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Unveiling the Faunal Diversity in the Water Column Adjacent to Two Seamounts in the Deep Arabian Sea Using Environmental DNA Metabarcoding

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Abstract: The diversity of organisms inhabiting deep-sea ecosystems, such as seamounts, has hitherto remained under-studied. In this study, we report on the faunal diversity in the water column adjacent to the summit and periphery of two seamounts (SMS2 and SMS3) and an oxygen minimum zone site located away from the seamounts in the southeast Arabian Sea. Environmental DNA (eDNA) in the water column was metabarcoded using the Cytochrome Oxidase C subunit I (COI) gene marker and Oxford Nanopore sequencing technology. Hydrographic conditions revealed that the summits of the seamounts intersect with the core oxygen minimum zone between depths of 300 and 600 m. Comparisons of COI gene sequences with those in available databases, MIDORI and BOLD, indicated the existence of a diverse group of novel organisms in the study area. Crustaceans dominated (75–95%) in the summit and periphery of the SMS2 and the OMZ site, while Cnidaria (56–63%) and Chordata (55%), respectively, dominated the summit and periphery of SMS3. Overall, the current study highlights the broad diversity of organisms living in the water column around the seamounts and underscores the potential of eDNA for exploring them.

Keywords: seamount; oxygen minimum zone; deep-sea; environmental DNA; metabarcoding; biodiversity

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1. Introduction

Seamounts are vulnerable ecosystems formed through intense oceanic volcanism and are known for harbouring a diverse group of organisms [1–3]. The biodiversity of over 99% of the 33,000-plus seamounts documented in the world's oceans has yet to be explored, primarily due to logistical challenges in obtaining morphologically intact samples [4]. The seamounts are recognized as zones of significant endemic diversity and are often identified as critical habitats for breeding, feeding and nurturing of fishes, corals and endangered organisms [2,3,5–8]. The distinctiveness in diversity between seamounts is influenced by regional oceanographic processes. The bathymetric studies conducted in the last decade identified the existence of 14 seamounts in the southeast Arabian Sea [9]. Arabian Sea holds one of the significant oxygen minimum zones (OMZs) of the world, where the dissolved oxygen (DO) concentration is generally lower than 1 mgL^{-1} [10]. Pioneering studies on the diverse group of organisms residing in these seamounts reported the existence of two species of brittle stars (*Ophiozonella moesta* and *Ophiothamnus venustus*) in seamount S3 (coordinates 72.212° E, 12.087° N) by our team in 2023 [11]. The summit of the studied seamounts in the southeast Arabian Sea [9] are intercepted by OMZ, characterized by low oxygen concentrations (<0.5 mL L^{-1}) [12,13]. Although the summit of seamounts is rich with partially degraded organic materials that sink from the surface waters, hypoxic conditions may play a selective role in the biodiversity of organisms living around the seamounts [2]. Oxygen minimum zones and their formation are attributed to a combination of factors, including the consumption of oxygen during the degradation of organic

matter and inadequate oxygen supply [14] that may influence the life of organisms in such regions [15].

Classical approaches to studying the biodiversity of an ecosystem demand the availability of morphologically intact specimens, which is logistically difficult in deep-sea ecosystems. This forms one of the major reasons for a smaller number of reports on the biodiversity of seamounts in particular and deep-sea ecosystems in general. The introduction of environmental DNA (eDNA) techniques overcomes this difficulty because the genetic material of animals living in a particular area can be amplified from the DNA traces they release through the shedding of tissue, faecal matter, gametes and mucus, which are collectively called eDNA [16]. The metabarcoding of eDNA is a rapid and cost-effective technique, which has proven to be a promising tool for studying the biodiversity of an ecosystem, especially deep-sea ecosystems, where access to live samples is very limited [17,18]. While there is a wealth of eDNA-based studies on marine biodiversity, including some pioneering studies on fishes and mammals in deep marine ecosystems, there is an exciting opportunity for further exploration and research in this area [19–21]. The benefits of using eDNA metabarcoding in deep ocean water have also been accurate in the identification of specific communities like metazoans, cephalopods and zooplankton [22–25]. A recent review on eDNA metabarcoding for deep-sea biodiversity monitoring in conjugation with optoacoustics proposes it as a comprehensive marine biodiversity monitoring tool that is revolutionising this field [26]. A wide range of taxa (including vertebrates) that are otherwise inaccessible by direct capture or observation could be studied with recent eDNA advancements [19,26–31]. The method is especially useful to investigate microscopic eukaryotic taxa which are abundant and diverse in the ecosystems in comparison with traditional approaches [32].

We attempted to delineate the faunal diversity prevailing in the water. Here, we report the first eDNA metabarcoding study of organisms from seamounts of the deep Arabian Sea. This study purposed for a preliminary eDNA biodiversity assessment of marine fauna present in the water column adjacent to the summit and periphery of two seamounts in the southeast Arabian Sea, which were geologically described by Bijesh et al. in 2018 [9]. The study was broadly targeted to identify all metazoans present in the sample by barcoding the eukaryotic Cytochrome Oxidase C subunit I (COI) region of mitochondrial DNA, a universal marker gene used for the species identification of animals, followed by high throughput long-read sequencing by Oxford Nanopore Technologies.

2. Materials and Methods

2.1. Study Area

Samples were collected during the cruise #SSK148 aboard the RV Sindhu Sankalp (21 November 2022 to 5 December 2022) from the water column adjacent to the summits (S) and periphery (P) of two seamounts (SMS2 and SMS3) and an OMZ site (Table 1, Figure 1A). The seamounts SMS2 and SMS3 emerge from a depth of ~1400 m, and their summits are intercepted by OMZ at 340 and 440 m, respectively (Figure 1B). Further details on the geological features of these seamounts have already been described in the past study [9]. Three water samples were collected from the summit of SMS2 (SMS2S), having a broad surface area of 15.11 km^2, and one each was collected from the narrow summit of SMS3 (SMS3S), having a surface area of 3.71 km^2, water column adjacent to the periphery of both seamounts (SMS2P and SMS3P) and an OMZ site far away from these seamounts.

Table 1. Location of sampling stations.

Station	Sample Details	Sample No	Coordinates	Water Column Depth (m)	Sample Collection Depth (m)
SMS2	SMS2S: Water column adjacent to the summit	A	13°55.508′ N, 72°38.395′ E	334	330

Table 1. *Cont.*

Station	Sample Details	Sample No	Coordinates	Water Column Depth (m)	Sample Collection Depth (m)
SMS2	SMS2S: Water column adjacent to the summit	B	13°55.360′ N, 72°38.002′ E	360	347
		C	13°54.532′ N, 72°38.156′ E	400	370
	SMS2P: Water column adjacent to the periphery	A	13°59.190′ N, 72°38.238′ E	1604	350
SMS3	SMS3S: Water column adjacent to the summit	A	12°6.043′ N, 72°12.451′ E	554	550
	SMS3P: Water column adjacent to the periphery	A	12°6.759′ N, 72°10.515′ E	1430	500
OMZ site	OMZ site: Water column far away from seamounts	A	14°19.090′ N, 73°1.408′ E	972	350

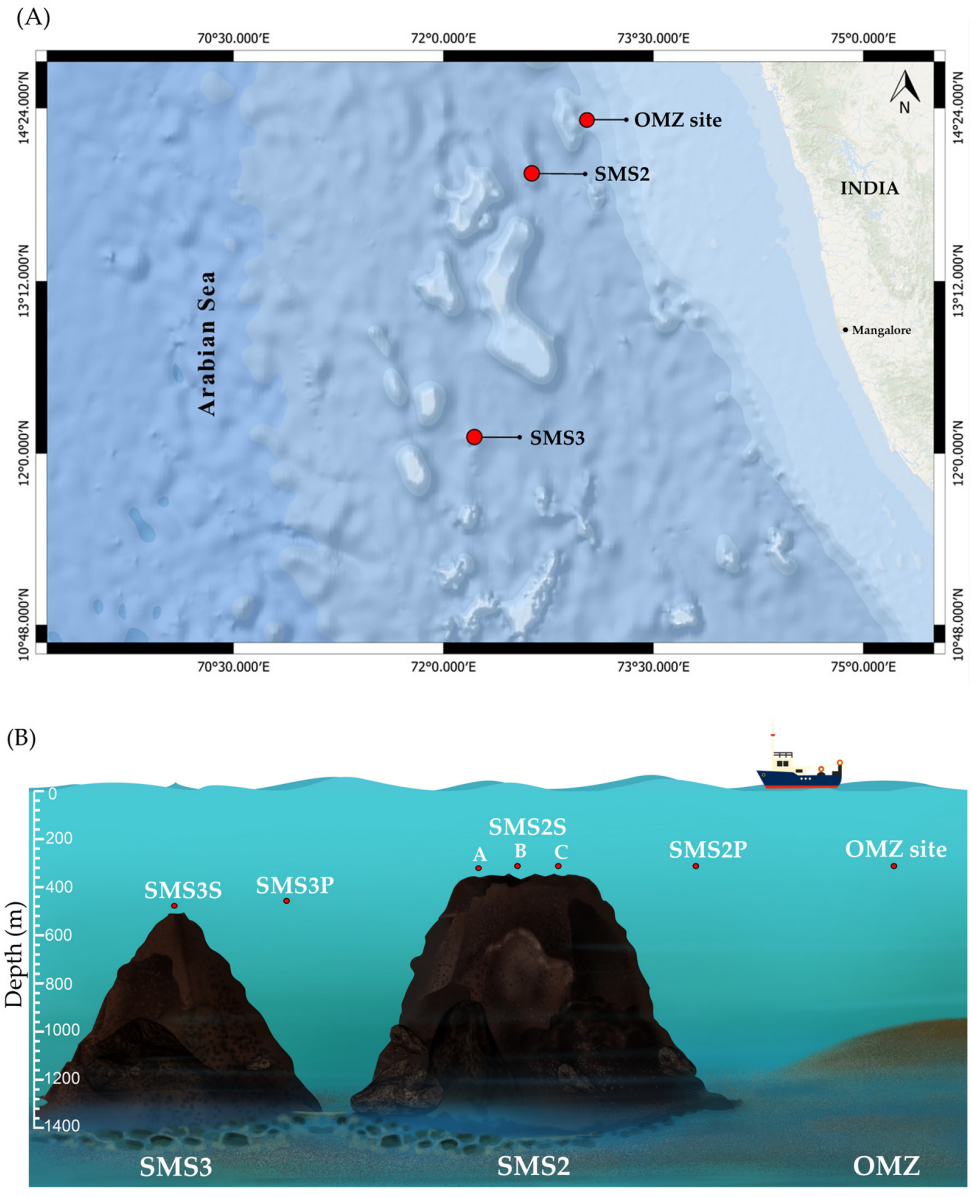


Figure 1. (**A**) Geographic map of station location (**B**) schematic representation of sampling stations.

2.2. Sample Collection, Preservation and Analysis of Hydrographic Variables

The hydrographic variables such as salinity, temperature, and dissolved oxygen of the water column above the summit and periphery of the seamounts (SMS2 and SMS3) and OMZ site were measured using sensors attached to a Conductivity, Temperature and Depth (CTD) rosette. The water samples collected using 10 L capacity Niskin water sampler rosette were distributed to sterile bottles (5 L) and processed immediately. The water samples (1 L) for the extraction of eDNA were passed through mixed cellulose ester membrane filter (Cytiva Life Sciences, Marlborough, MA, USA) with a pore size of 0.2 µm, dehydrated by washing with absolute ethanol (5 mL) and preserved at −80 °C [33,34]. Triplicate samples were collected and preserved from each station.

2.3. Extraction of eDNA and Eukaryotic COI Gene Amplification

The eDNA was extracted from filter paper using NucleoSpin eDNA water kit (Macherey-Nagel, Düren, Germany), following the method prescribed in the product manual. The eDNA was preserved in elution buffer (100 µL) provided along with the kit, and the concentration was measured using a Nanodrop spectrophotometer (BioTEK, Agilent Technologies Inc., Santa Clara, CA, USA). The Cytochrome Oxidase subunit I (COI) gene (~650 bp) of eukaryotic organisms present in the eDNA samples were amplified in a PCR using a primer combination of dgLCO (5′ GGT CAA CAA ATC ATA AAG AYA TYG G 3′) and dgHCO (5′ TAA ACT TCA GGG TGA CCA AAR AAY CA 3′) [35]. The PCR reaction mixture (25 µL) containing 1 µL of template eDNA (50 ng μL^{-1}), 1 µL each of forward and reverse primers, 12.5 µL of EmeraldAmp GT Master Mix (Takara Bio Inc., Kusatsu, Shiga, Japan) and Milli-Q water (14.5 µL) were exposed to the standard cycling conditions to amplify COI gene. The PCR cycling conditions consisted of initial denaturation at 94 °C for 2 min, followed by 25 cycles of denaturation (94 °C for 30 s), annealing (45 °C for 30 s) and extension (72 °C for 12 s). Then, the final extension at 72 °C for 7 min and infinity hold at 4 °C. The COI gene was amplified in separate tubes from the eDNA extracted from each subsample, and the PCR products of each sample were pooled for nanopore sequencing analysis to reduce the cost of analysis.

2.4. Nanopore Sequencing of COI Gene Amplicons

The amplicons of COI gene were purified using 1.6× Sera-Mag Select beads (Cytiva Life Sciences, Marlborough, MA, USA). The purified amplicons were quantified and used for preparing the library using Ligation sequencing amplicons—Native Barcoding Kit 24 V14 (SQK-NBD114.24) (Oxford Nanopore Technologies, Oxford, UK). Precisely, 50 ng of purified amplicon from each sample was end-repaired by NEBNext Ultra II End Repair/dA-Tailing Module (New England Biolabs, Ipswich, MA, USA) and further cleaned using 1× Sera-Mag Select beads. Subsequently, the purified amplicons were ligated with Native Barcodes using Blunt/TA Ligase Master Mix (New England Biolabs, Ipswich, MA, USA) and cleaned again with 1× Sera-Mag Select beads. Subsequently, the barcoded samples were pooled in equimolar concentrations and ligated with sequencing adaptor (AMII) using NEBNext Quick T4 DNA Ligase (New England Biolabs, Ipswich, MA, USA). The library was further cleaned using 0.4× Sera-Mag Select beads and finally eluted into 15 µL of elution buffer. Final library yield was quantified using Qubit dsDNA HS assay kit (Invitrogen, Thermo Fisher Scientific Inc., Waltham, MA, USA). The adapter-ligated, barcoded library was loaded into a SpotON flow cell R9.4 (FLO-MIN106) on a Mk1C device (Oxford Nanopore Technologies, Oxford, UK), and sequencing was performed using MinKNOW v22.08.9 in a 48 hr sequencing protocol.

2.5. Bioinformatic Analysis

The long-read raw sequence data ('fast5' format) generated from Nanopore sequencer were base-called to 'fastq' format and de-multiplexed using Guppy v2.3.4, a basecaller provided by Oxford Nanopore Technologies [36]. The quality of the raw reads was checked with Nanoplot v1.41.0 [37]. The sequencing adapters and Native Barcodes were removed using Porechop v0.2.4 [38]. Further, the sequences of desired length (500–750 bp) and quality (Phred quality score, q-score > 12) were filtered using Nanofilt v2.8.0 [37]. The quality-filtered nanopore sequences were imported to QIIME2-2023.2 pipeline [39], and the sequences were dereplicated by '*dereplicate-sequences*' method by using VSEARCH [40] plugin in QIIME2. The COI gene amplicon sequences were clustered into Molecular Operational Taxonomic Units (MOTUs) [41] using VSEARCH plugin by '*çluster-features-denovo'* method. The sequences were clustered into MOTUs at different identity thresholds (80%, 85%, 90%, 95% and 97%) in order to mitigate the impact of erroneous sequences generated by Nanopore sequencing while maximising the genetic diversity information for COI gene sequences. Chimeric sequences were removed from the MOTUs by '*uchime-denovo'* method in VSEARCH plugin.

Taxonomy-free diversity assessments (alpha and beta diversity) of the metazoan community were performed by using the MOTUs rarefied to minimum sampling depth (54,562 for the present study). Alpha diversity (within the sample) was described by observed OTUs (MOTUs), Shannon entropy [42] and Chao index [43] to determine within-sample diversity from the MOTU abundance table. Beta diversity was explored by incidence (presence or absence) based on Jaccard distance [44] to compare the diversity between samples.

Taxonomic assignments to the MOTUs were given with BLAST+ [45] consensus taxonomy classification method ('*classify-consensus-blast'*) by 'feature-classifier' plugin in the QIIME2 pipeline itself. MOTUs were aligned against the QIIME2 formatted COI gene sequence reference databases of Barcode of Life Data (BOLD) systems (version 168) [46] and MIDORI-UNIQUE [47] created from NCBI-Genbank repository (Genbank259_2023-12-17). An initial run using BLAST+ to classify the MOTUs with standard identity threshold of 96% resulted in excluding most clusters, leaving them taxonomically "unassigned". Taxonomy assignment was performed with varying identity thresholds [48,49] (from 100% to 70%) with at least 80% query coverage [50] to evaluate BLAST alignments across different taxonomic levels. Phylum level reliability threshold was chosen at minimum identity of 80% for COI gene [48].

3. Results

3.1. Hydrographic Settings of the Study Area

The temperature of the water column in all the stations showed a rapid decline from 29 °C at the surface to 14 °C at 200 m depth with an average reduction of approximately 0.1 °C per m (Figure 2A). Temperature showed a gradual decrease further down in stations outside the summit of seamounts and reached 5 °C at a 1400 m depth. The temperature of water at the summit of seamounts and the same depth adjacent to the periphery and OMZ site were between 11 °C and 12 °C. Dissolved oxygen concentration also showed the characteristic trend of the Arabian Sea OMZ, with a maximum of 4.6 mL L^{-1} at the surface, which reduced to a minimum of 0.007 mL L^{-1} at 313 m depth. The oxygen minimum conditions were maintained at all the stations with DO concentrations of 0.02 mL L^{-1} (SMS3S), 0.2 mL L^{-1} (SMS3P), 0.12 mL L^{-1} (SMS2S), 0.07 mL L^{-1} (SMS2P) and 0.01 mL L^{-1} (OMZ site) (Figure 2B). The salinity of the water column was in the range of 34.5 to 35.5 PSU (Figure 2C).

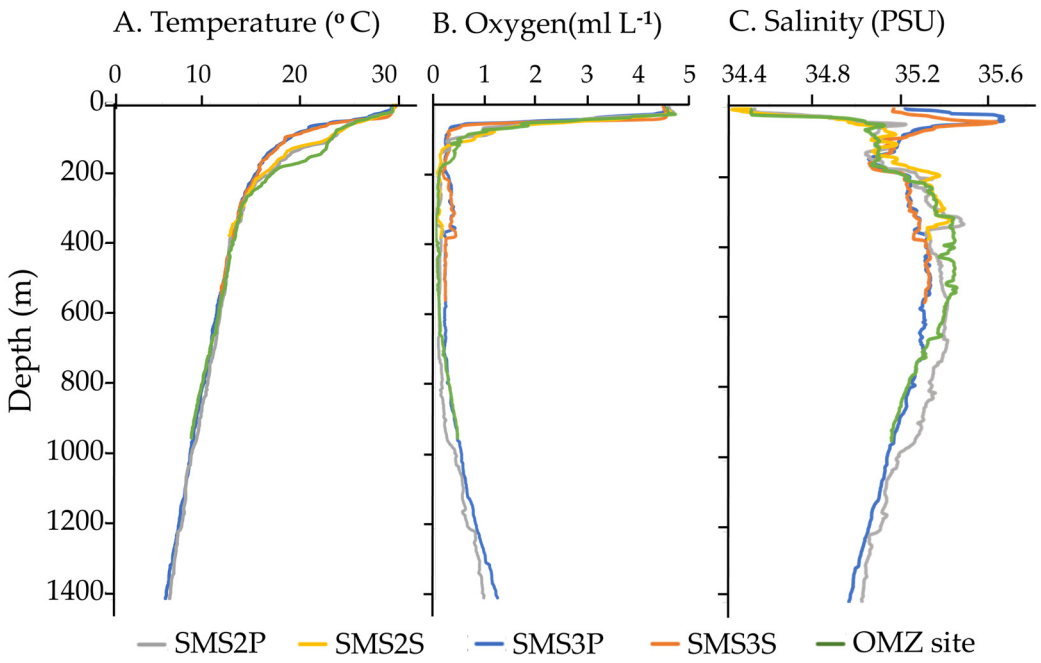


Figure 2. Hydrographic profile of (**A**) temperature, (**B**) oxygen, (**C**) salinity of the study area.

3.2. eDNA Sequences and Data Processing

The nanopore sequencing of the COI gene amplicon library, built from the eDNA samples, generated a total of 737,324 raw reads, further processing (demultiplexing, adapter removal, length and quality filtering) of which resulted in 675,767 good-quality reads. The number of good-quality reads was distributed as 125,355 for SMS2P, 133,603 for SMS2S, 104,430 for SMS3P, 134,705 for SMS3S and 177,674 for OMZ site (Supplementary Table S1). MOTU clustering resulted in varying numbers of clusters, with the maximum number of 367,797 at a 97% threshold and a minimum of 5434 at 80%. We considered the 85% threshold that generated 12,702 clusters as optimum, considering the reliability of true sequence cluster (MOTUs) formation (frequency > 1) and reduced chances of sequencing errors contributing to the formation of clusters at higher stringency (Supplementary Table S2). Thus, community diversity can be resolved with minimum data loss (while excluding singletons). A total of 7833 MOTU clusters, after removing chimera and singletons, were formed at 85% similarity from the dereplicated sequences of the COI gene from five eDNA samples. These clusters were taxonomically classified. Additional filters were applied on the MOTUs after taxonomic classification as follows. Fully unassigned MOTUs were removed along with non-target clusters (Protists or fungi). Probable contaminant MOTUs belonging to non-marine taxa (aves and terrestrial mammals) comprising < 2% were manually excluded [48]. The curation finally picked up marine metazoan-only MOTUs for the phylum and class-level community characterisation.

3.3. Alpha and Beta diversity

Alpha and Beta diversity analysis from a rarefied sequence data set estimated the diversity of both identified and unidentified organisms from the eDNA sequences from the water column near seamounts in the deep Arabian Sea. Maximum number of MOTUs were observed at SMS2S (4070 no.s), followed by SMS2P (2929 no.s), OMZ site (2843 no.s), SMS3S (2784 no.s) and SMS3P (2774 no.s). The alpha diversity index and Shannon entropy that considered both the richness and evenness of MOTUs were also higher in the summits of seamount SMS2 (SMS2S: 9.5, SMS2P: 9.1) compared to SMS3 (SMS3S: 7.7, SMS3P: 7.1). The Shannon entropy at the OMZ site (8.1) is closer to that of SMS2. The mean species diversity described by the Chao index is highest at SMS2S (4842.9), followed by OMZ site (3675.5), SMS2P (3654.9), SMS3S (3253.5) and SMS3P (3144.0). We also analysed beta

diversity by Jaccard distance to compare the diversity of organisms between different stations (Supplementary Table S3). The Jaccard distance between all stations was above 0.5, which indicated a difference of more than 50% in their biodiversity.

3.4. Taxonomic Distribution of Organisms

The analysis of sequence data indicates that 613 (7.8%) of MOTUs were shared among all stations. There were also MOTUs that were exclusive to a particular station and were in the order SMS2S (788), OMZ site (742), SMS2P (693), SMS3P (624) and SMS3S (618). A significant number of the MOTUs in the seamounts and OMZ site did not show similarity with COI gene sequences available in both BOLD Systems and MIDORI databases, and they remained as taxonomically 'unassigned'. The percentage of unassigned MOTUs were higher in OMZ site (BOLD: 98.7%, MIDORI: 98.5%), followed by the summit of SMS3 (SMS3S—BOLD: 97.4%, MIDORI: 97.7%), water column adjacent to the periphery of SMS3 (SMS3P—BOLD: 90.5%, MIDORI: 87.2%), the summit of SMS2 (SMS2S—BOLD: 88%, MIDORI: 87.5%) and the water column adjacent to the periphery of SMS2 (SMS2P—BOLD: 62.9%, MIDORI: 60.75%). The assigned group of MOTUs was classified into nine and eight phyla, respectively, in the BOLD Systems and MIDORI database. Common phyla across both databases include Crustacea, Chordata, Cnidaria, Mollusca, Chaetognatha, Echinodermata, Porifera and Annelida. Both databases identified Crustacea as the dominant phylum, comprising 87.50% of MOTUs in BOLD Systems and 88.16% of MOTUs in the MIDORI database, respectively. A minor fraction of MOTUs classified as Bryozoa (0.01%) by BOLD Systems were not detected in the MIDORI database. The relative abundance of these phylum in each station, classified by both databases, is given in Figure 3A.

In the study area, organisms belonging to the phylum Crustacea exhibited dominance across various zones in both MIDORI and BOLD systems: OMZ site (75%), the summit (SMS2S: 95%), and the periphery (SMS2P: 95%) of SMS2 (Figure 3). However, the remaining 25% of the assigned group of MOTUs in the OMZ site showed minor variations in relative abundance as classified by the MIDORI database and BOLD systems. Chordata emerged as the second dominant phylum, with relative abundances of 22% (according to the MIDORI database) and 21% (according to BOLD systems). Following this, we observed Cnidaria (1.4% in MIDORI and 2% in BOLD), Chaetognatha (0.35% in MIDORI and 1.7% in BOLD) and Annelida (0.2% in both databases). Additionally, the BOLD systems also identified MOTUs within Porifera (0.3%) and Bryozoa (0.2%) in the OMZ site. Among the remaining 5% of MOTUs in SMS2S, both the MIDORI and BOLD systems followed similar classification, which included Chordata (3%), Cnidaria (2%), Porifera, Annelida and Chaetognatha (together contributing < 1%). Analysis of the data in the BOLD system also assigned a minor fraction of MOTUs to molluscs (0.06%).

The assigned group of MOTUs in the summit and periphery of SMS3 were distributed into different phylum with visible differences in classification between MIDORI and BOLD databases. As per the analysis in the MIDORI database, the MOTUs from SMS3S are in the order of Cnidaria (63%) > Chordata (26%) > Crustacea (10%) > Porifera (1%). On analysing the same datasets of MOTUs in BOLD systems reported a change in the dominance of different phylum, which is in the order of Cnidaria (56%) > Crustacea (26%) > Chordata (16.5%) > Mollusca (0.9%) > Porifera (0.5%). A similar difference was also observed upon analysing the datasets of MOTUs retrieved from SMS3P, wherein the order of dominance observed in the MIDORI and BOLD database were Chordata (55%) > Crustacea (32%) > Cnidaria (10%) > Echinodermata (2%) > Mollusca (0.2%) and Crustacea (50%) > Chordata (39%) > Cnidaria (7%) > Mollusca (2%) > Echinodermata (1.6%) > Chaetognatha (1%) > Annelida (0.05%), respectively. Results of the current study indicate that the Bryozoans were present in the eDNA library of the OMZ site, while the Chaetognaths and molluscs were absent there.

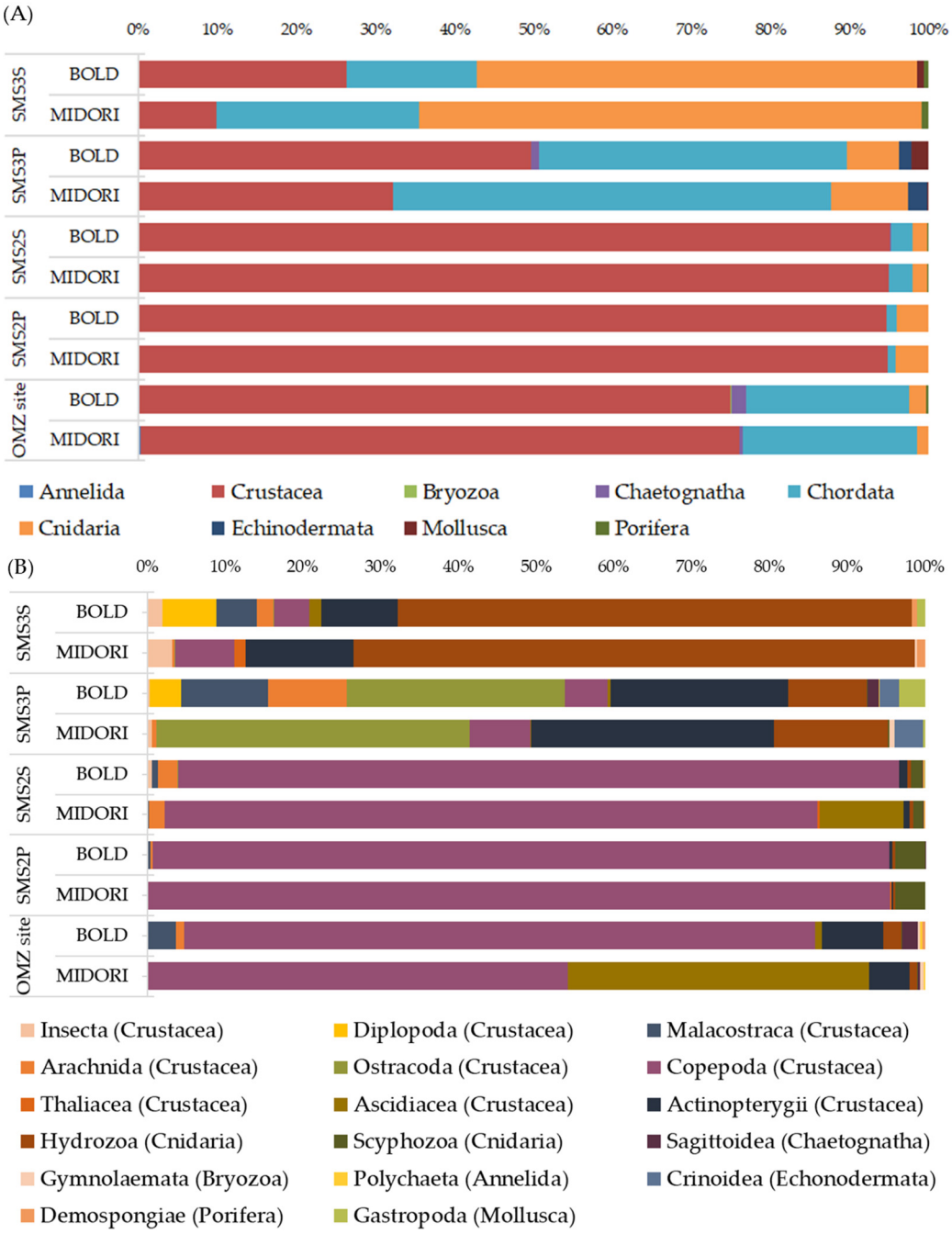


Figure 3. Relative abundance at (**A**) phylum (**B**) class level of metazoans in the stations, assigned by BOLD systems and MIDORI databases.

Further, the relative abundance of MOTUs at different classes was analysed (Figure 3B), which showed the dominance of copepods in the summit (83.9% in MIDORI and 92.7% in BOLD) and periphery (95.5% in MIDORI and 94.7% in BOLD) of SMS2 and OMZ site (53.9% in MIDORI and 81.1% in BOLD). Within the copepods, calanoids were the dominant Order at the OMZ site (86.2% in MIDORI and 77% in BOLD) and SMS2S (92.5% in MIDORI and 89.2% in BOLD), whereas cyclopoids were dominant at SMS2P (83.4% in MIDORI and 93.3%). Comparison of datasets with MIDORI database classified 10.8% and 38.8% of MOTUs in the summit of SMS2 and OMZ site, respectively, under Ascidiaceae, while a similar result was not observed on comparing with the database of BOLD. The MOTUs in the summit and periphery of SMS3 were different, with the dominance of Hydrozoa (72.2% in MIDORI and 66.1% in BOLD) in the former and Ostracoda (40.3% in MIDORI and 28.1%

in BOLD) in the latter. Significant number of MOTUs belonging to Actinopterygi (13.8% in MIDORI and 9.8% in BOLD) and Copepoda (7.6% in MIDORI and 4.5% in BOLD) were also observed in the summit of SMS3, whereas Actinopterygi (31.2% in MIDORI and 22.8% in BOLD), Hydrozoa (14.7% in MIDORI and 10.1% in BOLD) and Copepoda (7.8% in MIDORI and 5.4% in BOLD) were the other dominant class of organisms in the periphery of SMS3.

4. Discussion

The current study reports the faunal diversity in the water column adjacent to two seamounts in the deep Arabian Sea. The hydrographic conditions of the study area indicate the existence of a typical OMZ with an oxygenated water mass at the surface, and the core OMZ intercepts the summit of seamounts between 300 and 600 m in depth. The rapid reduction of oxygen concentrations in the intermediate levels leading to the formation of OMZ in the Arabian Sea is well studied and is attributed to the high oxygen uptake by microorganisms for the degradation of sinking organic matter and lack of ventilation [51–57]. Seamounts are generally considered as the oases of life in the deep ocean, which enhances primary productivity, concentrates local productivity, and provides refugia for some continental slope species. The hypoxic water, which has experienced significant biological consumption, covering the seamounts in the current study could serve as a chemical sealing over the diversity of organisms [58]. The current study forms one of the first eDNA metabarcoding reports on the faunal biodiversity of the water column adjacent to the summits and periphery of seamounts in the southeast Arabian Sea, which hitherto remains less studied using conventional techniques, too. The eDNA-based metabarcoding offers a powerful and efficient approach to exploring biodiversity, particularly in deep-sea ecosystems. There are several reasons, such as its high sensitivity that allows the identification with a small quantity of DNA, avoids sacrificing the animal life for documentation, avoids the dependency on specialized taxonomic experts, and possibilities of automation propose it as a valuable tool for ecological research [59].

We collected three samples from the summit of SMS2, averaging one sample per 5 km^2, and one sample from SMS3 at a density of one sample per 3.71 km^2. Technical difficulties precluded any further increase in sampling frequency. Nonetheless, the number of samples obtained in this study is comparable to those in prior seamount research conducted in other parts of the world [23,60]. The alpha diversity analysis by Shannon entropy and Chao index indicated the high richness and evenness of biodiversity in the water column adjacent to the summit and periphery of SMS2 and OMZ site, compared to that of SMS3. The Jaccard distance, which considered the presence and absence of MOTUs without considering their abundance, indicated that the pair-wise diversity of organisms in the water column across all the stations varied above 50%. Since the entire study area falls within an OMZ, the hypoxia may not significantly affect the differences in biodiversity indices between stations. However, the role of sinking organic matter (which may include dead organisms and their faecal matter) settling onto the seamounts and contributing to the eDNA, along with local currents that retain more food material in the proximity of the summit, attracting a greater number of live organisms, represents another avenue for investigation [61]. Previous studies indicated the existence of localized water currents in and around seamounts that could influence the distribution of nutrients and oxygen levels in seamounts [62]. A survey based on specimens collected using various tools (such as trawls, dredges, hauls, bongo and fishing nets) recorded seamounts as hotspots of pelagic biodiversity in the Western and Central Pacific Ocean [3,63], while a lower diversity was recorded in seamounts penetrating OMZ in the Eastern Tropical Pacific Ocean, obviously due to oxygen stress [62]. The eDNA-based approach can provide a higher representation of diversity as it depends on the genetic material released through the faecal matter, shedding of tissues, mucus, and eggs released from the organisms instead of an intact organism for identification. In addition to the DNA of true inhabitants of the region, eDNA-based diversity of seamounts may also include DNA of those organisms that visited the area

during the sampling time for feeding, as well as other pelagic organisms in the form of sinking organic material from the top, apart from true inhabitants [64,65].

The COI gene sequence data generated from the eDNA revealed the existence of a diverse group of organisms in the study area. However, two limitations arose due to the lack of an adequate number of gene-based reports on the molecular phylogeny of marine organisms: (1) nearly 95% of the sequences generated in the study did not have any relatives (at the phylum level) in the molecular databases used (MIDORI and BOLD), and (2) differences existed in the assignment of organisms at the class level between the databases. Due to the scarcity of molecular phylogeny data for deep-sea taxa in databases, it is indeed possible for their closest relatives to exhibit nucleotide divergences of up to 20% [66]. This divergence can occur because the available genetic information may not fully capture the diversity and evolutionary relationships of these organisms in the deep-sea environment. Researchers often encounter such challenges when studying less explored or specialized ecosystems like the deep sea. The advantage of the current COI gene sequence dataset over the conventional morphology-based one lies in its future utility, which will increase as these databases strengthen. Additionally, biodiversity comparisons based on MOTUs and taxonomic assignments, even with a few sequences, prove useful in identifying spatial differences in biodiversity between seamounts. In a nutshell, the results of the current study, which showed that more than 95% of MOTUs do not match those available in the database, indicate that most of the organisms in this region are unknown to us.

Further, we investigated the global scenario of the biodiversity of seamounts, which is reviewed in Supplementary Table S4. Most of these studies used physical specimens and reported Arthropoda, Mollusca, Cnidaria, Crustacea and Chordata (fishes) as the major phyla represented on the seamounts. In the case of the seamounts in the southeastern Arabian Sea, the oxygen minimum conditions may allow only those organisms that have adaptations for hypoxia to survive in this ecosystem. Many organisms with a calcified exoskeleton, except foraminiferans [67] and mussel *Amygdalum* sp., Ref. [68] avoid oxygen-minimum waters, while nematodes [69–72]; polychaetes [68] and crustaceans, such as ostracods [73,74], copepods [52] and malacostracans [75,76], are adapted to live in the hypoxic conditions.

The current results showed that stations near the continental shelf (i.e., SMS2 and OMZ site) had a higher dominance of copepods in terms of the number of reads and OTUs. Copepods of Cyclopoida and Calanoida were reported from the Arabian Sea OMZ. Calanoids are reported as temporary inhabitants of the OMZ region, showing an ontogenetic movement [77]. It is known that the density of non-calanoid species in the Arabian Sea decreases as depth increases [78], and an intermediate abundance maxima of non-calanoid copepods, especially cyclopoid genus *Oncaea*, occurs in the mesopelagic zone (100–1000 m depth, which coincides with the OMZ) of Arabian Sea. This explains the differences in the abundance of copepods between two seamounts in the current study. In agreement with the finding of Fabian et al. [79] that ostracod density showed a distinct minimum between 200 and 600 m, we also found that the representation of ostracods was minimal in all the stations except SMS3P. Likewise, malacostracan and chaetognath abundance were low as the stations were in the mesopelagic zone, and the representation of these groups in the eDNA could be from the dead remains that fell from the epipelagic zone, where these organisms are aplenty.

A more diverse group of organisms was found in the water column adjacent to the summit and periphery of SMS3, which were characterized by a high abundance of Hydrozoa and Actinopterygii. Hydrozoans are the dominant group of invertebrates found on the flanks of many seamounts [70]. They follow a life cycle that includes a benthic polyp (hydroid) stage and a pelagic medusan (jellyfish-like) stage [69–72]. However, the molecular phylogeny of these organisms remains poorly explored [80], which hinders our ability to make complete taxonomic assignments for these groups in seamounts. The low oxygen conditions are not favourable for the growth of hydrozoans, and therefore, the presence of hydrozoans in the eDNA from the summit of seamounts could originate from the sinking

organic matter from the oxygenated surface waters. Some of the members of Actinopterygii, such as ray fish, also have adaptations to survive in hypoxic conditions and were reported from the continental slopes of the Bay of Biscay [19] oxygen minimum zones [81] and seamounts of [82,83]. In addition to hydrozoans, the presence of attached megabenthos, such as Scyphozoa in the water column adjacent to the summit and periphery of SMS2 and crinoids in the periphery of SMS3, suggest that the nature of the substratum of seamounts influence the benthic assemblage. Gooday et al. [67] have reported that substratum made of carbonate rocks may be colonized by deep-endemic fauna (mainly provannid gastropods, limpets, cnidarians in the OMZ) and a diversity of more widespread annelids, whereas basalt substrates and manganese crusts on seamounts that protrude into OMZs are covered by strongly zoned polychaetes, sponges, crustaceans and echinoderms [62,67]. Previous studies using physical specimens showed a high abundance of myctophids in the oxygen minimum zones [84]. Our results indicate that these organisms contribute to less than 1% of the MOTUs in the seamounts.

5. Conclusions

The current study reports the faunal biodiversity of seamounts of the Arabian Sea, studied using an eDNA-based approach. The summit of the studied seamounts is exposed to the oxygen minimum zone and is characterized by rich biodiversity. The existence of a large number of unknown organisms is confirmed by the observation that 95% of the OTUs in the current study did not have any similarity with organisms reported in the available database. The remaining 5% of OTUs showed a dominance of copepods in the seamount (SMS2) and the OMZ site near the shelf, while it was more diverse in SMS3 in the deeper ocean. Our results also highlight the importance of eDNA metabarcoding techniques for understanding the diversity of organisms in deep-sea ecosystems where morphological studies are difficult to practice. The study also discusses the possible artifacts, i.e., sinking organic matter, that could mislead the eDNA-based approach in studying the faunal diversity of seamounts. The identification of sessile benthic forms like cnidarians opens further research avenues on the nature of the substratum of seamounts and their ecology. The lesser availability of COI gene molecular data in marine organisms is a limitation to the widespread application of the eDNA-based approach and can be improved by further depositions of COI gene data by taxonomists worldwide.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jmse12060971/s1, Supplementary Table S1: Read data statistics; Table S2: Sequence clustering; Supplementary Table S3: Beta diversity-Jaccard distance; Supplementary Table S4: Biodiversity of seamounts across the world. References [85–92] are cited in the Supplementary Materials.

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References

1. Iyer, S.D.; Mehta, C.M.; Das, P.; Kalangutkar, N.G. Seamounts—Characteristics, formation, mineral deposits and biodiversity. *Geol. Acta* **2012**, *10*, 295–308. [CrossRef]
2. McClain, C.R. Seamounts: Identity crisis or split personality? *J. Biogeogr.* **2007**, *34*, 2001–2008. [CrossRef]
3. Morato, T.; Hoyle, S.D.; Allain, V.; Nicol, S.J. Seamounts are hotspots of pelagic biodiversity in the open ocean. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 9707–9711. [CrossRef] [PubMed]
4. Xu, K. Exploring seamount ecosystems and biodiversity in the tropical Western Pacific Ocean. *J. Oceanol. Limnol.* **2021**, *39*, 1585–1590. [CrossRef]
5. Jeunen, G.-J.; Knapp, M.; Spencer, H.G.; Lamare, M.D.; Taylor, H.R.; Stat, M.; Bunce, M.; Gemmell, N.J. Environmental DNA (eDNA) metabarcoding reveals strong discrimination among diverse marine habitats connected by water movement. *Mol. Ecol. Resour.* **2019**, *19*, 426–438. [CrossRef] [PubMed]
6. Rogers, A.D.; Baco, A.; Griffiths, H.; Hart, T.; Hall-Spencer, J.M. Corals on seamounts. In *Seamounts: Ecology, Fisheries & Conservation*; Blackwell Publishing: Oxford, UK, 2007; pp. 141–169. [CrossRef]
7. Bineesh, K.K.; Akhilesh, K.V.; Abdussamad, E.M.; Prakasan, D. Seamount associated fishery of south-west coast of India—A preliminary assessment. *Indian J. Fish.* **2014**, *61*, 29–34.
8. Garrigue, C.; Clapham, P.J.; Geyer, Y.; Kennedy, A.S.; Zerbini, A.N. Satellite tracking reveals novel migratory patterns and the importance of seamounts for endangered South Pacific humpback whales. *R. Soc. Open Sci.* **2015**, *2*, 150489. [CrossRef] [PubMed]
9. Bijesh, C.M.; John Kurian, P.; Yatheesh, V.; Tyagi, A.; Twinkle, D. Morphotectonic characteristics, distribution and probable genesis of bathymetric highs off southwest coast of India. *Geomorphology* **2018**, *315*, 33–44. [CrossRef]
10. Paulmier, A.; Ruiz-Pino, D. Oxygen minimum zones (OMZs) in the modern ocean. *Prog. Oceanogr.* **2009**, *80*, 113–128. [CrossRef]
11. Jaleel, A.K.; Parameswaran, U.V.; Anagha, B.; Parthasarathi, S.; Anas, A. First report of brittle stars (Echinodermata: Ophiuroidea) from a seamount in the eastern Arabian Sea. *Zootaxa* **2023**, *5285*, 578–584. [CrossRef]
12. Cline, J.D.; Richards, F.A. Oxygen deficient conditions and nitrate reduction in the eastern tropical North Pacific Ocean. *Limnol. Oceanogr.* **1972**, *17*, 885–900. [CrossRef]
13. Levin, L.A. Oxygen minimum zone benthos: Adaptation and community response to hypoxia. *Oceanogr. Mar. Biol. Annu. Rev.* **2003**, *41*, 1–45.
14. Fennel, K.; Testa, J.M. Biogeochemical controls on coastal hypoxia. *Annu. Rev. Mar. Sci.* **2019**, *11*, 105–130. [CrossRef] [PubMed]
15. Gooday, A.J.; Bett, B.J.; Escobar, E.; Ingole, B.; Levin, L.A.; Neira, C.; Raman, A.V.; Sellanes, J. Habitat heterogeneity and its influence on benthic biodiversity in oxygen minimum zones. *Mar. Ecol.* **2010**, *31*, 125–147. [CrossRef]
16. Taberlet, P.; Coissac, E.; Hajibabaei, M.; Rieseberg, L.H. Environmental DNA. *Mol. Ecol.* **2012**, *21*, 1789–1793. [CrossRef]
17. Ruppert, K.M.; Kline, R.J.; Rahman, M.S. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. *Glob. Ecol. Conserv.* **2019**, *17*, e00547. [CrossRef]
18. Pawlowski, J.; Christen, R.; Lecroq, B.; Bachar, D.; Shahbazkia, H.R.; Amaral-Zettler, L.; Guillou, L. Eukaryotic richness in the abyss: Insights from pyrotag sequencing. *PLoS ONE* **2011**, *6*, e18169. [CrossRef] [PubMed]
19. Canals, O.; Mendibil, I.; Santos, M.; Irigoien, X.; Rodríguez-Ezpeleta, N. Vertical stratification of environmental DNA in the open ocean captures ecological patterns and behavior of deep-sea fishes. *Limnol. Oceanogr. Lett.* **2021**, *6*, 339–347. [CrossRef]
20. Brodnicke, O.B.; Meyer, H.K.; Busch, K.; Xavier, J.R.; Knudsen, S.W.; Møller, P.R.; Hentschel, U.; Sweet, M.J. Deep-sea sponge derived environmental DNA analysis reveals demersal fish biodiversity of a remote Arctic ecosystem. *Environ. DNA* **2023**, *5*, 1405–1417. [CrossRef]
21. Joydas, T.V.; Manokaran, S.; Gopi, J.; Rajakumar, J.P.; Lin, Y.-J.; Heinle, M.; Nazal, M.K.; Manikandan, K.P.; Qashqari, M.; Mohandas, S.P.; et al. Advancing ecological assessment of the Arabian Gulf through eDNA metabarcoding: Opportunities, prospects, and challenges. *Front. Mar. Sci.* **2024**, *11*, 1276956. [CrossRef]
22. Govindarajan, A.F.; Francolini, R.D.; Jech, J.M.; Lavery, A.C.; Llopiz, J.K.; Wiebe, P.H.; Zhang, W. Exploring the use of environmental DNA (eDNA) to detect animal taxa in the mesopelagic zone. *Front. Ecol. Evol.* **2021**, *9*, 574899. [CrossRef]
23. Laroche, O.; Kersten, O.; Smith, C.R.; Goetze, E. Environmental DNA surveys detect distinct metazoan communities across abyssal plains and seamounts in the western Clarion Clipperton Zone. *Mol. Ecol.* **2020**, *29*, 4588–4604. [CrossRef] [PubMed]
24. Merten, V.; Bayer, T.; Reusch, T.B.H.; Puebla, O.; Fuss, J.; Stefanschitz, J.; Lischka, A.; Hauss, H.; Neitzel, P.; Piatkowski, U.; et al. An integrative assessment combining deep-sea net sampling, in situ observations and environmental DNA analysis identifies cabo verde as a cephalopod biodiversity hotspot in the Atlantic Ocean. *Front. Mar. Sci.* **2021**, *8*, 760108. [CrossRef]
25. Visser, F.; Merten, V.J.; Bayer, T.; Oudejans, M.G.; de Jonge, D.S.W.; Puebla, O.; Reusch, T.B.H.; Fuss, J.; Hoving, H.J.T. Deep-sea predator niche segregation revealed by combined cetacean biologging and eDNA analysis of cephalopod prey. *Sci. Adv.* **2021**, *7*, eabf5908. [CrossRef] [PubMed]
26. Stefanni, S.; Mirimin, L.; Stanković, D.; Chatzievangelou, D.; Bongiorni, L.; Marini, S.; Modica, M.V.; Manea, E.; Bonofiglio, F.; del Rio Fernandez, J.; et al. Framing cutting-edge integrative deep-sea biodiversity monitoring via environmental DNA and optoacoustic augmented infrastructures. *Front. Mar. Sci.* **2022**, *8*, 797140. [CrossRef]
27. Gargan, L.M.; Morato, T.; Pham, C.K.; Finarelli, J.A.; Carlsson, J.E.L.; Carlsson, J. Development of a sensitive detection method to survey pelagic biodiversity using eDNA and quantitative PCR: A case study of devil ray at seamounts. *Mar. Biol.* **2017**, *164*, 112. [CrossRef]

28. Kitahashi, T.; Sugime, S.; Inomata, K.; Nishijima, M.; Kato, S.; Yamamoto, H. Meiofaunal diversity at a seamount in the pacific ocean: A comprehensive study using environmental DNA and RNA. *Deep Sea Res. Part I Oceanogr. Res. Pap.* **2020**, *160*, 103253. [CrossRef]
29. Cowart, D.A.; Matabos, M.; Brandt, M.I.; Marticorena, J.; Sarrazin, J. Exploring environmental DNA (eDNA) to assess biodiversity of hard substratum faunal communities on the lucky strike vent field (mid-Atlantic ridge) and investigate recolonization dynamics after an induced disturbance. *Front. Mar. Sci.* **2020**, *6*, 783. [CrossRef]
30. Lacoursière-Roussel, A.; Howland, K.; Normandeau, E.; Grey, E.K.; Archambault, P.; Deiner, K.; Lodge, D.M.; Hernandez, C.; Leduc, N.; Bernatchez, L. eDNA metabarcoding as a new surveillance approach for coastal Arctic biodiversity. *Ecol. Evol.* **2018**, *8*, 7763–7777. [CrossRef]
31. Laroche, O.; Kersten, O.; Smith, C.R.; Goetze, E. From sea surface to seafloor: A benthic allochthonous eDNA survey for the Abyssal Ocean. *Front. Mar. Sci.* **2020**, *7*, 682. [CrossRef]
32. Bik, H.M.; Porazinska, D.L.; Creer, S.; Caporaso, J.G.; Knight, R.; Thomas, W.K. Sequencing our way towards understanding global eukaryotic biodiversity. *Trends Ecol. Evol.* **2012**, *27*, 233–243. [CrossRef] [PubMed]
33. Hinlo, R.; Gleeson, D.; Lintermans, M.; Furlan, E. Methods to maximise recovery of environmental DNA from water samples. *PLoS ONE* **2017**, *12*, e0179251. [CrossRef] [PubMed]
34. Goldberg, C.S.; Pilliod, D.S.; Arkle, R.S.; Waits, L.P. Molecular detection of vertebrates in stream water: A demonstration using rocky mountain tailed frogs and idaho giant salamanders. *PLoS ONE* **2011**, *6*, e22746. [CrossRef]
35. Meyer, C.P.; Geller, J.B.; Paulay, G. Fine scale endemism on coral reefs: Archipelagic differentiation in turbinid gastropods. *Evolution* **2005**, *59*, 113–125. [CrossRef] [PubMed]
36. Kahlke, T. *Guppy Basecaller*; Oxford Nanopore Technologies: Oxford, UK, 2019.
37. De Coster, W.; D'Hert, S.; Schultz, D.T.; Cruts, M.; Van Broeckhoven, C. NanoPack: Visualizing and processing long-read sequencing data. *Bioinformatics* **2018**, *34*, 2666–2669. [CrossRef]
38. Wick, R. Porechop: Adapter Trimmer for Oxford Nanopore Reads. 2017. Available online: https://github.com/rrwick/Porechop/ (accessed on 10 May 2024).
39. Bolyen, E.; Rideout, J.R.; Dillon, M.R.; Bokulich, N.A.; Abnet, C.C.; Al-Ghalith, G.A.; Alexander, H.; Alm, E.J.; Arumugam, M.; Asnicar, F.; et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **2019**, *37*, 852–857. [CrossRef]
40. Rognes, T.; Flouri, T.; Nichols, B.; Quince, C.; Mahé, F. VSEARCH: A versatile open source tool for metagenomics. *PeerJ* **2016**, *4*, e2584. [CrossRef]
41. Floyd, R.; Abebe, E.; Papert, A.; Blaxter, M. Molecular barcodes for soil nematode identification. *Mol. Ecol.* **2002**, *11*, 839–850. [CrossRef] [PubMed]
42. Shannon, C.E. A mathematical theory of communication. *Bell Syst. Tech. J.* **1948**, *27*, 379–423. [CrossRef]
43. Jaccard, P. The distribution of the flora in the Alpine Zone. *New Phytol.* **1912**, *11*, 37–50. [CrossRef]
44. Chao, A. Nonparametric Estimation of the Number of Classes in a Population. *Scand. J. Stat.* **1984**, *11*, 265–270. Available online: http://www.jstor.org/stable/4615964 (accessed on 10 May 2024).
45. Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W.; Lipman, D.J. Basic local alignment search tool. *J. Mol. Biol.* **1990**, *215*, 403–410. [CrossRef] [PubMed]
46. Ratnasingham, S.; Hebert, P.D. BOLD: The barcode of life data system. *Mol. Ecol. Notes* **2007**, *7*, 355–364. [CrossRef]
47. Machida, R.J.; Leray, M.; Ho, S.L.; Knowlton, N. Metazoan mitochondrial gene sequence reference datasets for taxonomic assignment of environmental samples. *Sci. Data* **2017**, *4*, 170027. [CrossRef]
48. Stefanni, S.; Stanković, D.; Borme, D.; de Olazabal, A.; Juretić, T.; Pallavicini, A.; Tirelli, V. Multi-marker metabarcoding approach to study mesozooplankton at basin scale. *Sci. Rep.* **2018**, *8*, 12085. [CrossRef]
49. Holovachov, O. Metabarcoding of marine nematodes—Evaluation of reference datasets used in tree-based taxonomy assignment approach. *Biodivers. Data J.* **2016**, *4*, e10021. [CrossRef]
50. Di Capua, I.; Piredda, R.; Mazzocchi, M.G.; Zingone, A. Metazoan diversity and seasonality through eDNA metabarcoding at a Mediterranean long-term ecological research site. *ICES J. Mar. Sci.* **2021**, *78*, 3303–3316. [CrossRef]
51. Rangamaran, V.R.; Sankara Subramanian, S.H.; Balachandran, K.R.S.; Gopal, D. Vertical microbial profiling of arabian sea oxygen minimal zone reveals complex bacterial communities and distinct functional implications. *Microb. Ecol.* **2022**, *85*, 357–371. [CrossRef] [PubMed]
52. Vidhya, V.; Jyothibabu, R.; Jagadeesan, L.; Rashid, C.; Alok, K.T.; Arunpandi, N.; Thirumurugan, R. Oxygen minimum zone copepods in the Arabian Sea and the Bay of Bengal: Their adaptations and status. *Prog. Oceanogr.* **2022**, *206*, 102839. [CrossRef]
53. Stramma, L.; Johnson, G.C.; Sprintall, J.; Mohrholz, V. Expanding oxygen-minimum zones in the tropical oceans. *Science* **2008**, *320*, 655–658. [CrossRef]
54. Bandekar, M.; Ramaiah, N.; Jain, A.; Meena, R.M. Seasonal and depth-wise variations in bacterial and archaeal groups in the Arabian Sea oxygen minimum zone. *Deep Sea Res. Part II Top. Stud. Oceanogr.* **2018**, *156*, 4–18. [CrossRef]
55. Cavan, E.L.; Trimmer, M.; Shelley, F.; Sanders, R. Remineralization of particulate organic carbon in an ocean oxygen minimum zone. *Nat. Commun.* **2017**, *8*, 14847. [CrossRef] [PubMed]
56. Lam, P.; Kuypers, M.M.M. Microbial nitrogen cycling processes in oxygen minimum zone. *Annu. Rev. Mar. Sci.* **2011**, *3*, 317–345. [CrossRef] [PubMed]

57. Anas, A.; Tharakan, B.; Jasmin, C.; Divya, B.; Ameri, S.; Narayanan, S. Microbial distribution and diversity along a vertical profile in the water column of a seasonal Oxygen minimum zone in the Southeast Arabian Sea. *Reg. Stud. Mar. Sci.* **2023**, *67*, 103206. [CrossRef]
58. Ma, J.; Li, X.; Song, J.; Wen, L.; Wang, Q.; Xu, K.; Dai, J.; Zhong, G. The effects of seawater thermodynamic parameters on the oxygen minimum zone (OMZ) in the tropical western Pacific Ocean. *Mar. Pollut. Bull.* **2023**, *187*, 114579. [CrossRef] [PubMed]
59. Miya, M. Environmental DNA metabarcoding: A novel method for biodiversity monitoring of marine fish communities. *Annu. Rev. Mar. Sci.* **2022**, *14*, 161–185. [CrossRef] [PubMed]
60. Clark, M.R.; Bowden, D.A. Seamount biodiversity: High variability both within and between seamounts in the Ross Sea region of Antarctica. *Hydrobiologia* **2015**, *761*, 161–180. [CrossRef]
61. White, M.; Bashmachnikov, I.; Arístegui, J.; Martins, A. Physical processes and seamount productivity. In *Seamounts: Ecology, Fisheries & Conservation*; Blackwell Publishing: Oxford, UK, 2007; pp. 62–84. [CrossRef]
62. Wishner, K.; Levin, L.; Gowing, M.; Mullineaux, L. Involvement of the oxygen minimum in benthic zonation on a deep seamount. *Nature* **1990**, *346*, 57–59. [CrossRef]
63. Morato, T.; Pauly, D. *Seamounts: Biodiversity and Fisheries*; Fisheries Centre, University of British Columbia: Vancouver, BC, Canada, 2004.
64. Foote, A.D.; Thomsen, P.F.; Sveegaard, S.; Wahlberg, M.; Kielgast, J.; Kyhn, L.A.; Salling, A.B.; Galatius, A.; Orlando, L.; Gilbert, M.T.P. Investigating the potential use of environmental DNA (eDNA) for genetic monitoring of marine mammals. *PLoS ONE* **2012**, *7*, e41781. [CrossRef]
65. Harrison, J.B.; Sunday, J.M.; Rogers, S.M. Predicting the fate of eDNA in the environment and implications for studying biodiversity. *Proc. Biol. Sci.* **2019**, *286*, 20191409. [CrossRef]
66. Brandt, M.I.; Pradillon, F.; Trouche, B.; Henry, N.; Liautard-Haag, C.; Cambon-Bonavita, M.-A.; Cueff-Gauchard, V.; Wincker, P.; Belser, C.; Poulain, J.; et al. Evaluating sediment and water sampling methods for the estimation of deep-sea biodiversity using environmental DNA. *Sci. Rep.* **2021**, *11*, 7856. [CrossRef] [PubMed]
67. Gooday, A.J.; Bernhard, J.M.; Levin, L.A.; Suhr, S.B. Foraminifera in the Arabian Sea oxygen minimum zone and other oxygen-deficient settings: Taxonomic composition, diversity, and relation to metazoan faunas. *Deep Sea Res. Part II Top. Stud. Oceanogr.* **2000**, *47*, 25–54. [CrossRef]
68. Levin, L.A.; Gage, J.D.; Martin, C.; Lamont, P.A. Macrobenthic community structure within and beneath the oxygen minimum zone, NW Arabian Sea. *Deep Sea Res. Part II Top. Stud. Oceanogr.* **2000**, *47*, 189–226. [CrossRef]
69. Gu, Z.; Ruthensteiner, B.; Moura, C.J.; Liu, L.; Zhang, R.; Song, X. Systematic affinities of zygophylacidae (Cnidaria: Hydrozoa: Macrocolonia) with descriptions of 15 deep-sea species. *Zool. J. Linn. Soc.* **2022**, *196*, 52–87. [CrossRef]
70. Gil, M.; Ramil, F. Hydroids (Cnidaria, Hydrozoa) from the vema and valdivia seamounts (SE Atlantic). *Eur. J. Taxon.* **2021**, *758*, 49–96. [CrossRef]
71. Moura, C.J.; Harris, D.J.; Cunha, M.R.; Rogers, A.D. DNA barcoding reveals cryptic diversity in marine hydroids (Cnidaria, Hydrozoa) from coastal and deep-sea environments. *Zool. Scr.* **2008**, *37*, 93–108. [CrossRef]
72. Maggioni, D.; Terraneo, T.I.; Chimienti, G.; Marchese, F.; Pica, D.; Cairns, S.D.; Eweida, A.A.; Rodrigue, M.; Purkis, S.J.; Benzoni, F. The first deep-sea stylasterid (Hydrozoa, Stylasteridae) of the Red Sea. *Diversity* **2022**, *14*, 241. [CrossRef]
73. Wilson, B.; Coimbra, J.C.; Hayek, L.-A.C. Ostracoda (Arthropoda, Crustacea) in a miocene oxygen minimum zone, Trinidad, West Indies: A test of the platycopid signal hypothesis. *J. S. Am. Earth Sci.* **2014**, *54*, 210–216. [CrossRef]
74. Graves, C.; Angel, M. Bathymetric distribution of planktonic ostracods (Ostracoda; Crustacea) in the Gulf of Oman (Northwest Indian Ocean) in relation to the Oxygen Minimum Zone. *Int. J. Mar. Biol. Res.* **2017**, *2*, 1–14. [CrossRef]
75. Papiol, V.; Hendrickx, M.E. Community structure of deep-water decapod crustaceans below the oxygen minimum zone in the south-east Gulf of California and analysis of environmental drivers. *Mar. Freshw. Res.* **2016**, *67*, 1862–1879. [CrossRef]
76. Catul, V.; Gauns, M.; Karuppasamy, P.K. A review on mesopelagic fishes belonging to family Myctophidae. *Rev. Fish Biol. Fish.* **2011**, *21*, 339–354. [CrossRef]
77. Koppelmann, R.; Weikert, H. Temporal and vertical distribution of two ecologically different calanoid copepods (*Calanoides carinatus* Krøyer 1849 and *Lucicutia grandis* Giesbrecht 1895) in the deep waters of the central Arabian Sea. *Mar. Biol.* **2005**, *147*, 1173–1178. [CrossRef]
78. Böttger-Schnack, R. Vertical structure of small metazoan plankton, especially noncalanoid copepods. I. Deep Arabian Sea. *J. Plankton Res.* **1996**, *18*, 1073–1101. [CrossRef]
79. Fabian, H.; Koppelmann, R.; Weikert, H. Full-depths zooplankton composition at two deep sites in the western and central Arabian Sea. *Indian J. Mar. Sci.* **2005**, *34*, 174–187.
80. Prudkovsky, A.; Rybakova, E.; Sanamyan, N.; Sanamyan, K. Deep-sea hydrozoans in the western part of the Bering Sea: Some hydroids associated with methane seep communities at upper bathyal depths and abyssal hydroids with a description of two new species. *Deep Sea Res. Part II Top. Stud. Oceanogr.* **2023**, *207*, 105230. [CrossRef]
81. Lau, G.Y.C. Adaptive Variation of Mitochondrial Function in Response to Oxygen Variability in Intertidal Sculpins (Cottidae, Actinopterygii). Ph.D. Thesis, University of British Columbia, Vancouver, BC, Canada, 2017.
82. McClain, C.R.; Lundsten, L.; Barry, J.; DeVogelaere, A. Assemblage structure, but not diversity or density, change with depth on a northeast Pacific seamount. *Mar. Ecol.* **2010**, *31*, 14–25. [CrossRef]

83. Okamoto, M.; Motomura, H.; Hoshino, K.; Yanagimoto, T.; Hayashibara, T. New records of the noline scorpionfish, *Phenacoscorpius megalops* (Actinopterygii: Scorpaeniformes: Scorpaenidae), from the Emperor Seamounts, central North Pacific. *Biogeography* **2012**, *14*, 77–81. [CrossRef]
84. Karuppasamy, P.K.; LaluRaj, C.M.; Muraleedharan, K.R.; Nair, M. Myctophid and pelagic shrimp assemblages in the oxygen minimum zone of the Andaman Sea during the winter monsoon. *Indian J. Mar. Sci.* **2011**, *40*, 535–541.
85. Cardigos, F.; Colaço, A.; Dando, P.R.; Ávila, S.P.; Sarradin, P.M.; Tempera, F.; Conceição, P.; Pascoal, A.; Santos, R.S. Shallow water hydrothermal vent field fluids and communities of the d. João de castro seamount (azores). *Chem. Geol.* **2005**, *224*, 153–168. [CrossRef]
86. Samadi, S.; Bottan, L.; Macpherson, E.; De Forges, B.R.; Boisselier, M.-C. Seamount endemism questioned by the geographic distribution and population genetic structure of marine invertebrates. *Mar. Biol.* **2006**, *149*, 1463–1475. [CrossRef]
87. Parin, N.V.; Mironov, A.N.; Nesis, K.N. Biology of the nazca and sala y gòmez submarine ridges, an outpost of the indo-west pacific fauna in the eastern pacific ocean: Composition and distribution of the fauna, its communities and history. In *Advances in Marine Biology*; Blaxter, J.H.S., Southward, A.J., Gebruk, A.V., Southward, E.C., Tyler, P.A., Eds.; Academic Press: Cambridge, MA, USA, 1997; pp. 145–242.
88. Richer de Forges, B.; Koslow, J.A.; Poore, G.C.B. Diversity and endemism of the benthic seamount fauna in the southwest pacific. *Nature* **2000**, *405*, 944–947. [CrossRef] [PubMed]
89. Moore, J.A.; Vecchione, M.; Gibbons, R.; Galbraith, J.K.; Turnipseed, M.; Southworth, M.; Watkins, E. Biodiversity of bear seamount, new england seamount chain: Results of exploratory trawling. *J. Northwest Atl. Fish. Sci.* **2003**, *31*, 363–372. Available online: https://scholarworks.wm.edu/vimsarticles/1970 (accessed on 10 May 2024). [CrossRef]
90. Clarke, M. Seamounts and cephalopods. In *Seamounts: Ecology, Fisheries & Conservation*; Blackwell Publishing: Oxford, UK, 2007; pp. 207–229.
91. McClain, C.R.; Lundsten, L.; Ream, M.; Barry, J.; DeVogelaere, A. Endemicity, biogeography, composition, and community structure on a northeast pacific seamount. *PLoS ONE* **2009**, *4*, e4141. [CrossRef] [PubMed]
92. Cherel, Y.; Romanov, E.V.; Annasawmy, P.; Thibault, D.; Ménard, F. Micronektonic fish species over three seamounts in the southwestern indian ocean. *Deep. Sea Res. Part II Top. Stud. Oceanogr.* **2020**, *176*, 104777. [CrossRef]

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