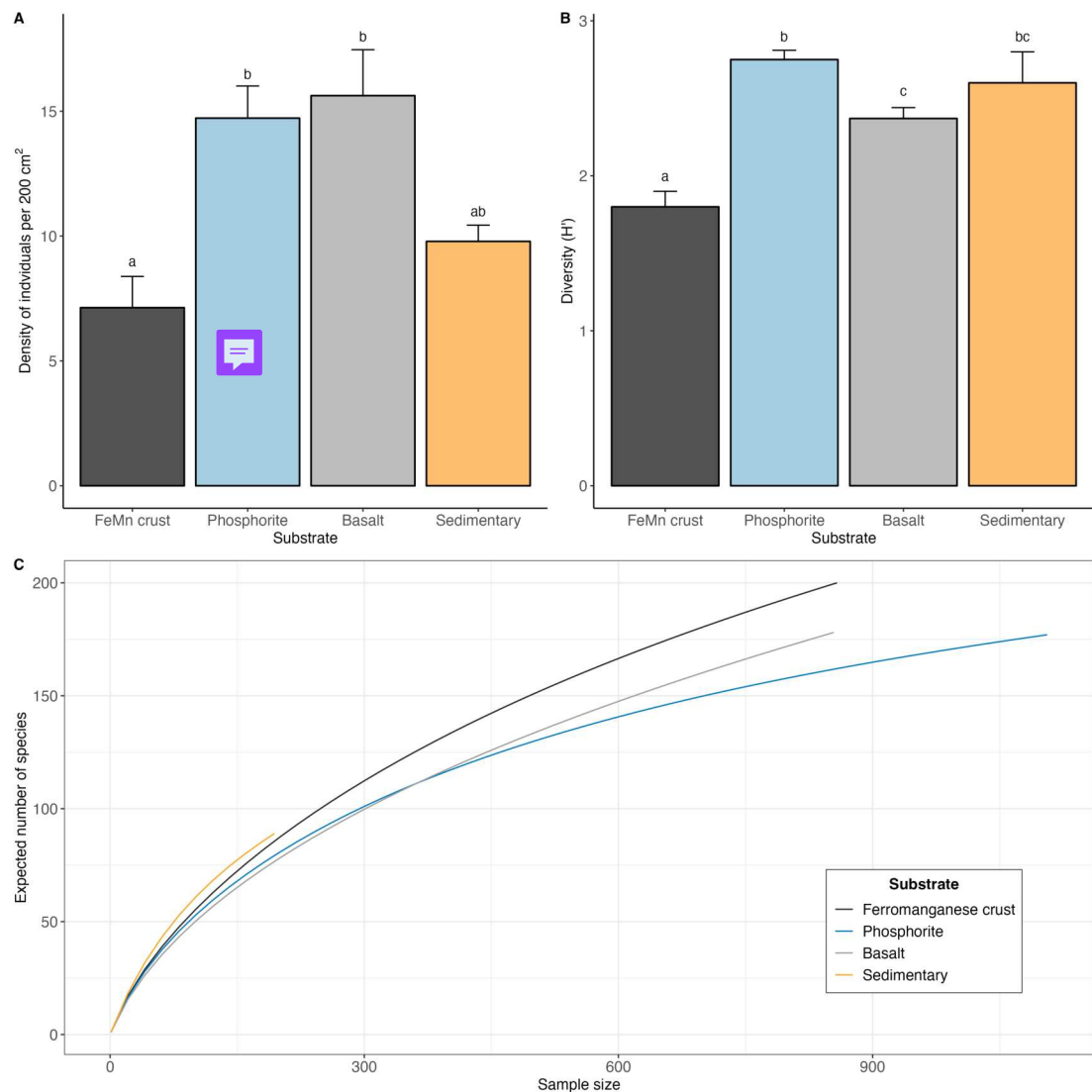
Figure 5. A) Average (± 1 standard error) density of macrofauna per 200 cm^2, B) average Shannon-Weiner diversity index (H'$_{[loge]}$) (±1 standard error), C) rarefaction curve (ES) for macrofaunal diversity for each substrate type derived from data pooled for rocks of the same substrate type.

Table 2. Average macrofaunal species richness (S), diversity (H'), evenness (J'), and $ES_{(20)}$ for each substrate type, using rocks as replicates and substrate comparison results from the Kruskal-Wallis test. The letters in parenthesis next to each value represent the substrates that are statistically different from one another in terms of each diversity metric.

Substrate	Species Richness (S)	Shannon Index (H'_{log_e})	Shannon Index ($H'_{log_{10}}$)	Evenness (J')	$ES_{(20)}$
FeMn crust	10.08 ± 1.48 (a)	1.80 ± 0.10 (a)	0.78 ± 0.04 (a)	0.89 ± 0.02 (a)	7.65 ± 0.62 (a)
Phosphorite	23.26 ± 1.67 (b)	2.75 ± 0.06 (b)	1.19 ± 0.02 (b)	0.88 ± 0.01 (a)	12.74 ± 0.43 (b)
Basalt	16.68 ± 1.24 (b)	2.37 ± 0.07 (c)	1.03 ± 0.03 (c)	0.86 ± 0.02 (a)	11.15 ± 0.53 (b)
Sedimentary	17.57 ± 3.08 (b)	2.60 ± 0.20 (bc)	1.13 ± 0.08 (bc)	0.94 ± 0.01 (a)	12.78 ± 1.44 (b)
Chi-Squared	34.91	32.43	32.43	8.95	28.49
df	3	3	3	3	3
p-value	1.27e-07	4.23e-07	4.23e-07	0.029	2.86e-06

basalt rocks were similar to one another. The dominant phyla across all substrates were Annelida and Echinodermata with the highest percentage of Annelida ($\sim 35\%$) on sedimentary rocks, and an equal proportion of Annelida ($\sim 27\%$) and Echinodermata ($\sim 27\%$) on phosphorite rocks (Figure 7A). FeMn crust and basalt rocks had a similar percentage of Annelida present ($\sim 23\%$ and $\sim 25\%$, respectively). However, FeMn crust is the only substrate with Porifera in their top ten taxa, along with three Ophiuroidea, two Amphipoda and four Polychaeta. Phosphorite rocks exhibited five Ophiuroidea, two Amphipoda, one Tanaidacea, one Isopoda and one Polychaeta in their top ten taxa, whereas basalt rocks were the only substrate with a Bivalvia and a Holothuroidea within the top ten (Table 3).

Benthic macrofaunal community composition differed across substrate type (PERMANOVA: F = 1.65, p = 0.001, df = 3) between FeMn crust and sedimentary rocks (t: 1.19, p: 0.043), phosphorite and basalt rocks (t: 1.61, p: 0.001), and phosphorite and sedimentary rocks (t: 1.53, p: 0.001) (Table A5). Community composition also varied within a substrate, particularly among FeMn crusts (SIMPER, average similarity = 5.39%) when compared to phosphorite rocks (SIMPER, average similarity = 20.98%) (Figure 7B). The taxa that occurred on the most number of FeMn crusts were: *Ophiocten* cf. *centobi*, *Pseudotanais* sp. 1, *Ophioleuce* cf. *gracilis*, and Ophiuroidea sp. 5 (postlarvae) (present on 12, 9, 9, and 8 out of 37 rocks, respectively). For a list of the 135 taxa that occurred once on any FeMn crust, see Table A6. Of the 95 unique taxa on FeMn crusts, 31 taxa were Annelida, 17 taxa were Arthropoda, 1 taxon was branching Bryozoa, 1 taxon was Chordata, 2 taxa were Cnidaria, 9 taxa were Echinodermata, 22 taxa were Mollusca, and 12 taxa were Porifera (Table A7). Of the 72 unique taxa on phosphorite rocks, 39 taxa were Annelida, 13 taxa were Arthropoda, 3 taxa were branching Bryozoa, 1 taxon was a Cnidaria, 5 taxa were Echinodermata, 6 taxa were Mollusca, and 4 taxa were Porifera (Table A8). The six most important taxa contributing to the dissimilarity between FeMn crust and sedimentary rock communities (SIMPER, average dissimilarity = 94.08%) were: *Spirorbinae* Sp. 1, *Ophiocten* cf. *centobi*, Sabellidae sp. 1, Ophiuroidea sp. 11, *Pseudotanais* sp. 1, and Ophiuroidea sp. 5 (postlarvae), which were less abundant on FeMn crust. The six most important taxa contributing to the dissimilarity between phosphorite and basalt rocks (SIMPER, average dissimilarity = 86.47%) were: Ophiuroidea sp. 5 (postlarvae), *Sphaerosyllis* nr. *ranunculus*, *Amphipholis pugetana*? (juvenile), *Munnopsorus* sp. 1, Ophiuroidea sp. 7 (postlarvae), *Ophiocten* cf. *centobi*, which were nearly absent on phosphorite rocks. The six most important taxa contributing to the dissimilarity between phosphorite and sedimentary rocks (SIMPER, average dissimilarity = 88.14%) were: Ophiuroidea sp. 5, *Spirorbinae* sp. 1, *Ophiocten* cf. *centobi*, *Sphaerosyllis* nr. *ranunculus*, *Amphipholis pugetana*? (juvenile), Ophiuroidea sp. 7 (postlarvae), which were nearly absent on sedimentary rocks.
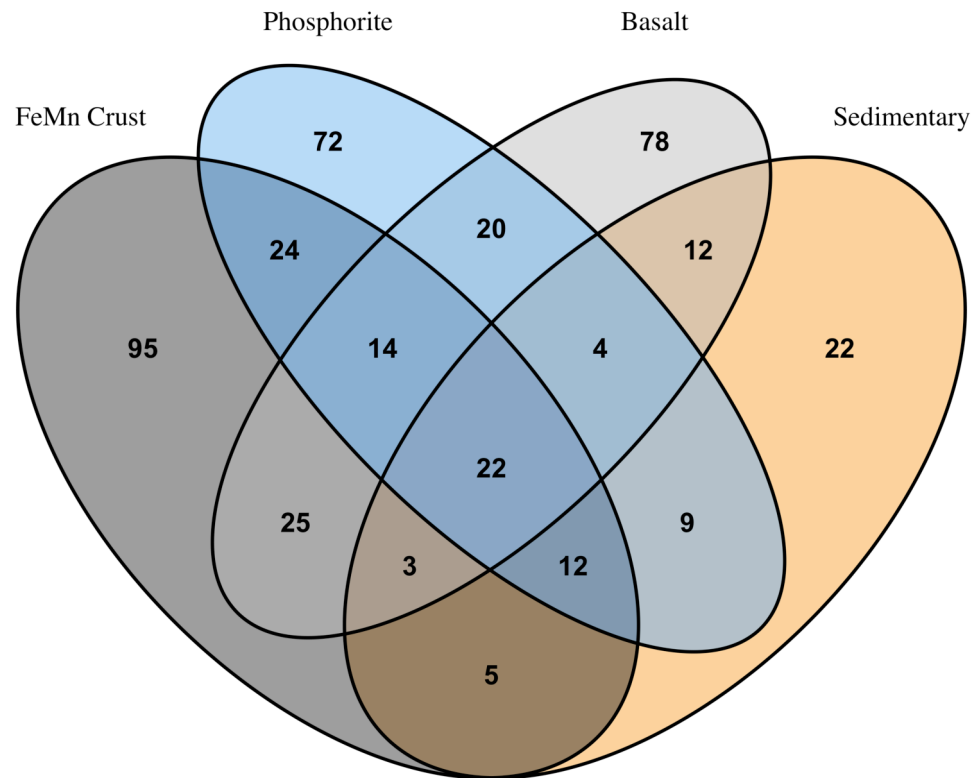
Figure 6. Venn diagram showing numbers of overlapping invertebrate morphospecies among macrofaunal communities on different substrate types.
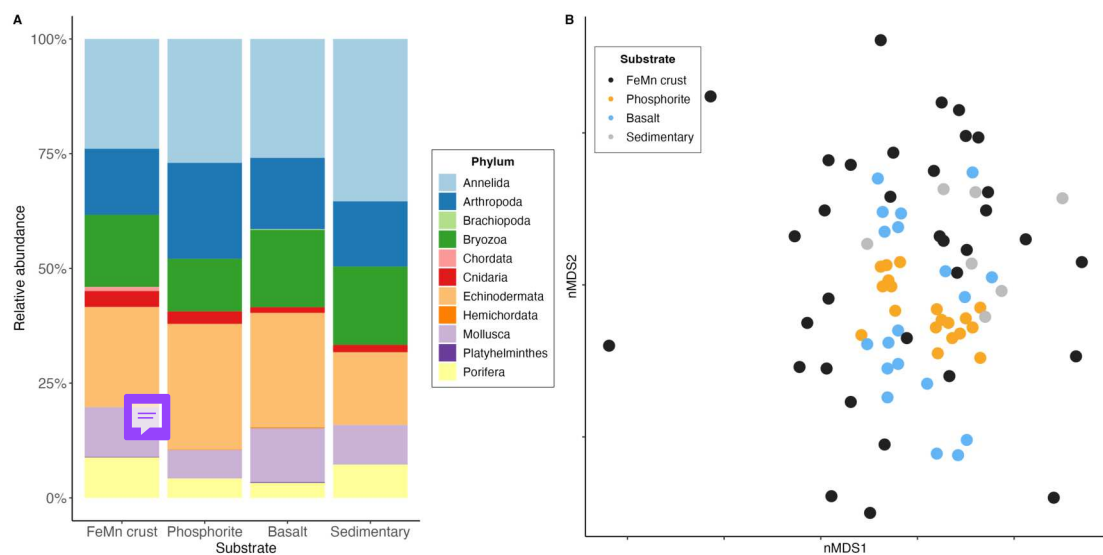

Figure 7. A) Community composition of macrofauna by phylum, and B) Multi-dimensional scaling analysis of macrofauna community composition across different substrate types. Each point represents the assemblage of morphospecies on a single rock.

Table 3. Top 10 taxa by substrate type with corresponding percentages of total macrofaunal individuals per substrate.

FeMn crust	%	Phosphorite rock	%	Basalt rock	%	Sedimentary rock	%
Ophiocten cf. *centobi*	6.6	Ophiuroidea sp. 5 (postlarvae)	12.6	*Protocirrineris* nr. *socialis*	14.2	*Spirorbinae* sp. 1	5.2
Porifera sp. 5	5.2	*Astrophiura marionae*	5.2	Ophiuroidea sp. 5 (postlarvae)	9.6	*Ophiocten* cf. *centobi*	5.2
Ophiuroidea sp. 5 (postlarvae)	4.4	*Sphaerosyllis* nr. *ranunculus*	3.3	*Ophiocten* cf. *centobi*	3.7	*Sphaerosyllis* nr. *ranunculus*	3
Stenothoidae sp. 3	4	*Ophiocten* cf. *centobi*	3.2	Placopecten sp. 1	3	Sabellidae sp. 1	2.6
Spirorbinae sp. 1	3.6	*Amphipholis pugetana*? (juvenile)	2.8	*Ophryotrocha* spp.	2.7	*Rhachotropis inflata*	2.6
Photidae sp. 1	2.6	Ophiuroidea sp. 7 (postlarvae)	2.6	*Astrophiura marionae*	2.6	Stegocephalidae sp. 3	2.2
Serpulidae spp. (juvenile)	2.6	*Munnopsurus* sp. 1	2.5	Ophiuroidea sp. 7 (postlarvae)	2.2	*Pseudotanais* sp. 1	2.2
Ophioleuce cf. *gracilis*	2.4	*Metopa* nr. *dawsoni*	2.2	*Amphipholis pugetana*? (juvenile)	1.9	Ampharetidae sp. 2	1.7
Sabellidae sp. 1	1.5	Stenothoe sp. 1	2	*Psolus* sp.	1.8	Cirratulidae sp. 1	1.7
Gyptis sp. 1	1.4	*Pseudotanais* sp. 1	2	Bivalvia sp. 3 (juvenile)	1.8	Ophiuroidea sp. 11	1.7

2.3 Macrofauna Relationships with Oxygen and Depth as a Function of Substrate Type

Oxygen and depth exhibited multicollinearity ($r = 0.82$, $p = 1.6e-34$). This complicates the interpretation of relationships with the macrofauna community, as changes in one variable may be confounded by the influence of others. Therefore, the following results are presented for each substrate to test the effects of each variable among comparable samples.

Densities on FeMn crust decreased with increasing oxygen (Figure 8A), however on phosphorite, basalt and sedimentary rocks (which were found at shallower depths), densities showed no relationship with oxygen (Figure 8B, C). On FeMn crust and phosphorites, macrofaunal densities decreased with increasing water depth (Figure 8D, E) but no trend was present for the other substrates (Figure 8F). On FeMn crust and phosphorites, macrofaunal diversity also decreased with increasing oxygen (Figures 9A, B) but no trend was observed on the other substrates (Figure 9C). Macrofaunal diversity on FeMn crust decreased with increasing depth (Figure 9D), however exhibited no relationship on the other substrates (Figure 9E, F).

3 DISCUSSION

3.1 Macrofaunal Relationships to Substrate Type and Environmental Variables

In line with previous studies on FeMn crust on seamounts, our results from the SCB revealed lower fauna abundance on FeMn crust compared to non-FeMn substrates (Grigg et al., 2013; Schlacher et al., 2014). In the SCB, phosphorite rocks had 50% more macrofauna than FeMn crusts on average. This trend is driven by phosphorite rocks retrieved from Coronado Escarpment, Cortes Bank and Patton Ridge South at depths <700 m. Among the surveyed sites, FeMn crust from Little Joe Seamount ($\sim$2700 m) exhibited
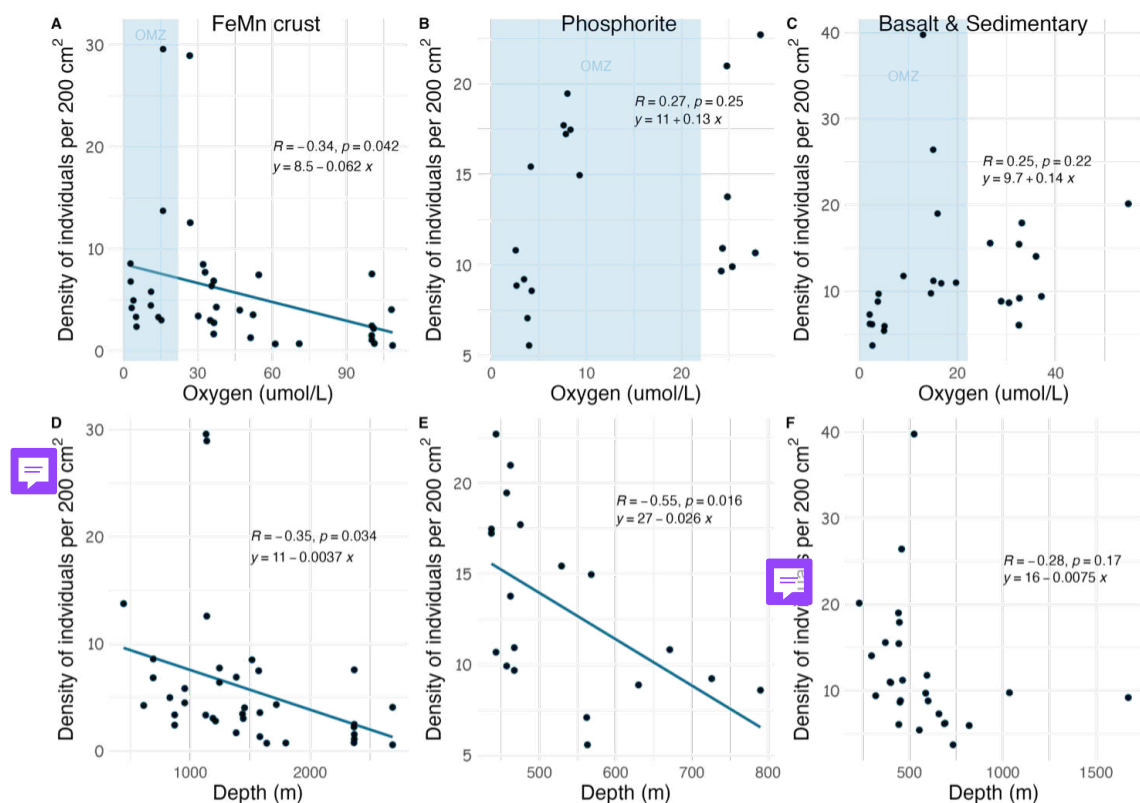
Figure 8. Relationship between macrofaunal density and environmental variables (oxygen and depth) by substrate type (FeMn crust: A, D; phosphorite: B, E; basalt and sedimentary: C, F). The blue shaded areas in the top row represent the oxygen minimum zone.
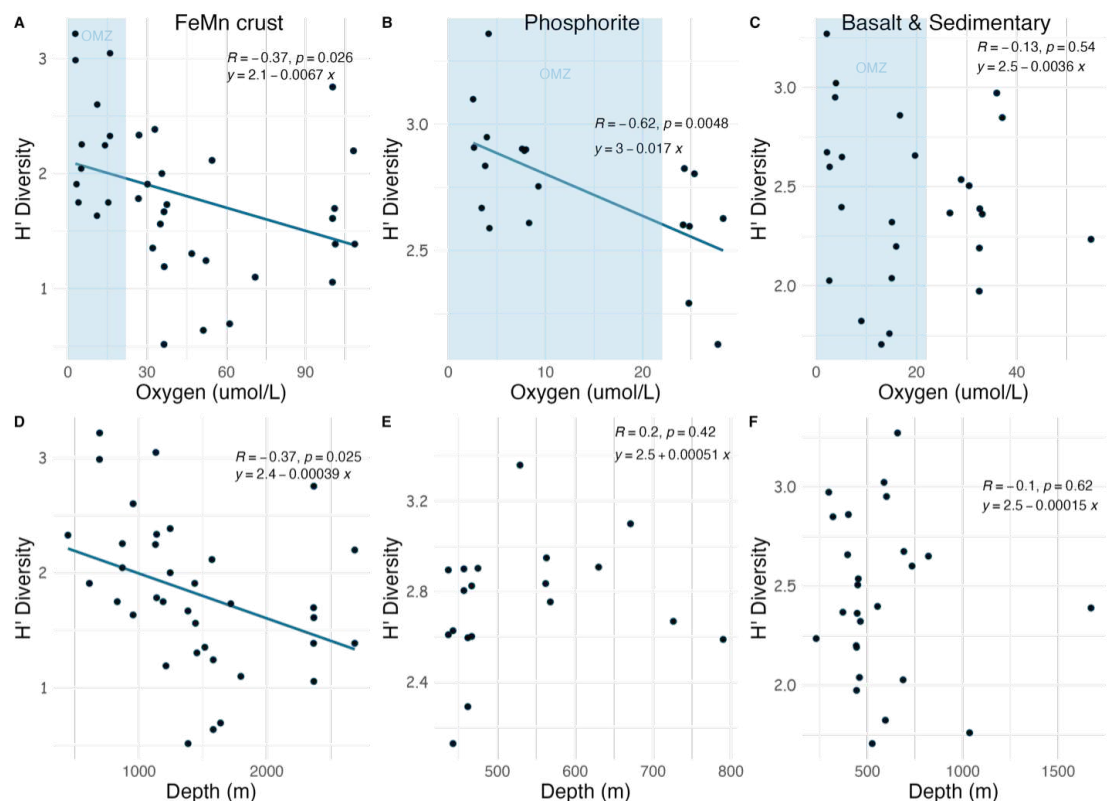
Figure 9. Relationship between macrofaunal diversity and environmental variables (oxygen and depth) by substrate type (FeMn crust: A, D; phosphorite: B, E; basalt and sedimentary: C, F). The highlighted areas in the top row represent the oxygen minimum zone.

the lowest macrofaunal density and the highest diversity when pooled by substrate. Vlach (2022) reported the same pattern for megafauna, which exhibited the lowest density and highest diversity on FeMn crust at Little Joe Seamount in the SCB.

Results presented here show that the community composition of macrofaunal assemblages on FeMn crust in the SCB have a higher number of distinct taxa and their pooled rarefaction diversity is higher compared to non-FeMn substrates. Corrêa et al. (2022) and Schlacher et al. (2014) also noted distinct biological communities on FeMn crust compared to non-FeMn substrates at the Rio Grande Rise (seamount region in the Southwest Atlantic) and at a Hawaiian Seamount Chain in the Central North Pacific, respectively. Vlach (2022) reported that megafaunal communities on FeMn crust exhibit higher rarefaction diversity compared to phosphorite and other rock types in the SCB.

The microbial communities associated with FeMn crust at different depths could promote high diversity among ~~microbial grazing macrofauna communities~~. Kato et al. (2018) showed that FeMn crust from water depths of 1,150 to 5,520 m on a seamount of the northwestern Pacific have distinct microbial communities across depths. Although Bergo et al. (2021) showed that FeMn substrates of the Rio Grande Rise exhibit no difference in their microbial community compared to other substrates in the same region, they found that sampling depth was correlated with differences in microbial community structure. Therefore, water depth might be influencing the uniformity of the microbial community and may be contributing to variations in the macrofauna community (that rely on these microbes as a food source) on FeMn crust at various depths. Further research is needed to determine whether microbes are distinct across different substrates in the SCB.

Variation in macrofauna community composition among FeMn crusts may also be influenced by faunal tolerance to metal concentrations, impacting their settlement (Schlacher et al., 2014). For instance, although it is unclear which aspect of the rocks is supporting the macrofaunal communities, Verlaan (1992) observed higher Foraminifera densities on FeMn crust compared to basalt rocks. On the other hand, studies like Veillette et al. (2007) found no clear relationship between the geochemical composition of FeMn crusts and associated fauna. These conflicting results suggest the need for more comprehensive research on interactions of mineral composition, microbes, and fauna (Clark, 2011; Schlacher et al., 2014). The chemical composition of FeMn crust varies with depth, distance from shore, surface productivity, and distance from the OMZ (Usui et al., 2017; Mizell et al., 2020; Benites et al., 2023). Benites et al. (2023) identified higher concentrations of certain metals, such as Mn, Co, V, As, Mo, Tl, U, Zn, and Sb in FeMn crusts collected at depths exceeding 2000 m in the Southwest Atlantic Ocean. Given the diverse depth range of FeMn crust in this study, there could be potential variations in metal concentrations among rocks. If geochemistry influences faunal distribution based on metal tolerance, it could contribute to the observed community composition differences among FeMn crust (Figure 7B) compared to phosphorites found at similar depths.

The relationship between substrate type and macrofaunal density, diversity and composition may be best explained by environmental factors occurring where each of the substrate types were collected, rather than by substrate type alone. Oxygen and depth were found to be significantly correlated in the SCB study, and the relationship of oxygen with density and diversity of the macrofaunal assemblages is influenced by the depth categories as per a covariate statistical test. Most FeMn crusts were collected from deeper waters (>600 m) and a broad depth range. In contrast, all phosphorite rocks, and most of the basalt and sedimentary rocks were collected from shallower waters and a smaller depth range (231 – 800 m) (Figure 10). FeMn crusts were the only substrate collected from the most oxygenated waters (O_2 >90 μmol L^{-1}) at the deepest depths (>2,200 m). Most phosphorite rocks (12 out of 19) were found within the OMZ above 800 m water depth (Figure 10).

Across all substrates, density decreased with increasing water depth in the SCB. This trend has been observed in other deep-ocean studies from sediments (Levin et al., 2000; Wei et al., 2012; Baldrighi et al., 2014) presumably as a result of decreasing food availability with depth (Ramirez-Llodra et al., 2010). Density of macrofauna on FeMn crust was negatively correlated with oxygen and depth. Macrofaunal densities on FeMn crust were lowest at depths >2000 m and highest within the OMZ (400 – 1100 m). Decomposition of organic matter within the OMZ happens at a slower rate (Ma et al., 2021), thus, more food may arrive at the seafloor within the OMZ, which could explain the pattern of high density on FeMn crusts, phosphorite, basalt and sedimentary rocks in this zone. On a per rock basis, FeMn crusts had lower diversity than the other substrates (Table 2). However, when data were pooled by substrate, FeMn crusts exhibited the highest diversity as shown by rarefaction diversity (Figure 5C). This is likely due to the
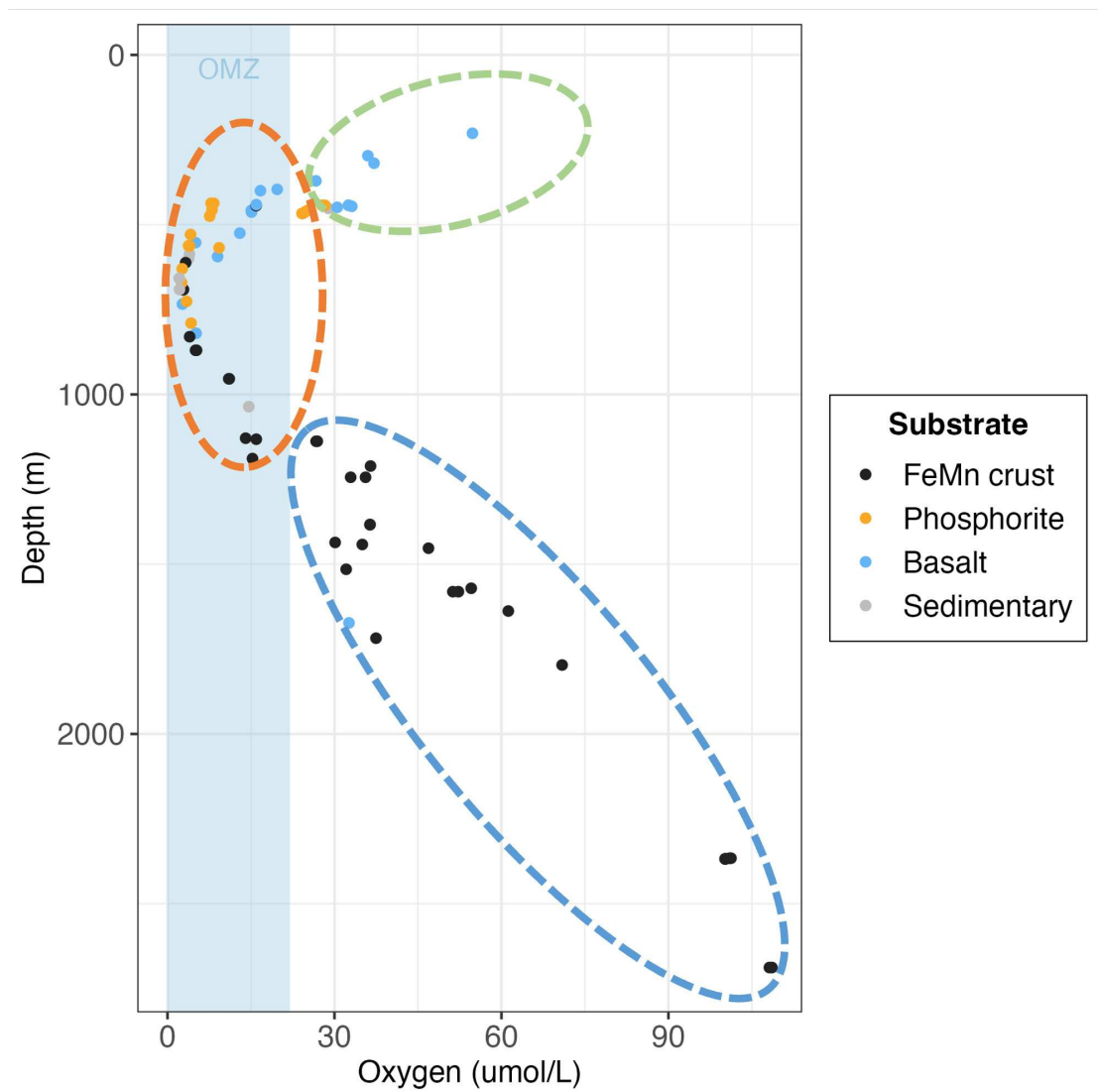
Figure 10. Scatterplot of individual rock samples as a function of oxygen and depth at collection site, colored by substrate type. Ellipses represent the OMZ categories (green = above OMZ, orange = within OMZ and blue = below OMZ).

greater depth range they were collected from (Figure 10). This mirrors the high diversity recently reported for fauna in the polymetallic nodule zone in the Clarion Clipperton Zone (Rabone et al., 2023).

Schlacher et al. (2014), found that faunal assemblages on Pacific seamounts may vary from one site to another within a single seamount (separated by 1 - 2 km), from seamount to seamount, and due to depth variations. The dissimilarity of macrofauna among FeMn crusts sampled in this study may thus be explained by the different depth ranges where the crusts were found (ranging from 600 m to 2700 m). To illustrate, FeMn crusts exhibited high dissimilarity in community composition at different sites on San Juan Seamount. Since all FeMn crusts were found at depths of >600 m and most of them were at >1000 m (Figure 11), the high diversity could be linked to low dominance due to diminishing supply of organic matter with depth (Wei and Rowe, 2019; Levin et al., 2001). Levin et al. (2000), also reported highest rarefaction richness for pooled samples at depths >600 m deep for deep-ocean sediments.

Unexpectedly, there was a significant negative correlation of diversity with oxygen concentration on phosphorite rocks (Figure 9B). Instead of the commonly reported lowest species richness within OMZs (Levin et al., 2001), diversity of phosphorite rocks was highest within the OMZ, and as oxygen increased, diversity decreased. When considering individual rocks, average diversity metrics were highest for phosphorites compared to all other substrates analyzed in this study (Table 2). Leduc et al. (2015), found that macro-infaunal diversity in a phosphorite nodule ecosystem in the regions where the nodules were sitting, is correlated with topographic heterogeneity and variability. Similarly, Veillette et al. (2007) found higher species richness in hard substrates with more complex surfaces. All the phosphorite rocks from this study were characterized by uneven surfaces, including depressions, crevices, and holes, which could explain the high macrofaunal diversity on these rocks. The other substrates studied, particularly FeMn crusts, tended to be smoother and flatter without as many depressions and crevices (Figure 2).

3.2 Density, Diversity and Community Composition of the SCB

Only a limited number of studies provide quantitative data for macrofauna (> 0.3mm) on hard substrates in the deep ocean; the most comparable are data for carbonate rocks. The average macrofaunal densities (ind. 200 cm^{-2}) in the SCB ranged from 15.6 on basalt, 14.7 on phosphorite, 9.8 on sedimentary rocks, and 7.1 on ferromanganese crust; with an overall average of 11.08 ± 0.87 ind. 200 cm^{-2}. These densities are similar to those found on carbonate rocks at inactive sites near methane seeps on the Oregon margin (12.7 and 10.9 ind. 200 cm^{-2} at ~ 600 m and 800 m, respectively) (Levin et al., 2017); and on the Costa Rica margin (14.3 and 11.5 ind. 200 cm^{-2} at ~ 400 and 740 m, respectively) (Levin et al., 2015). However, the SCB macrofaunal densities are notably lower ($< 25\%$) than those reported for inactive carbonates at ~ 1000 m off Costa Rica at Mound 11 (46.7 ind. 200 $^{-2}$) and Mound 12 (43-87 ind. 200 $^{-2}$) by Levin et al. (2015) and Pereira et al. (2021). Relative to carbonates experiencing active seepage, average densities from the SCB were about 2% that of macrofauna on seep carbonates off Costa Rica at 1000 m (610 $\pm$ 123 ind. 200 cm^{-2}) (Pereira et al., 2022) and 18-37% of average densities on carbonates at active methane seeps on the Oregon margin (61.5 and 29.8 ind. 200 cm^{-2} at 600 and 800 m, respectively) (Levin et al., 2017). SCB densities reported here are also much lower than on organic falls: 5% that of macrofauna on whale skeletons from Southern California (average of 223 ind. 200 cm^{-2}) (Baco and Smith, 2003); and 40% that of macrofauna on experimental wood deployed away from seepage in Costa Rica (26 ind. 200 cm^{-2}) (Pereira et al., 2022). Higher macrofaunal densities are expected at active methane seeps as a result of bacterial production stimulated by the availability of methane and hydrogen sulfide. Bacteria provide a primary food source for heterotrophic macrofauna at seeps (Levin et al., 2013, 2015).

In terms of diversity ($H'_{[log_e]}$), the SCB macrofauna on basalt ($H'_{[log_e]} = 2.37$), sedimentary ($H'_{[log_e]} = 2.60$), and phosphorite rocks ($H'_{[log_e]} = 2.75$) from the SCB had a higher average Shannon-Wiener index than the inactive carbonates from the Oregon ($H'_{[log_e]} = 2.05$ at 600 m and 1.76 at 800 m) and Costa Rica margins ($H'_{[log_e]} = 1.80$) and active methane seep sites off Oregon ($H'_{[log_e]} = 1.95$ at 600 m and 1.86 at 800 m) (Levin et al., 2015, 2017), whereas the FeMn crusts had comparable diversity ($H'_{[log_e]} = 1.80$). Diversity at active seeps across the Costa Rica margin ($H'_{[log_e]} = 2.3$) is similar to that on SCB basalt.

Non-reducing hard substrates such as those studied here, phosphorites in New Zealand (Leduc et al., 2015), and inactive carbonates in Costa Rica (Levin et al., 2015) appear to exhibit similar proportions of annelids, gastropods, arthropods, and cnidarians. In contrast, macrofaunal communities exposed to active seepage are dominated by gastropods and annelids which may be grazing chemoautotrophic bacteria (Levin et al., 2015, 2017; Pereira et al., 2022). The proportion of macrofaunal species found per phylum in
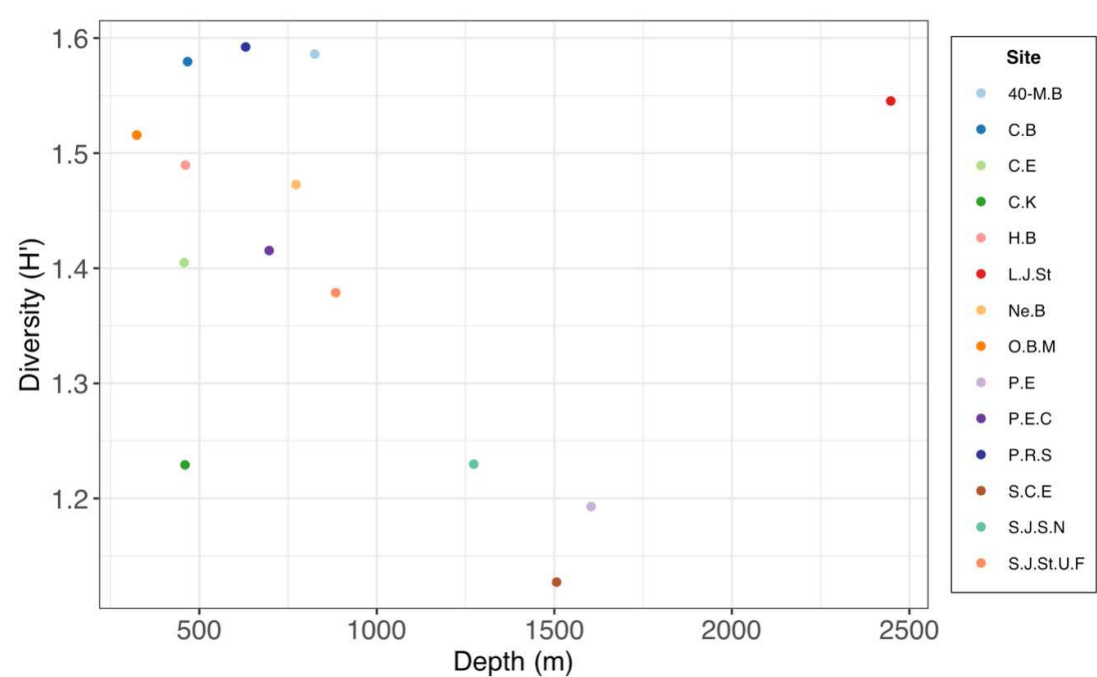
Figure 11. Scatterplot of macrofaunal diversity (H'$_{[loge]}$) on rocks pooled by site and average depth per site.

455 this study was similar to that found by Leduc et al. (2015) in sediments surrounding phosphorite deposits,
456 with arthropods and annelids being the most diverse groups overall.

457 **3.3 Do paradigms developed for deep-sea sediments apply to SCB hard substrates?**
458 Most deep-sea paradigms, such as those involving depth gradients, have evolved based on the study of
459 sediment ecosystems. This study offers an opportunity to examine these paradigms for hard substrates.
460 Researchers initially proposed the concept of peak diversity occurring at mid to lower bathyal depths (1500
461 to 2000 m), establishing the unimodal hypothesis for deep-sea diversity (Rex, 1981). However, the SCB
462 hardground macrofauna does not exhibit the typical unimodal diversity pattern observed in sediments (Rex,
463 1981). In the SCB this pattern is inverted with highest diversity at shallower depths (500 to 1000 m) and
464 deeper depths (2500 m), and low at intermediate depths (1250 to 1600 m) (Figure 11). This finding aligns
465 with other studies that counter universal applicability of the unimodal hypothesis for deep-sea ecosystems
466 (Levin et al., 2001). The SCB study also contributes to a growing understanding of the high heterogeneity
467 and complexity of continental margins and the deep sea in general (Levin and Sibuet, 2012; Danovaro
468 et al., 2014), and the California margin in particular (Kuhnz et al., 2022, Vlach et al., submitted). The
469 original paradigm of a homogeneous, desert-like sediment covered ecosystem has been replaced by one
470 of heterogeneous substrates, topographic features and environmental conditions supporting diverse biota.
471 The SCB escarpments, seamounts, knolls, and ridges comprised of ferromanganese crusts, phosphorites,
472 basalts and sedimentary rocks spanning a range of depths, temperatures and oxygen regimes reflect this
473 heterogeneity.

474 One paradigm that may be be supported by this study involves rarity. As observed in deep-sea
475 ecosystems (Bax, 2011), our SCB samples were dominated by rare species, with one or two individuals
476 appearing in the whole study. Such species (singletons or doubletons) accounted for 56.35% of all
477 individuals sampled. This finding suggests that macrofauna in mineral-rich hard substrates may resemble
478 macrofauna of deep-sea sediments in being comprised largely of rare species (Carney, 1997). This rarity
479 trend has also been observed for manganese nodules considered for deep-sea mining in the Clarion
480 Clipperton Zone located in the Pacific (Christodoulou et al., 2019; Macheriotou et al., 2020; Pape et al.,
481 2021), and it highlights the potential loss of unique biodiversity within deep-sea ecosystems with the
482 exploitation of associated marine minerals.

3.4 Edge Effects

According to Levin (2003) and Gooday et al. (2010), large abundances of ophiuroids are common at OMZ edge zones because of more food availability. Five of the 10 most abundant taxa on phosphorites, which dominated near OMZ boundaries, were Echinodermata morphospecies, including Ophiuroidea sp.5 (postlarvae) and *Astrophiura marionae*, *Ophiocten* cf. *centobi*, *Amphipholis pugetana*? (juvenile), and Ophiuroidea sp.7 (postlarvae). The most abundant Annelida species was *Sphaerosyllis* nr. *ranunculus* and the other most abundant morphospecies were within Isopoda, Amphipoda and Tanaidacea. Ophiuroidea morphospecies reached their highest densities on phosphorite rocks at shallower depths (0 - 500 m), and at the lower transition zone of the OMZ where oxygen concentrations ranged from 6 - 22 and 22 - 40 μmol L^{-1}. Earlier studies on the margin off Central and Southern California also found high densities of ophiuroids at oxygen concentrations of 22.3 μmol L^{-1} and 17 μmol L^{-1} (Smith and Hamilton, 1983; Thompson et al., 1985). A similar trend was observed for the megafauna of the SCB (Vlach, 2022).

3.5 Relevance for Seafloor Management.

The seafloor of the California continental margin is vulnerable to multiple stressors, including from pollution (Schmidt et al., 2024), warming, acidification and deoxygenation (Evans et al., 2020), offshore wind infrastructure, and resource extraction (bottom fishing, oil and gas extraction, and potentially seabed mining) (Ramirez-Llodra et al., 2011). Studies are lacking on how these different stressors interact with communities on different substrate types. The examination of all FeMn crusts revealed that in aggregate they host a high level of diversity and 47% of the species examined were exclusive to this substrate. Similarly, phosphorite rocks exhibited notable diversity on an individual rock basis and 40% of the species found were exclusive to this substrate. Because diversity is strongly influenced by depth and substrate type it will be important that protections aimed at preserving biodiversity cover a broad range of depths and substrates in the SCB.

Protections against harmful commercial fishing gear have been implemented by NOAA Fisheries on San Juan Seamount, 40-Mile Bank, and Northeast Bank due to their classification as Habitat Areas of Particular Concern (HAPC) (PFMC, 2023; NOAA, 2021). HAPCs are conservation priority areas considered for their rarity, ecosystem function importance and sensitivity to human activities (NOAA, 2021). Although oil and gas extraction occurs off Southern California, there is a ban on new leases in State waters, and no new federal leases have occurred off California. In addition, California state waters (0 to 3 nautical miles from shore), have been protected from mining under the California Seabed Mining Prevention Act in 2022 AB1832 (2022). The sites studied in this paper are in federal waters and could be susceptible to disturbance from mining, should it ever occur. However, at present, there are no mining contracts under consideration off California and metal grades suggest it is not likely soon (Conrad et al., 2017). The baseline findings presented here, concerning heterogeneity, rarity and high diversity on mineral-rich substrates, can help us understand other regions where FeMn crust and phosphorite rocks are present and which are being considered for exploitation.

CONCLUSIONS

1) This study highlights the under-researched macrofauna of SCB hardgrounds, revealing a diverse fauna with few highly dominant species and many rare taxa.

2) Macrofauna on hardgrounds in the SCB are highly heterogeneous with respect to density, diversity, and composition as a result of varied substrate type, depth, and seawater oxygen concentration.

3) SCB hard substrate macrofauna at bathyal depths exhibit patterns that counter several deep-sea paradigms, including the absence of unimodal diversity (mid-slope maximum) and lack of depressed diversity in the oxygen minimum zone, but they resemble deep-sea sediments in having high diversity comprised of rare species.

4) FeMn crusts in the SCB exhibit high macrofaunal diversity, likely due to the heterogeneous environmental conditions at the different study sites, including varying temperatures, water depth, oxygen levels, and food supply.

5) Additional research is needed on (a) how and if the microbial community differs among substrate types and moderates macrofaunal community structure, through settlement cues, chemical mediation, or food provision and (b) if disparity in surface texture among substrate types contributes

534 to differences in macrofaunal density and diversity across the studied substrates. The phosphorite
535 rocks were characterized by holes, crevices and depressions, which could account for the observed
536 high macrofaunal diversity on a per rock basis, when compared to the other smoother substrates in
537 this study.

538 6) Biodiversity data for the SCB reported here can inform offshore resource management regarding
539 fishing, pollution, climate change, energy infrastructure and potentially seabed mining.

ACKNOWLEDGMENTS

3.6 Declaration of funding.

REFERENCES

559 AB1832, C. (2022). Waters subject to tidal influence: hard mineral extraction.
560 Amon, D. J., Ziegler, A. F., Dahlgren, T. G., Glover, A. G., Goineau, A., Gooday, A. J., Wiklund,
561 H., and Smith, C. R. (2016). Insights into the abundance and diversity of abyssal megafauna in a
562 polymetallic-nodule region in the eastern clarion-clipperton zone. *Scientific Reports*, 6:30492.
563 Baco, A. and Smith, C. (2003). High species richness in deep-sea chemoautotrophic whale skeleton
564 communities. *Marine Ecology Progress Series*, 260:109–114.
565 Baker, M., Ramirez-Llodra, E., and Tyler, P. (2020). *Natural Capital and Exploitation of the Deep Ocean*.
566 Oxford University Press.
567 Baldrighi, E., Lavaleye, M., Aliani, S., Conversi, A., and Manini, E. (2014). Large spatial scale variability
568 in bathyal macrobenthos abundance, biomass, α- and β-diversity along the mediterranean continental
569 margin. *PLoS ONE*, 9:e107261.
570 Bax, N. (2011). Book review • deep-sea biodiversity: Pattern and scale. *Oceanography*, 24:183–185.
571 Benites, M., González, J., Hein, J., Marino, E., Reyes, J., Millo, C., and Jovane, L. (2023). Controls on
572 the chemical composition of ferromanganese crusts from deep-water to the summit of the rio grande
573 rise, south atlantic ocean. *Marine Geology*, 462:107094.
574 Benjamini, Y. and Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful
575 approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)*,
576 57(1):289–300.
577 Bergo, N. M., Bendia, A. G., Ferreira, J. C. N., Murton, B. J., Brandini, F. P., and Pellizari, V. H. (2021).
578 Microbial diversity of deep-sea ferromanganese crust field in the rio grande rise, southwestern atlantic
579 ocean. *Microbial Ecology*, 82:344–355.
580 Bograd, S. J., Buil, M. P., Lorenzo, E. D., Castro, C. G., Schroeder, I. D., Goericke, R., Anderson,
581 C. R., Benitez-Nelson, C., and Whitney, F. A. (2015). Changes in source waters to the southern
582 california bight. *Deep Sea Research Part II: Topical Studies in Oceanography*, 112:42–52. CCE-LTER:
583 Responses of the California Current Ecosystem to Climate Forcing.

Bourque, J. R., Robertson, C. M., Brooke, S., and Demopoulos, A. W. (2017). Macrofaunal communities associated with chemosynthetic habitats from the u.s. atlantic margin: A comparison among depth and habitat types. *Deep Sea Research Part II: Topical Studies in Oceanography*, 137:42–55.

Breitburg, D., Levin, L. A., Oschlies, A., Grégoire, M., Chavez, F. P., Conley, D. J., Garçon, V., Gilbert, D., Gutiérrez, D., Isensee, K., Jacinto, G. S., Limburg, K. E., Montes, I., Naqvi, S. W. A., Pitcher, G. C., Rabalais, N. N., Roman, M. R., Rose, K. A., Seibel, B. A., Telszewski, M., Yasuhara, M., and Zhang, J. (2018). Declining oxygen in the global ocean and coastal waters. *Science*, 359.

Buhl-Mortensen, L., Vanreusel, A., Gooday, A. J., Levin, L. A., Priede, I. G., Buhl-Mortensen, P., Gheerardyn, H., King, N. J., and Raes, M. (2010). Biological structures as a source of habitat heterogeneity and biodiversity on the deep ocean margins. *Marine Ecology*, 31(1):21–50.

Carney, R. S. (1997). Basing conservation policies for the deep-sea ¯oor on current-diversity concepts: a consideration of rarity.

Checkley, D. M. and Barth, J. A. (2009). Patterns and processes in the california current system. *Progress in Oceanography*, 83:49–64.

Christodoulou, M., O'Hara, T. D., Hugall, A. F., and Arbizu, P. M. (2019). Dark ophiuroid biodiversity in a prospective abyssal mine field. *Current Biology*, 29:3909–3912.e3.

Chuar, C. H., Tong, S. J. W., Chim, C. K., Wong, H. P. S., and Tan, K. S. (2020). Abyssal macrofaunal community structure in the polymetallic nodule exploration area at the easternmost region of the clarion-clipperton fracture zone, pacific ocean. *Deep-Sea Research Part I: Oceanographic Research Papers*, 161.

Clark, M. R. (2011). *Fauna of cobalt-rich ferromanganese crust seamounts*. International Seabed Authority.

Conrad, T., Hein, J. R., Paytan, A., and Clague, D. A. (2017). Formation of fe-mn crusts within a continental margin environment. *Ore Geology Reviews*, 87:25–40.

Corrêa, P. V. F., Jovane, L., Murton, B. J., and Sumida, P. Y. G. (2022). Benthic megafauna habitats, community structure and environmental drivers at rio grande rise (sw atlantic). *Deep Sea Research Part I: Oceanographic Research Papers*, 186:103811.

Danovaro, R., Fanelli, E., Aguzzi, J., Billett, D., Carugati, L., Corinaldesi, C., Dell'Anno, A., Gjerde, K., Jamieson, A. J., Kark, S., McClain, C., Levin, L., Levin, N., Ramirez-Llodra, E., Ruhl, H., Smith, C. R., Snelgrove, P. V. R., Thomsen, L., Dover, C. L. V., and Yasuhara, M. (2020). Ecological variables for developing a global deep-ocean monitoring and conservation strategy. *Nature Ecology and Evolution*, 4:181–192.

Danovaro, R., Gambi, C., Dell'Anno, A., Corinaldesi, C., Fraschetti, S., Vanreusel, A., Vincx, M., and Gooday, A. J. (2008). Exponential decline of deep-sea ecosystem functioning linked to benthic biodiversity loss. *Current Biology*, 18:1–8.

Danovaro, R., Snelgrove, P. V., and Tyler, P. (2014). Challenging the paradigms of deep-sea ecology. *Trends in ecology and evolution (Amsterdam)*, 29(8):465–475.

De Smet, B., Pape, E., Riehl, T., Bonifácio, P., Colson, L., and Vanreusel, A. (2017). The community structure of deep-sea macrofauna associated with polymetallic nodules in the eastern part of the clarion-clipperton fracture zone. *Frontiers in Marine Science*, 4.

De Smet, B., Simon-Lledó, E., Mevenkamp, L., Pape, E., Pasotti, F., Jones, D. O., and Vanreusel, A. (2021). The megafauna community from an abyssal area of interest for mining of polymetallic nodules. *Deep-Sea Research Part I: Oceanographic Research Papers*, 172.

Dong, D., Li, X., Yang, M., Gong, L., Li, Y., Sui, J., Gan, Z., Kou, Q., Xiao, N., and Zhang, J. (2021). Report of epibenthic macrofauna found from haima cold seeps and adjacent deep-sea habitats, south china sea. *Marine Life Science and Technology*, 3.

Evans, N., Schroeder, I. D., Buil, M. P., Jacox, M. G., and Bograd, S. J. (2020). Drivers of subsurface deoxygenation in the southern california current system. *Geophysical Research Letters*, 47.

Gage, J. D. and Tyler, P. A. (1991). *Deep-Sea Biology*. Cambridge University Press.

Gooday, A. J., Bett, B. J., Escobar, E., Ingole, B., Levin, L. A., Neira, C., Raman, A. V., and Sellanes, J. (2010). Habitat heterogeneity and its influence on benthic biodiversity in oxygen minimum zones. *Marine Ecology*, 31:125–147.

Grigg, R. W., Malaboff, A., Chave, E. H., and Landahl, J. (2013). *Seamount Benthic Ecology and Potential Environmental Impact from Manganese Crust Mining in Hawaii*, pages 379–390.

Grischenko, A., Gordon, D. P., and Melnik, V. P. (2018). Bryozoa (cyclostomata and ctenostomata) from

polymetallic nodules in the russian exploration area, clarion-clipperton fracture zone, eastern pacific ocean-taxon novelty and implications of mining. *Zootaxa*, 4484(1):1–91.

Halbach, P., FT, M., and Otten, P. (1982). Co-rich ferromanganese deposits in the marginal seamount regions of the central pacific basin: Results of the midpac 81.

Hein, J., Koschinsky, A., Mikesell, M., Mizell, K., Glenn, C., and Wood, R. (2016). Marine phosphorites as potential resources for heavy rare earth elements and yttrium. *Minerals*, 6:88.

Hein, J. R., Mizell, K., Koschinsky, A., and Conrad, T. A. (2013). Deep-ocean mineral deposits as a source of critical metals for high- and green-technology applications: Comparison with land-based resources.

Jones, D. O. B., Amon, D. J., and Chapman, A. S. A. (2018). Mining deep-ocean mineral deposits: What are the ecological risks? *Elements*, 14:325–330.

Kato, S., Okumura, T., Uematsu, K., Hirai, M., Iijima, K., Usui, A., and Suzuki, K. (2018). Heterogeneity of microbial communities on deep-sea ferromanganese crusts in the takuyo-daigo seamount. *Microbes and Environments*, 33:366–377.

Kersken, D., Janussen, D., and Martínez Arbizu, P. (2018). Deep-sea glass sponges (hexactinellida) from polymetallic nodule fields in the clarion-clipperton fracture zone (ccfz), northeastern pacific: Part i – amphidiscophora. *Marine biodiversity*, 48(1):545–573.

Kuhnz, L. A., Gilbane, L., Cochrane, G. R., and Paull, C. K. (2022). Multi-factor biotopes as a method for detailed site characterization in diverse benthic megafaunal communities and habitats in deep-water off morro bay, california. *Deep Sea Research Part I: Oceanographic Research Papers*, 190:103872.

Le, J. T., Levin, L. A., and Carson, R. T. (2017). Incorporating ecosystem services into environmental management of deep-seabed mining. *Deep Sea Research Part II: Topical Studies in Oceanography*, 137:486–503.

Leduc, D., Rowden, A. A., Torres, L. G., Nodder, S. D., and Pallentin, A. (2015). Distribution of macro-infaunal communities in phosphorite nodule deposits on chatham rise, southwest pacific: Implications for management of seabed mining. *Deep-Sea Research Part I: Oceanographic Research Papers*, 99:105–118.

Levin, L., Gutiérrez, D., Rathburn, A., C, C. N., Sellanes, J., Muñoz, P., Gallardo, V., and Salamanca, M. (2002). Benthic processes on the peru margin: a transect across the oxygen minimum zone during the 1997-98 el niño.

Levin, L. A. (2003). Oxygen minimum zone benthos: Adaptation and community response to hypoxia.

Levin, L. A., Etter, R. J., Rex, M. A., Gooday, A. J., Smith, C. R., Jes´, J., Pineda, J., Stuart, C. T., Hessler, R. R., and Pawson, D. (2001). Environmental influences on regional deep-sea species diversity *.

Levin, L. A., Gage, J. D., Martin, C., and Lamont, P. A. (2000). Macrobenthic community structure within and beneath the oxygen minimum zone, nw arabian sea.

Levin, L. A., Huggett, C. L., and Wishner, K. F. (1991). Previously published as cynthia l. thomas, 3. graduate school of oceanography.

Levin, L. A., Mendoza, G. F., and Grupe, B. M. (2017). Methane seepage effects on biodiversity and biological traits of macrofauna inhabiting authigenic carbonates. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 137:26–41.

Levin, L. A., Mendoza, G. F., Grupe, B. M., Gonzalez, J. P., Jellison, B., Rouse, G., Thurber, A. R., and Waren, A. (2015). Biodiversity on the rocks: Macrofauna inhabiting authigenic carbonate at costa rica methane seeps. *PLoS ONE*, 10.

Levin, L. A., Mengerink, K., Gjerde, K. M., Rowden, A. A., Dover, C. L. V., Clark, M. R., Ramirez-Llodra, E., Currie, B., Smith, C. R., Sato, K. N., Gallo, N., Sweetman, A. K., Lily, H., Armstrong, C. W., and Brider, J. (2016). Defining "serious harm" to the marine environment in the context of deep-seabed mining. *Marine Policy*, 74:245–259.

Levin, L. A. and Sibuet, M. (2012). Understanding continental margin biodiversity: A new imperative. *Annual Review of Marine Science*, 4:79–112.

Levin, L. A., Ziebis, W., F. Mendoza, G., Bertics, V. J., Washington, T., Gonzalez, J., Thurber, A. R., Ebbe, B., and Lee, R. W. (2013). Ecological release and niche partitioning under stress: Lessons from dorvilleid polychaetes in sulfidic sediments at methane seeps. *Deep Sea Research Part II: Topical Studies in Oceanography*, 92:214–233. Deep-Sea Biodiversity and Life History Processes.

Li, X. (2017). Taxonomic research on deep-sea macrofauna in the south china sea using the chinese deep-sea submersible ¡i¿jiaolong¡/i¿. *Integrative Zoology*, 12:270–282.

Lins, L., Zeppilli, D., Menot, L., Michel, L. N., Bonifácio, P., Brandt, M., Pape, E., Rossel, S., Uhlenkott, K., Macheriotou, L., Bezerra, T. N., Sánchez, N., Alfaro-Lucas, J. M., Arbizu, P. M., Kaiser, S., Murakami, C., and Vanreusel, A. (2021). Toward a reliable assessment of potential ecological impacts of deep-sea polymetallic nodule mining on abyssal infauna.

Ma, J., Song, J., Li, X., Wang, Q., Zhong, G., Yuan, H., Li, N., and Duan, L. (2021). The omz and its influence on poc in the tropical western pacific ocean: Based on the survey in march 2018. *Frontiers in Earth Science*, 9.

Macheriotou, L., Rigaux, A., Derycke, S., and Vanreusel, A. (2020). Phylogenetic clustering and rarity imply risk of local species extinction in prospective deep-sea mining areas of the clarion–clipperton fracture zone. *Proceedings of the Royal Society B: Biological Sciences*, 287:20192666.

Mizell, K., Hein, J. R., Lam, P. J., Koppers, A. A. P., and Staudigel, H. (2020). Geographic and oceanographic influences on ferromanganese crust composition along a pacific ocean meridional transect, 14 n to 14s. *Geochemistry, Geophysics, Geosystems*, 21.

Montserrat, F., Guilhon, M., Corrêa, P. V. F., Bergo, N. M., Signori, C. N., Tura, P. M., de los Santos Maly, M., Moura, D., Jovane, L., Pellizari, V., Sumida, P. Y. G., Brandini, F. P., and Turra, A. (2019). Deep-sea mining on the rio grande rise (southwestern atlantic): A review on environmental baseline, ecosystem services and potential impacts.

Mullineaux, L. S. (1987). Organisms living on manganese nodules and crusts: distribution and abundance at three north pacific sites.

Mullineaux, L. S. (1989). Vertical distributions of the epifauna on manganese nodules: Implications for settlement and feeding. *Limnology and Oceanography*, 34:1247–1262.

NOAA (2021). Habitat areas of particular concern on the west coast.

Oliver, T. H., Heard, M. S., Isaac, N. J., Roy, D. B., Procter, D., Eigenbrod, F., Freckleton, R., Hector, A., Orme, C. D. L., Petchey, O. L., Proença, V., Raffaelli, D., Suttle, K. B., Mace, G. M., Martín-López, B., Woodcock, B. A., and Bullock, J. M. (2015). Biodiversity and resilience of ecosystem functions.

Oschlies, A., Brandt, P., Stramma, L., and Schmidtko, S. (2018). Drivers and mechanisms of ocean deoxygenation. *Nature Geoscience*, 11:467–473.

Pape, E., Bezerra, T. N., Gheerardyn, H., Buydens, M., Kieswetter, A., and Vanreusel, A. (2021). Potential impacts of polymetallic nodule removal on deep-sea meiofauna. *Scientific Reports*, 11:19996.

PCMSC (2022). Researching seafloor mineral resources that occur within the u.s. exclusive economic zone and areas beyond national jurisdictions.

Pereira, O. S., Gonzalez, J., Mendoza, G., Le, J., McNeill, M., Ontiveros, J., Lee, R. W., Rouse, G. W., Cortés, J., and Levin, L. A. (2022). Does substrate matter in the deep sea? a comparison of bone, wood, and carbonate rock colonizers. *PLoS ONE*, 17.

Pereira, O. S., Gonzalez, J., Mendoza, G. F., Le, J., Coscino, C. L., Lee, R. W., Cortés, J., Cordes, E. E., and Levin, L. A. (2021). The dynamic influence of methane seepage on macrofauna inhabiting authigenic carbonates. *Ecosphere*, 12.

PFMC (2023). Pacific coast groundfish fishery management plan.

Rabone, M., Wiethase, J. H., Simon-Lledó, E., Emery, A. M., Jones, D. O., Dahlgren, T. G., Bribiesca-Contreras, G., Wiklund, H., Horton, T., and Glover, A. G. (2023). How many metazoan species live in the world's largest mineral exploration region? *Current Biology*, 33:2383–2396.e5.

Ramirez-Llodra, E., Brandt, A., Danovaro, R., Mol, B. D., Escobar, E., German, C. R., Levin, L. A., Arbizu, P. M., Menot, L., Buhl-Mortensen, P., Narayanaswamy, B. E., Smith, C. R., Tittensor, D. P., Tyler, P. A., Vanreusel, A., and Vecchione, M. (2010). Deep, diverse and definitely different: Unique attributes of the world's largest ecosystem. *Biogeosciences*, 7:2851–2899.

Ramirez-Llodra, E., Tyler, P. A., Baker, M. C., Bergstad, O. A., Clark, M. R., Escobar, E., Levin, L. A., Menot, L., Rowden, A. A., Smith, C. R., and Dover, C. L. V. (2011). Man and the last great wilderness: Human impact on the deep sea. *PLoS ONE*, 6:e22588.

Rex, M. A. (1981). Community structure in the deep-sea benthos.

Schlacher, T. A., Baco, A. R., Rowden, A. A., O'Hara, T. D., Clark, M. R., Kelley, C., and Dower, J. F. (2014). Seamount benthos in a cobalt-rich crust region of the central ¡scp¿p¡/scp¿ acific: conservation challenges for future seabed mining. *Diversity and Distributions*, 20:491–502.

Schmidt, J. T., Wu, M. S. C., Kittner, H. E., Arey, J. S., Hammond, D. E., , and Valentine, D. L. (2024). Disentangling the history of deep ocean disposal for ddt and other industrial waste off southern california. *Environmental Science & Technology*, 58(9):4346–4356. PMID: 38380834.

Simon-Lledó, E., Bett, B. J., Huvenne, V. A. I., Schoening, T., Benoist, N. M. A., and Jones, D. O. B. (2019). Ecology of a polymetallic nodule occurrence gradient: Implications for deep-sea mining. *Limnology and Oceanography*, 64:1883–1894.

Smith, C. R. and Hamilton, S. C. (1983). Epibenthic megafauna of a bathyal basin off southern california: patterns of abundance, biomass, and dispersion. *Deep Sea Research Part A. Oceanographic Research Papers*, 30:907–928.

Stramma, L., Schmidtko, S., Levin, L. A., and Johnson, G. C. (2010). Ocean oxygen minima expansions and their biological impacts. *Deep Sea Research Part I: Oceanographic Research Papers*, 57:587–595.

Thompson, J. B., Mullins, H. T., Newton, C. R., and Vercoutere, T. L. (1985). Alternative biofacies model for dysaerobic communities. *Lethaia*, 18:167–179.

Thurber, A. R., Sweetman, A. K., Narayanaswamy, B. E., Jones, D. O. B., Ingels, J., and Hansman, R. L. (2014). Ecosystem function and services provided by the deep sea. *Biogeosciences*, 11:3941–3963.

Tilot, V. (2006a). *Biodiversity and Distribution of the Megafauna: The polymetallic nodule ecosystem of the eastern equatorial Pacific Ocean*, volume 1. Unesco.

Tilot, V. (2006b). *Biodiversity and Distribution of the Megafauna. Vol. 2: Annotated Photographic Atlas of the Echinoderms of the Clarion-Clipperton Fracture Zone*. Intergovernmental Oceanographic Commission.

Toscano, F. and Raspini, A. (2005). Epilithozoan fauna associated with ferromanganese crustgrounds on the continental slope segment between capri and li galli islands (bay of salerno, northern tyrrhenian sea, italy). *Facies*, 50:427–441.

Usui, A., Nishi, K., Sato, H., Nakasato, Y., Thornton, B., Kashiwabara, T., Tokumaru, A., Sakaguchi, A., Yamaoka, K., Kato, S., Nitahara, S., Suzuki, K., Iijima, K., and Urabe, T. (2017). Continuous growth of hydrogenetic ferromanganese crusts since 17 myr ago on takuyo-daigo seamount, nw pacific, at water depths of 800–5500 m. *Ore Geology Reviews*, 87:71–87.

Vanreusel, A., Fonseca, G., Danovaro, R., Silva, M. C. D., Esteves, A. M., Ferrero, T., Gad, G., Galtsova, V., Gambi, C., Genevois, V. D. F., Ingels, J., Ingole, B., Lampadariou, N., Merckx, B., Miljutin, D., Miljutina, M., Muthumbi, A., Netto, S., Portnova, D., Radziejewska, T., Raes, M., Tchesunov, A., Vanaverbeke, J., Gaever, S. V., Venekey, V., Bezerra, T. N., Flint, H., Copley, J., Pape, E., Zeppilli, D., Martinez, P. A., and Galeron, J. (2010). The contribution of deep-sea macrohabitat heterogeneity to global nematode diversity. *Marine Ecology*, 31:6–20.

Veillette, J., Sarrazin, J., Gooday, A. J., Galéron, J., Caprais, J.-C., Vangriesheim, A., Étoubleau, J., Christian, J. R., and Juniper, S. K. (2007). Ferromanganese nodule fauna in the tropical north pacific ocean: Species richness, faunal cover and spatial distribution. *Deep Sea Research Part I: Oceanographic Research Papers*, 54:1912–1935.

Verlaan, P. A. (1992). Marine biology benthic recruitment and manganese crust formation on.

Vla D. S. (2022). Characterizing megafaunal communities on ferromanganese and phosphorite-rich hardgrounds in the southern california borderland.

Wei, C.-L. and Rowe, G. T. (2019). Productivity controls macrofauna diversity in the deep northern gulf of mexico. *Deep Sea Research Part I: Oceanographic Research Papers*, 143:17–27.

Wei, C.-L., Rowe, G. T., Escobar-Briones, E., Nunnally, C., Soliman, Y., and Ellis, N. (2012). Standing stocks and body size of deep-sea macrofauna: Predicting the baseline of 2010 deepwater horizon oil spill in the northern gulf of mexico. *Deep Sea Research Part I: Oceanographic Research Papers*, 69:82–99.

Yan, R., Shen, C., Zhang, D., Li, Z., Fang, L., and Wang, C. (2024). Predicting the distribution of megabenthic communities on deep-water seamounts with cobalt-rich crusts in the magellan seamount chain in the northwestern pacific ocean. *Deep Sea Research Part I: Oceanographic Research Papers*, 208:104303.