SENCKENBERG

REVIEW

Diversity, distribution and composition of abyssal benthic Isopoda in a region proposed for deep-seafloor mining of polymetallic nodules: a synthesis

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Abstract

Due to the increasing challenge of meeting human demands for metals from land-based resources, interest in extracting mineral ores from the deep sea has gained momentum in recent years. Targeted mining of deep-seabed minerals could have adverse effects on the associated ecosystems, but knowledge on the biological communities found there, their structure and functions is still limited. The focus of this study is to provide an overview on isopod crustaceans from the Clarion Clipperton Fracture Zone (CCFZ), an area well-known for its abundance of high-grade polymetallic nodules. Isopods generally comprise an important part of the macrofaunal communities of soft deep-sea sediments and indeed are one of the most dominant macrobenthic groups in the CCFZ. In this review, we have compiled all available data and information on isopod diversity and distribution in the CCFZ in a hybrid manner, which includes published data from the literature as well as the analysis of previously unpublished sources and newly collected data. Although isopods are one of the more prevalent and better-known groups of the CCFZ fauna, this study shows that it is still remarkably difficult to obtain a clear perception of isopod diversity and distribution, as well as the factors that could be responsible for the observed patterns. In many places, knowledge remains incomplete, which is largely due to the low sampling and taxonomic effort, non-standardised sample protocols and the limited taxonomic inter-calibration between studies. The latter is pivotal due to the high proportion of undescribed and presumably new species that typically occur there. An important starting point would therefore be to increase sampling effort and its spatial and temporal coverage in a standardised way, to intensify (integrative) taxonomic work as well as to facilitate sample and data exchange between scientists and contractors. These are fundamental requirements to improve our understanding of the biodiversity of isopods, but also of other faunal groups, in the CCFZ, before mining operations begin.

Abbreviations

APEI	Area of Particular Environmental Interest
ABYSSLINE	Abyssal Baseline Programme of UKSRL
BOLD	Barcode of Life Database
BGR	Bundesanstalt für Geowissenschaften und Rohstoffe (Federal Institute for Geosciences and Natural Resources)
CCFZ	Clarion Clipperton Fracture Zone
COI	Cytochrome c oxidase subunit I
DISCOL	Disturbance and recolonisation experiment in a manganese nodule area of the deep South Pacific
DOMES A	Deep Ocean Mining Environmental study site A
DZMB	German Centre for Marine Biodiversity Research

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| Echo 1	Site sampled during ECHO expedition (Spiess et al. 1984) corresponds to DOMES site C (Wilson 2017)
GSR	Global Sea Mineral Resources NV, DEME group
IFREMER	L'Institut Français de Recherche pour l'Exploitation de la Mer (French Research Institute for Exploitation of the Sea)
IOM	Interoceanmetal Joint Organization
IRZ	Impact Reference Zone
ISA	International Seabed Authority
JPIO	Joint Programming Initiative Healthy and Productive Seas and Oceans (JPI Oceans) pilot action "MiningImpact"
KR	Contract area of the Korean Republic
MOTU	Molecular taxonomic unit
NOAA	National Oceanic and Atmospheric Administration
OMS	Ocean Mineral Singapore Pte. Ltd.
PA	Prospective Area
PRA	Preservational Reserve Area Site (termed by NOAA; Wilson 1990)
PRZ	Preservation Reference Zone
ROV	Remotely operated vehicle
UKSRL	UK Seabed Resources Ltd

Introduction

Deep-seabed mining

In search of new avenues to tap precious metal and metalloid sources, a number of deep-sea habitats that hold commercially interesting mineral ores were brought into the limelight. The latter include, *inter alia*, seafloor massive sulphides near hydrothermal vents, cobalt-rich ferromanganese crusts associated with seamounts and abyssal polymetallic nodule fields, which are considered as promising suppliers of many kinds of sought-for metals such as copper, nickel, cobalt, zinc, lead, titanium, lithium, tellurium, silver and gold. However, these mineral deposits are not only of considerable economic value. They also have great ecological and environmental significance as biodiversity reservoirs and for the provision of a range of ecosystem services and functions (Danovaro et al. 2008; Thurber et al. 2014; Fritz 2016). Nodules and crusts increase habitat heterogeneity at multiple spatial scales and concomitantly promote species abundance and richness (Amon et al. 2016; Vanreusel et al. 2016; Gollner et al. 2017). At the same time, they provide important habitats for numerous organisms that may be unique to these areas and are believed to be very susceptible to mining perturbations (Veillette et al. 2007; Schlacher et al. 2014; Gollner et al. 2015, 2017; Singh et al.

2016, 2019; Amon et al. 2016; Vanreusel et al. 2016; Jones et al. 2017). To this end, conservation and exploitation efforts will need to be reconciled to devise environmentally sustainable and economically feasible strategies for seabed mining (Van Dover et al. 2017; Cuvelier et al. 2018; Niner et al. 2018; Jones et al. 2019).

The Clarion Clipperton Fracture Zone (CCFZ) in the eastern tropical Pacific is the primary area of interest for the exploitation of polymetallic (or manganese) nodules. The interplay of different geological, topographical and hydrographic factors and processes has been shown to influence the spatial distribution of polymetallic nodules in the region (Peukert et al. 2018) but also affects community structure and distribution of the sediment and encrusting fauna (Janssen et al. 2015; Vanreusel et al. 2016). On larger spatial scales, gradients in surface productivity and depth across the CCFZ are important drivers of benthic biodiversity and community structure, while small-scale variations in topography and nodule density are likely to explain local patterns (e.g. Bonifacio et al. 2020). Similar environmental gradients were put forward to identify a network of nine large Areas of Particular Environmental Interest (APEIs) bordering the CCFZ to the north and south (Wedding et al. 2013) that are to remain pristine as protected areas and where no influence of mining may occur. The representativeness of these APEIs to fully capture biodiversity and habitats in the region has been under debate (McQuaid et al. 2020; Washburn et al. 2021a), and as a result, four further areas (APEIs #10–13) have recently been adopted into the Environmental Management Plan of the CCFZ (ISA 2021) (Fig. 1). In addition, international regulations set up in the draft "Mining Code" require the establishment of two different types of management zones within exploration contract areas: Impact Reference Zones (IRZ), where mining impacts are to be investigated, and Preservation Reference Zones (PRZ), as control sites where no mining or effects of mining may occur (ISA 2011; Jones et al. 2020).

Thus far, the International Seabed Authority (ISA) has issued 17 contracts for the exploration of polymetallic nodules in the CCFZ (as of December 2022; www.isa.org.jm), which are scheduled for a period of 15 years with the possibility of an extension for a further 5 years (Lodge et al. 2014). During this time, contractors are obliged to collect geological, oceanographic and biological information to characterise the baseline status of the ecosystem before mining can occur and additionally to assess the potential impact of mining activities on the marine environment in accordance with ISA regulations (ISA 2000; Lodge et al. 2014). Several exploration contracts have recently been extended for the second 5-year period (December 2021), and the ISA has been put under increasing pressure to adopt the Mining Code for exploitation by July 2023 (e.g. Singh 2021). Therefore, it is imperative to provide detailed baseline information
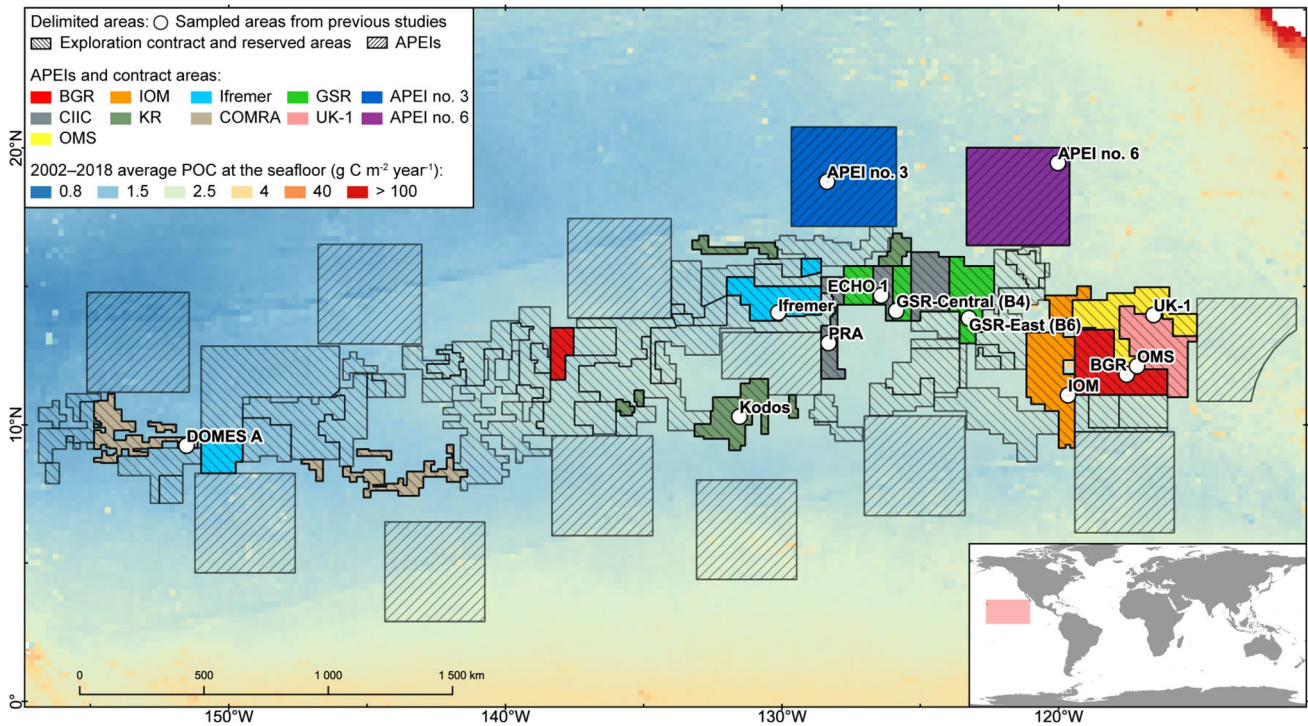
Fig. 1 Map of the polymetallic nodule exploration contract areas analysed in this study and Areas of Particular Environmental Interest (APEIs, large squares, APEI#3 and #6 marked in blue and purple, respectively) in the Clarion Clipperton Fracture Zone (CCFZ), superimposed on a map of POC flux at the seafloor (g C m^{-2} year^{-1}; average 2012–2018; from Bonifacio et al. 2020). In addition, sampled areas from previous studies are shown: DOMES A, Echo1 and PRA. Figure reproduced from Bonifácio et al. (2020) under the Creative Commons license CC BY 4.0

about the resident biota in order to build a comprehensive framework for the environmental management of seabed mining in the CCFZ (Wedding et al. 2013, 2015; Levin et al. 2016; Durden et al. 2017).

Deep-sea Isopoda

Isopod crustaceans are one of the most prevalent and diverse members of deep-sea macrobenthic soft-sediment communities, and due to their ubiquity across deep-sea environments, they are commonly used as target taxa to study variations in abundance, diversity and distribution across a range of temporal and spatial scales (Hessler and Wilson 1983; Rex et al. 1993; Brandt 1995; Wilson 1998, 2017; Stuart et al. 2003; Brandt et al. 2007; Kaiser et al. 2007, 2018; Janssen et al. 2015; Brix et al. 2018, 2020; Raupach et al. 2009). In the CCFZ, isopods also represent one of the most dominant macrofaunal taxa, comprising up to one-third of total macrofauna (Thistle and Wilson 1987, 1996; Kaiser et al. 2015; De Smet et al. 2017; Wilson 2017; Chuar et al. 2020; Pasotti et al. 2021). As with all peracarids, isopods lack planktonic larvae, which imply a restriction of their dispersal capacity. Potentially, this makes obligate brooders, such as isopods,

ideal for assessing changes in biodiversity and distributions as a result of environmental disturbances.

In the deep sea, the asellote superfamily Janiroidea Sars, 1897 is particularly successful in both evolutionary and ecological terms, accounting for > 90% of the entire deep-sea Isopoda (Kussakin 1973; Hessler et al. 1979; Hessler and Wilson 1983). Molecular phylogenetic reconstructions indicate an ancient colonisation history of Janiroidea in the deep sea, as evidenced by the likely deep-sea origin of several families (Wilson 1999; Lins et al. 2012; Raupach et al. 2009). Their great success in occupying many different habitats has been attributed to a highly specialised mating system, which is beneficial in low-density environments such as the abyss (Wilson 1991). For most asellote families, detritivory or foraminivory is assumed to be the norm (Svavarsson et al. 1993; Brökeland et al. 2010; Würzberg et al. 2011; Riehl et al. 2018a), but some have been reported to feed on meiofauna (e.g. *Munnopsurus* spp.; Cartes et al. 2002). Although most species are strictly benthic, following an in- or epifaunal lifestyle, there are transitions towards benthopelagic or even holo-pelagic forms (Osborn 2009).

Thus far, 23 janiroid families—consisting of > 1600 described species—from deep-sea environments have been recorded, with most of these families occurring

predominantly or exclusively in the deep sea (Boyko et al. 2008 onwards; Glover et al. 2021). Munnopsidae Lilljeborg, 1864 and Desmosomatidae G. O. Sars, 1897 are typically the most specious and abundant with > 330 and > 120 described species, respectively (WoRMS Editorial Board 2021), and numerous awaiting formal description (Malyutina and Brandt 2018, 2020; Brix et al. 2020, 2021; Jennings et al. 2020). While the majority of janiroidean families and genera appear to be cosmopolitan (with a few exceptions, such as *Micromesus* Birstein, 1963, *Microthambema* Birstein, 1961, *Pirinectes* Malyutina et al., 2020, and *Sugoniscus* Menzies & George, 1972 thus far only found in Pacific waters, and *Parvochelus* Brix et al., 2015 as a potential Atlantic endemic), most species seem to have more limited distributions (Brandt et al. 2012). In addition, the large number of suspected new species that are generally found in deep-sea collections (Brandt et al. 2007; Rex and Etter 2010; Kaiser et al. 2017; Golovan et al. 2019) indicates our poor level of knowledge for most deep-sea areas and challenges the evaluation of macroecological patterns of diversity and distributions.

Over the past two decades, advances in deep-sea isopod research have been made due to increased sampling efforts with complimentary sampling devices (Brandt et al. 2016; Kaiser and Brenke 2016) and the use of modern (molecular and modelling) techniques (Brix and Svavarsson 2010; Janssen et al. 2015; Schnurr et al. 2018; Riehl et al. 2018b; Kürzel et al. 2022; Paulus et al. 2022). In particular, application of an integrative taxonomy to derive species boundaries using multiple lines of evidence has led to a more robust identification of species. This, in turn, has facilitated the assessment of diversity patterns and geographic ranges (Brökeland and Raupach 2008; Brix et al. 2011, 2015, 2018; Riehl et al. 2018b; Janssen et al. 2015, 2019; Kaiser et al. 2018, 2021; Schnurr et al. 2018; Riehl and Kühn 2020; Riehl and De Smet 2020). Here, two opposing patterns have emerged; morphologically similar but genetically divergent (i.e. cryptic) species appear to be common (Brix et al. 2015; Janssen et al. 2015; Schnurr et al. 2018; Kaiser et al. 2021), but some isopod species, despite being brooders, are also found to be widespread even surmounting major topographic obstacles (the latter appearing to be the exception and not the rule though, Riehl and Kaiser 2012; Janssen et al. 2015; Bober et al. 2018; Riehl et al. 2018b; Brix et al. 2020; Kaiser et al. 2021). The mechanisms have yet to be clarified, but distance, environmental and topographic barriers as well as adult lifestyle are likely to play an important role in shaping the large-scale distribution of isopod species in the deep sea (Brix and Svavarsson 2010; Bober et al. 2018; Riehl et al. 2018b; Schnurr et al. 2018; Brix et al. 2020; Kaiser et al. 2021).

Study objectives

The aim of the present study is to present a summary of all available data on the diversity and distribution of benthic isopods from the CCFZ. The emphasis is on a literature review of published data, but previously unpublished data will also be considered. We selected three key areas in the study of CCFZ Isopoda and evaluated recent advances in each field. First, we investigate how the abundance and diversity of the isopod fauna is spatially distributed over the CCFZ and discuss the driving factors and processes behind those patterns. We then shift our focus to look at current patterns of species distributions (i.e. endemism and rarity vs. large geographic ranges). We analyse whether evidence from new taxonomic data and molecular techniques can help us to obtain more robust estimates of the geographic distribution and population structure of species in CCFZ Isopoda and to elucidate their primary mechanisms. Finally, we identify gaps in the study of CCFZ isopods in terms of taxonomy, geography or methodology and provide recommendations on how these might be tackled in the future. In the following, we address these questions based on advances in a number of biological disciplines and assess how isopods have helped to understand and answer them. We review the range of data and results from available taxonomic, faunistic and molecular genetic analyses of isopods across the CCFZ to identify gaps in knowledge and determine future research opportunities that will ultimately help to better understand the patterns and processes governing their biodiversity and distribution in the central Pacific nodule province.

Material and methods

Data compilation

We were primarily interested in differences in the abundance, diversity and community composition between different exploration contract areas across the CCFZ. It should be noted here that contract areas cannot be viewed as individual biotopes with specific geographical boundaries. However, for conservation management purposes, for which the data presented here can be used, these artificial limits apply. We extracted all available information on abyssal isopod abundances and occurrences as well as genetic data (COI) from the CCFZ. Some of this information has been published in peer-reviewed journals (published between 1987 and 2021), while a large amount of data is only available in expedition reports or is hitherto entirely unpublished. In addition, the ISA database DeepData (Rabone et al. 2022; https://www.isa.org.jm/deepdata) was queried for isopod occurrences in the CCFZ in order to identify and assess data gaps in the region. Details of the

sampling protocols and processing for the unpublished data are provided below.

Sampling and processing

Benthic samples derive from 12 expeditions carried out by the BGR, GSR and IFREMER as well as part of the JPIO MiningImpact and ABYSSLINE projects between 2010 and 2018 (Table S1 in the electronic supplementary). For assessing the abundance and diversity of macrobenthic communities, a box corer and an epibenthic sledge (sensu Brenke 2005) have been deployed (Rühlemann et al. 2010, 2012; Janssen et al. 2013, 2014; Menot et al. 2013; Martínez Arbizu and Haeckel 2015; Uhlenkott et al. 2016, 2017, 2018; Pape et al. 2017, 2018). Here, macrofauna is defined as the size class of animals that pass through a 2000-μm but are retained by a 300-μm mesh. Overall, samples from six contract areas (eastern part of the German contract area [BGR], French [hereinafter referred to as IFREMER], Singapore [OMS], UK [UKSRL, -1B], Belgium [GSR, B4 and B6] and jointly Bulgaria, Cuba, Czech Republic, Poland, Russian Federation and Slovakia as sponsoring states [IOM]) as well as one APEI (#3) were available for the present study (for a summary of the contract areas and station details, see Fig. 1 and Table S1 in the electronic supplementary). In addition, since 2013, two areas for the implementation of a biological time series analysis have been designated in the BGR contract area, one in an established IRZ (PA1-West) and one in a corresponding PRZ located ca. 60 km west of the IRZ. The PRZ was tentatively defined based on a limited dataset that was available at that time and has been sampled regularly since then to test for its suitability as such. For this study, isopod material was available from the PRZ taken by means of an epibenthic sledge in 2013, 2014 and 2015 (MANGAN 2013, 2014 and JPIO MiningImpact SO239 expeditions) and the IRZ taken in 2013, 2014, 2015 and 2016 (MANGAN 2013, 2014, 2016 and JPIO MiningImpact SO239 expeditions, see Table S1 in the supplementary material). Based on this, we were able to assess how the isopod communities have changed over a 3-year time period and whether PRZ and IRZ differed in terms of diversity and composition. The latter gives conclusions as to whether the PRZ is suitable as a preservation area within the BGR contract area. Furthermore, complementary data were available from the DISCOL (DISturbance and reCOLonisation experiment in a manganese nodule area of the deep South Pacific) site, an abyssal manganese nodule area in the Peru Basin, which were collected during the JPIO MiningImpact SO242-1 expedition (Brix et al. 2020; Table S1).

Box corer isopod material that was examined in this study derives from four expeditions: JPIO MiningImpact SO239, MANGAN 2018, GSRNOD15A and GSRNOD17 (Martínez Arbizu and Haeckel 2015; Pape et al. 2017, 2018;

Rühlemann et al. 2019; Table S1). All box corer deployments used for these studies had a surface area of 0.25 m^2. Unless stated otherwise, sample processing was performed using the following protocol: upon arrival on deck, the surface water above the sediment was removed with a hose and sieved through a 300-μm sieve. A photo was then taken of the box corer surface. Individual biota visible to the eye on the surface (either attached to the nodules or lying on the sediment) were removed and fixed separately (in DESS [Yoder et al. 2006] or 96% ethanol). Nodules were removed and carefully washed to remove surface sediment, measured and weighed for further analyses (analyses not relevant to this study). The sediment in the box corer was then sliced into three layers: 0–3 cm, 3–5 cm and 5–10 cm. Each layer (0–3 cm, 3–5 cm and 5–10 cm) was transferred into cold filtered sea water (2–4 °C), carefully elutriated and separately sieved (through a 300-μm mesh) and fixed. During MANGAN 2018, one additional subcore was taken from each box corer for sedimentological assessment prior to slicing (Rühlemann et al. 2019). During SO239 (Martínez Arbizu and Haeckel 2015), the top layer (0–3 cm) was immediately sorted, and specimens were separately fixed (80 and 96% undenatured ethanol). The remaining layers were fixed in 4% formalin and later transferred to 80% denatured ethanol. During GSRNOD15A (Pape et al. 2016), the top 0–3-cm layer was fixed in bulk in 96% undenatured EtOH at − 20 °C; the deeper layers were first stored for 48 h in 10% formaldehyde, after which these were transferred to 80% undenatured EtOH. During GSRNOD17 (Pape et al. 2018), all sediment layers were stored in 96% undenatured EtOH at − 20 °C. Sorting of the box corer material was partly conducted on board, but mainly back in the home laboratories (IFREMER in Plouzané [France], Senckenberg am Meer—DZMB in Wilhelmshaven [Germany], or the Marine Biology Research Group Laboratory facilities in Ghent [Belgium]).

A detailed description of the epibenthic sledge can be found in Brenke (2005). In short, it consists of two superimposed nets, i.e. a lower epinet and an upper supranet, each with a mesh size of 500 μm, and ending in 300-μm cod ends. A door, attached to an opening/closing mechanism, is located in front of the net, which remains closed in the water column and opens as soon as the epibenthic sledge touches the sea floor. In this way, contamination by planktonic organisms can be minimised. For sampling in warm, tropical nodule areas, an additional metal grid is placed in front of the mesh openings to prevent nodules from entering the nets and thus damaging the samples. Furthermore, a box is placed around the cod ends, which holds cold deep water. This is to ensure a continuous cooling chain, which is essential for genetic analyses (Riehl et al. 2014a). For JPIO MiningImpact SO239 deployments, the trawling distance (*d*) was calculated in order to enable standardisation of the trawled seabed to 1000 m and thus assessment of faunal

densities (ind. per 1000 m^2), as the epibenthic sledge sample unit is 1-m wide (Table S1). Therefore, the following formula was used:

$$d = (V_1 \times T_1) + (V_2 \times T_2) + (V_3 \times T_3),$$

where V_1 is ship velocity during trawling, T_1 is trawling time, V_2 is ship velocity during haul, T_2 is haul time (sledge off bottom), V_3 is winch velocity and T_3 is haul time (sledge off bottom).

For all expeditions, sample processing followed a standard procedure. As soon as the epibenthic sledge was back on board, the nets were rinsed with sea water up to the cod. The cod ends of both nets were removed and immediately transferred to a cold room (mostly at ~2–4 °C and 11 °C during BIONOD). Epi- and supranet samples were processed separately. The samples were elutriated with filtered pre-cooled sea water, and the macrofauna was placed on a 300-µm mesh. Each sample was preserved in 96% pre-cooled undenatured ethanol and kept at low temperature (−20 °C) for at least 48 h (Riehl et al. 2014a). Macrofauna samples were then sorted on board and back at DZMB using stereomicroscopes to a higher taxon level (phylum, class, order) and stored in 96% undenatured ethanol. Isopod individuals were only counted if the head was present. Isopod samples were usually further discriminated to family level and partly to morphospecies level. If species could be morphologically assigned to known species, species names were used, while presumably new species were assigned a unique numerical code. Isopod specimens that were too damaged to be assigned to a species were marked as sp. indet. All isopod specimens that were not used for taxonomic descriptions are deposited with the DZMB in Wilhelmshaven or at the Marine Biology Research Group of Ghent University. Type material of described species is either stored at the Senckenberg Museum, Frankfurt (SMF), or the Crustacean Collection of the Museum of Nature (Leibniz Institute for the Analysis of Biodiversity Change, LIB) (formerly Center of Natural History, CeNak) in Hamburg (Kaiser et al. 2018, 2021; Malyutina et al. 2020, see Table S2 in the electronic supplementary for accession numbers).

Since data collected by box corer and epibenthic sledge are not entirely comparable (Lins and Brandt 2020) for assessing diversity and community composition, we only report on data collected by means of the epibenthic sledge for these parameters. Isopod specimens from the box corer of the MANGAN 2018 and GSR expeditions were used for genetic analysis exclusively to assess species' geographic range size and diversity at the regional scale.

DNA extraction, amplification, sequencing and molecular species delimitation

Published COI sequences were taken from GenBank (Table S2; Janssen et al. 2015, 2019; Kaiser et al. 2018, 2021; Riehl and De Smet 2020; Brix et al. 2020). Additional COI sequences were obtained from 486 isopod individuals summing up to a total of COI sequences from 1522 (epibenthic sledge: 1471; box corer: 51) individuals (Table S2). From each specimen, 2–3 legs were dissected unilaterally to keep the voucher specimen intact for later morphological reassessment. All tissue samples were initially stored in 96% undenatured ethanol. Prior to extraction, all tissue samples were washed in distilled water to remove excess ethanol. DNA was then extracted using Chelex® 100 BioRad (Walsh et al. 1991) based on the protocol provided by Janssen et al. (2015). DNA from isopods from the GSR expeditions (GSRNOD15A and GSRNOD17, Table S2) was extracted using the NucleoSpin Tissue XS kit (Macherey–Nagel, Germany). Amplification of the COI barcode was conducted using universal primers introduced by forward: LCO1490 5′-ggtcaacaaatcataaagatattgg-3′ and reverse: HCO2198 5′-taaacttcagggtgaccaaaaaatca-3′ (Folmer et al. 1994) following previously published protocols (sensu Janssen et al. 2015). PCR products that produced light bands after electrophoresis on 1% agarose gel were sent to the MacroGen Europe Laboratory in Amsterdam, Netherlands, for sequencing using the same set of primers as used for the PCR. DNA from isopods of the JPIO MiningImpact SO239 and JPIO MiningImpact SO242-1 expeditions were extracted using the PCR protocols of Brix et al. (2011) and Riehl et al. (2014a). Extraction and sequencing of the individuals was conducted at the Laboratories of Analytical Biology (LAB), Smithsonian National Museum of Natural History, Washington DC, USA, with an ABI 3730xl 96-well capillary sequencer. A consensus sequence was produced for every individual, combining reverse and forward sequences using SeqTrace (Stucky 2012) or Geneious Prime 2020. The consensus sequences were aligned in the MEGA6 (Tamura et al. 2013) and Geneious 10.1.2 (MAFFT alignment) softwares.

To assess the number of species (or rather MOTUs) in the COI dataset, a number of species delimitation (SD) analyses were conducted. Multiple delimitation methods were employed on the COI dataset to access potential congruence (Carstens et al. 2013). Four different distance- and tree-based species delimitation analyses were performed to allocate sequences into genetic species. Distance-based approaches (Assemble Species by Automatic Portioning, ASAP: Puillandre et al. 2021; Barcode Index Number, BIN: Ratnasingham and Hebert 2013) detect the distance at which the "barcode gap" occurs and sort the sequences into putative species based on this distance. ASAP analysis was implemented on the web interface (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html) (Puillandre et al. 2021) with default setting and under the p-distance model. Barcode Index Numbers (BINs) were assigned on the registered DNA dataset automatically using the BOLD v.4 workbench (www.boldsystems.org). Tree-based approaches (multi-rate Poisson

Tree Processes, mPTP: Kapli et al. 2017; General Mixed Yule Coalescent, GMYC: Pons et al. 2006) use a phylogenetic tree from which the fit of speciation and coalescent processes are modelled to delineate species based on the branching rate of the tree (Carstens et al. 2013; Tang et al. 2014). The ultrametric phylogenetic tree was produced in BEAST v.2.5. Settings were as follows: strict clock, Yule speciation model, two independent MCMC chain runs for 20,000,000 generations, and sampled every 1000 steps (10% was discarded as burn-in period). The best-fitting model of nucleotide substitution was selected using jModelTest v.2.1.10 for each family separately under the Akaike information criterion (Posada 2008). The model selected for COI was either HKY + I + G (Dendrotionidae, Haplomunnidae, Mesosignidae) or GTR + I + G (Desmosomatidae, Haploniscidae, Ischnomesidae, Macrostylidae, Munnopsidae, Nannoniscidae). Convergence of BEAST runs was assessed with Tracer v1.7.1 (Rambaut et al. 2018) and burn-in was selected after all effective sample sizes (ESSs) were at least 200. The GMYC method was implemented using the R package SPLITS (Fujisawa and Barraclough 2013), under the single-threshold model (stGMYC), while the mPTP was implemented on the web server (https://mptp.h-its.org) using the multi-rate Poisson tree process model and following default settings.

Community and diversity analysis

Due to the heterogeneity of the data, we kept box corer and epibenthic sledge data separate and reduced the datasets to the lowest common taxonomic denominator in order to enable a comparison between the different studies. Family-level data were available for a number of epibenthic sledge samples (Table S3). In some cases, data from epi- and supranet were merged (SO205, 2014, SO239); in other cases, only supranet data were available (BIONOD, SO239 stations #192 and #197; Table S3). Since it is assumed that epi- and supranet have similar faunal compositions (e.g. Knox et al. 2012), a family station matrix was created for the community analysis based on relative abundances (%) including both merged (epi and supra) and supranet-only samples (Table S3). In addition, for the SO239 dataset, family-level data were available from both epibenthic sledge and box corer (Tables S3, S4), so a direct comparison in family composition between the two devices could be made. Since most collected species were putatively new to science, and taxonomic calibration exercises have not yet been achieved, species-level analyses were conducted using the number of MOTUs per area (Table S2).

To compare MOTU richness between different contract areas within the CCFZ, APEI#3 and DISCOL, as well as PRZ and IRZ within the BGR contract area (as part of the time series), individual-based rarefaction curves were

computed in PAST version 3.26 (Hammer et al. 2001), as these avoid bias caused by different sample sizes (Gotelli and Colwell 2001). In addition, abundance-based (Chao 1) and incidence-based (Chao 2, Jackknife 1 and 2 and Bootstrap) richness estimators were calculated in PAST to predict total richness per predefined area (Magurran 2004). Therefore, samples for each area (BGR, UKSRL, OMS, IOM, GSR, IFREMER, APEI#3, DISCOL) were pooled prior to analysis. Chao 2, Jackknife 1 and 2 and Bootstrap were only computed for all contract areas (BGR, UKSRL, OMS, IOM, GSR, IFREMER) combined to extrapolate isopod richness for the CCFZ as a whole.

To assess the spatial and temporal variation in isopod composition between different areas, four matrices were analysed based on (1) a family × station matrix for relative abundance (percentage) per family based on epibenthic sledge samples from across the CCFZ, (2) a family × station matrix for relative abundance (percentage) per family based on box corer and epibenthic sledge samples from the SO239 expedition, (3) a MOTU × area (including contract areas, APEI3 and DISCOL) matrix based on relative abundance per MOTU and (4) a MOTU × area (IRZ and PRZ within the BGR contract area) matrix based on relative abundance per MOTU. A nonmetric multidimensional scaling (nMDS) based on a Bray–Curtis similarity matrix was plotted to visualise differences between locations using Primer 6.0. A one-way ANOSIM was computed in Primer 6.0 to test for significant differences in family and MOTU composition between different areas.

Results and discussion

A brief history of isopod research in the CCFZ

Initial investigations of the CCFZ isopod fauna date back to US NOAA funded programs conducted between 1970 and 1992, with data collected from different sites—DOMES A, Echo 1 and PRA—in the central and western CCFZ (Thistle and Wilson 1987, 1996; Wilson 1990, 2017; Fig. 1). With the resumption of interest in seabed mining during the last two decades, a larger spatial spectrum of information for isopods from nine different contract areas in the CCFZ, as well as first collections from APEIs (#3, #6 and #9), has become available (Janssen et al. 2015, 2019; Kaiser et al. 2015, 2021; De Smet et al. 2017; Yu et al. 2018; Brix et al. 2020; Chuar et al. 2020; Pasotti et al. 2021; Washburn et al. 2021a,b; summarised in Table 1). In addition, the first genetic data from isopods were obtained from the CCFZ (Janssen et al. 2015, 2019; Brix et al. 2020; Riehl and De Smet 2020; Kaiser et al. 2018, 2021).

Despite the wealth of information that has been gathered over the years, comparison between studies is

Table 1 Summary of peer-reviewed faunal studies examining isopod material from the CCFZ. *BC*, box corer; *EBS*, epibenthic sledge; *APEI*, Area of Particular Environmental Interest; *BGR*, eastern German contract area; *GSR*, Global Sea Mineral Resources NV (Belgian contract area); *IFREMER*, L'Institut Français de Recherche pour l'Exploitation de la Mer (French contract area); *IOM*, Interoceanmetal Joint Organization contract area; *OMS*, Ocean Mineral Singapore Pte. Ltd. (Singapore contract area); *UKSRL*, UK Seabed Resources Ltd. (UK contract areas); *KR*, contract area of the Korean Republic; *YUZM*, JSC Yuzhmorgeologiya (contract area of the Russian federation); *DISCOL*, disturbance and recolonisation experiment in a manganese nodule area of the deep South Pacific; *DOMES A*, Deep Ocean Mining Environmental study site A; *Echo 1*, site sampled during ECHO expedition (Spiess et al. 1984); *PRA*, Preservational Reserve Area Site (Wilson 1990)

| Reference	Area(s)	Gear type	Mesh size (μm)	Layer (BC)	Taxonomic resolution	Indicator variable
Thistle and Wilson (1987)	DOMES A	BC	300	0–5 cm	Family, genus	Density, taxon richness
Thistle and Wilson (1996)	Echo 1, PRA	BC	300	0–10 cm	Morpho-species	Density, richness
Janssen et al. (2015)	BGR, IFREMER	EBS	300	n.a	MOTU/morpho-species	Composition, richness estimators
De Smet et al. (2017)	GSR	BC	300	0–10 cm	Morpho-species/genus	Density, taxon richness, richness estimators, rarefaction
Wilson (2017)	DOMES A, Echo 1, PRA	BC	300	0–10 cm	Morpho-species	Density, turnover, rarefaction
Yu et al. (2018)	KR	BC	250	0–10 cm	Morpho-species	Diversity, density, composition (total macrofauna)
Brix et al. (2020)	BGR, IOM, GSR, IFREMER, APEI#3, DISCOL	EBS	300	n.a	MOTU/morpho-species	Composition, richness estimators, rarefaction
Chuar et al. (2020)	OMS	BC	300/250	0–10 cm	Family	Density, taxon richness, composition
Pasotti et al. (2021)	GSR	BC	300	0–10 cm	Morpho-species	Density, taxon richness, rarefaction
Washburn et al. (2021b)	BGR, IOM, GSR, IFREMER, APEIs #3, -6, -9, KR, YUZM, UKSRL; OMS	BC	300/250	0–10 cm	Morpho-species	Density, taxon richness, rarefaction

challenged by the fact that different sampling devices have been used for data collection and that much of these data are only available as grey literature (e.g. Wilson 1992; Smith et al. 2008; Martínez Arbizu and Haeckel 2015). Isopod records extracted from the ISA DeepData database indicate that most of the isopod material derives from sampling with epibenthic sledge, multicorer and box corer (Fig. 2), but physical specimens and/or images were also collected, albeit to a much lesser extent, by ROV (e.g. Martínez Arbizu and Haeckel 2015). The use of box corer, as a quantitative tool, was recommended for macrofauna sampling by the Legal and Technical Commission of the ISA (ISBA/25/LTC/6Rev1; ISA 2020). As one box corer with a standard size of 50 × 50 cm usually has very low macrofaunal densities (e.g. Washburn et al. 2021b), the epibenthic sledge was introduced as an additional type of gear during the sampling campaigns of the BGR (e.g. Rühlemann et al. 2010, 2012, 2019), in the course of JPIO MiningImpact (Martínez Arbizu and Haeckel 2015), and later by UKSRL as part of the ABYSSLINE project (Dahlgren et al. 2016). The epibenthic sledge is more suitable to sample larger amounts of epi- and suprabenthic macrofaunal specimens compared to the box corer, even if only in a semi-quantitative manner (Kaiser and Brenke 2016; Lins et al. 2021).

Box corer and epibenthic sledge are known to sample different numbers and fractions of the fauna, and thus to result in different patterns of abundance, diversity and composition that are only comparable to a limited extent (e.g. Jóźwiak et al. 2020; Lins and Brandt 2020). In addition, even for one type of sampling gear such as the box corer, different sampling protocols and mesh sizes (250 vs. 300 μm) were used for the various studies (Table 1). This influences the sampling efficiency and thus, in turn, the comparability of the diversity and abundance of fauna between studies (e.g. De Smet et al. 2017; Yu et al. 2018; Chuar et al. 2020; Washburn et al. 2021b). Last but not least, differences in taxonomic resolution (family vs. species level) between studies derived from morphological and/or molecular data as well as considering the sample set as a whole or only subsets of

Fig. 2 Summary of the CCFZ isopod datasets (*n* = 3367 total) in the ISA DeepData database: **a** proportionally per family (note: ca. 80% of samples in the database were not identified to family level); **b** per contractor area and **c** per gear type. BC, box corer; EBS, epibenthic sledge; MUC, multicorer; BGR, eastern German contract area; GSR, Global Sea Mineral Resources NV (Belgian contract area); IFREMER, L'Institut Français de Recherche pour l'Exploitation de la Mer (French contract area); IOM, Interoceanmetal Joint Organization contract area; OMS, Ocean Mineral Singapore Pte. Ltd. (Singapore contract area); UKSRL, UK Seabed Resources Ltd. (UK contract areas); KR, contract area of the Korean Republic; DORD, Deep Ocean Resources Development Co. Ltd. (Japanese contract area); YUZM, JSC Yuzhmorgeologiya (contract area of the Russian federation)
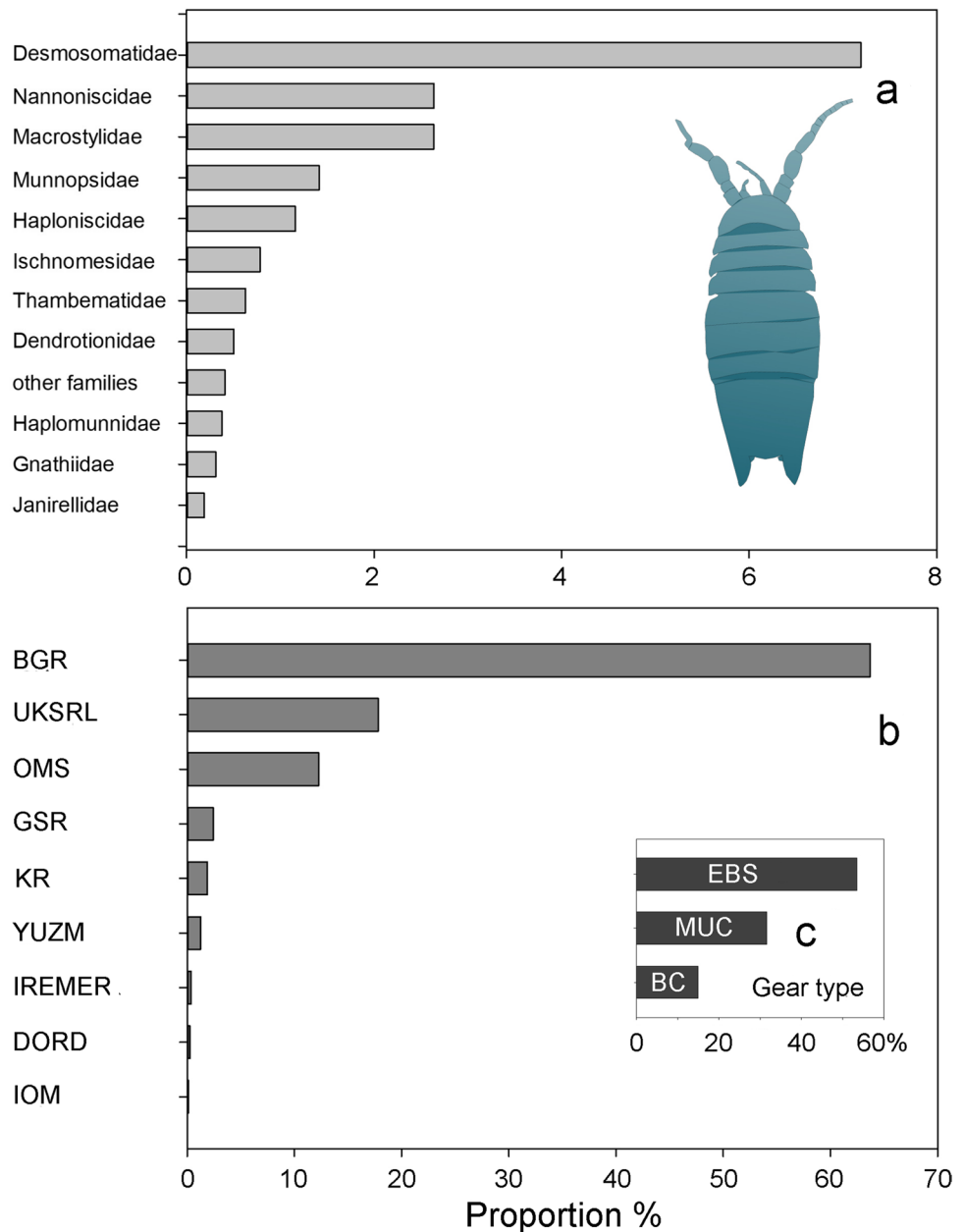
the data have provided a rather mixed picture of the isopod biodiversity in the CCFZ (see also Table 1).

Species delimitation

Reliable species delimitation is vital in order to be able to estimate the diversity of species, their distribution and ultimately the levels of impact from mining operations. In particular, the question of whether there are few species with a wide spatial distribution range or many species with only a limited range would have major implications for conservation strategies, including questions on the size of preservation areas and their distances from mining blocks.

Isopod species from the abyssal CCFZ were initially solely delimited by morphological means (Thistle and Wilson 1987, 1996; Wilson 1990, 2017; Malyutina 2011; Kaiser 2014; Riehl et al. 2014b; Malyutina et al. 2020). However, as in many other taxa, the morphological delineation of isopod species is complicated by the presence of morphologically very similar or identical, but genetically distinct species (Pfenninger and Schwenk 2007; Janssen et al. 2015; Kaiser et al. 2021). Also known from several families are a number of sexually strongly dimorphic species in which males are difficult to assign to the corresponding females (Riehl et al. 2012; Paulus et al. 2022). Small-sized crustaceans such as isopods are also prone to misidentification as they can easily

lose their legs during sampling or sample processing. Since legs often bear taxonomically important characters, individuals in this case could possibly not be assigned to a species or even genus level (Frutos et al. 2022). Therefore, an integrative approach that links multiple lines of evidence to derive species boundaries is now increasingly being pursued (Carstens et al. 2013; Kaiser et al. 2018, 2021; Brix et al. 2020; Riehl and De Smet 2020). For the CCFZ, the study by Janssen et al. (2015) was the first to combine mitochondrial DNA sequences (COI) and morphological data. Their results revealed a sometimes-large discrepancy between morphological and genetic diversity, with the number of MOTUs being two to three times higher than the number of morphological species (Janssen et al. 2015).

In our study, we defined MOTUs on the basis that the majority of SD methods applied were congruent. In addition, in most cases, molecular delimitation was backed up by additional morphological examination. For most species, this approach was unequivocal, i.e. all SD methods showed the same species demarcations, but some also gave incongruent results (Table S2 in the electronic supplementary). The latter could be cases where the genetic distances between clades fall within the barcode gap, as previously shown for CCFZ isopods (Kaiser et al. 2018, 2021). Overall, SD methods seem to behave differently, especially when looking at single-locus data (Dellicour and Flot 2018), as presented here, with genetic diversity, effective population size and speciation rates negatively affecting their performance (Dellicour and Flot 2018; Magoga et al. 2021). Tree-based approaches like GMYC tend to overestimate true species numbers, whereas distance-based approaches like Automatic Barcoding Gap Discovery (ABGD) or ASAP tend to lump species together and thus underestimate species richness (Dellicour and Flot 2018; Kaiser et al. 2018, 2021; Paulus et al. 2022). Therefore, the aim should be to use several SD methods in combination, the agreement of which then implies a correct species delimitation (Dellicour and Flot 2018).

Based on the above considerations, we assume that our differentiation is robust by combining different SD methods and linking them to morphology. On this basis, we could delineate 361 MOTUs, 345 of which were collected with the epibenthic sledge and 16 additional obtained from the box corer. Only eighteen MOTUs could be assigned to known species (e.g. *Acanthocope galatheae* Wolff, 1962, *Rectisura slavai* Malyutina, 2011, Table S2), while the majority appear to represent species new to science (~95%). Notably, seven of these species and two genera (*Ketosoma* Kaiser & Brix, 2018; *Pirinectes* Malyutina & Brix, 2020) have been newly described from this dataset (Kaiser et al. 2018; Malyutina et al. 2020; Riehl and De Smet 2020; Kaiser et al. 2021; Table 2). The wide distribution of *Acanthocope galatheae*, a species described from the Gulf of Panama and also

distributed in the Atlantic Ocean, has been molecularly confirmed (S. Bober, pers. comm.). Others, such as *Betamorpha fusiformis* (Barnard, 1920), occurring out of their presently known range, would need further examination. For the latter, a species complex has been identified that spans the Southern Ocean and the Atlantic (Raupach et al. 2007), making it very likely that the species occurring in the CCFZ represents a different and potentially new species. Our SD analysis also suggests that some species, in particular *Macrostylis metallicola* Riehl & De Smet, 2020 and *Rectisura slavai*, probably represent more than one species (Table S2). However, further analyses are required to confirm these findings.

The addition of molecular (barcoding) methods to morphology-based taxonomy has certainly helped advance the differentiation of isopod species, but the latter remains complex and challenging. The integrative approach, considering multiple lines of evidence, including morphological, molecular, ecological and/or biogeographical data, currently seems to be the best way forward to provide a stable taxonomy but requires significant time, financial resources and above all strong taxonomic expertise. With deep-seabed mining becoming imminent, however, methods must be found to assess and compare species richness and distribution more rapidly, while ensuring reliable species assignment. These include, on the one hand, the use of modern molecular methods such as proteomic fingerprinting, which has been successfully tested as a rather cheap and fast method for species delimitation of deep-sea isopods (Kürzel et al. 2022; Paulus et al. 2022). On the other hand, trained taxonomists are needed to identify and describe the many unnamed or new species, but also to produce identification keys and train future taxonomists to ensure thorough biodiversity assessment and monitoring in the CCFZ in the long term.

Spatial structure of isopod composition, abundance and diversity across the CCFZ

Composition

In many ways, macrobenthic communities of the CCFZ resemble other (nodule-free) abyssal areas, at least, when looking at supra-specific taxa, as similar groups exist and polychaetes and isopods typically dominate samples (e.g. Hessler and Jumars 1974; De Smet et al. 2017; Gollner et al. 2017). This is also reflected in isopods at the family level; CCFZ isopod communities mainly consist of typical deep-sea families (e.g. Munnopsidae, Desmosomatidae, Haploniscidae, Nannoniscidae, Macrostylidae; Fig. 3, Tables S3, S4), which is similar to ocean basins elsewhere (e.g. Brandt et al. 2007; Lörz et al. 2013; Elsner et al. 2015). Yet, there are profound differences in isopod family composition between box corer and epibenthic sledge collections. The families Munnopsidae and Desmosomatidae are the most dominant

Table 2 List of described isopod species known from the CCFZ; an asterisk (*) denotes the CCFZ as the type locality. The term cf. (confer) was used as a cautious attribution, when species could not be unambiguously identified due to damage to specimens, or for those found far removed from their type locality, thereby avoiding overestimation of their species ranges

| Family	Species	Type locality	Reference
Incertae sedis	*Sugoniscus* cf. *parasitus* Menzies & George, 1972	Peru–Chile Trench, 3909–4925 m	Menzies and George (1972); Kaiser unpubl. data
Nannoniscidae	*Hebefustis juansenii* Kaiser, 2014*	BGR CA, CCFZ, 4127–4360 m	Kaiser (2014)
Nannoniscidae	*Hebefustis vecino* Kaiser, 2014*	BGR CA, CCFZ, 4226 m	Kaiser (2014)
Nannoniscidae	*Ketosoma ruehlemanni* Kaiser & Janssen, 2018*	BGR CA, CCFZ, 4133–4358 m	Kaiser et al. (2018)
Nannoniscidae	*Micromesus* cf. *nannoniscoides* Birstein, 1963	NW Pacific, 4000–4150 m	Birstein (1963); Kaiser unpubl. data
Nannoniscidae	*Nannoniscus brenkei* Kaiser, Brix & Jennings, 2021*	BGR CA, CCFZ, 4093–4136 m	Kaiser et al. (2021)
Nannoniscidae	*Nannoniscus hilario* Kaiser & Kihara, 2021*	BGR CA, CCFZ, 4093–4259 m	Kaiser et al. (2021)
Nannoniscidae	*Nannoniscus magdae* Kaiser, Brix & Jennings, 2021*	French CA, CCFZ, 5017–5024 m	Kaiser et al. (2021)
Nannoniscidae	*Nannoniscus menoti* Kaiser, Janssen & Mohrbeck, 2021*	French CA, CCFZ, 4076–5024 m	Kaiser et al. (2021)
Nannoniscidae	*Nannoniscus pedro* Kaiser, Brix & Kihara, 2021*	GSR CA, CCFZ, 4093–5024 m	Kaiser et al. (2021)
Macrostylidae	*Macrostylis metallicola* Riehl & De Smet, 2020*	GSR CA, CCFZ, 4505 m	Riehl and De Smet (2020)
Munnopsidae	*Acanthocope galatheae* Wolff, 1962	Gulf of Panama, NE Pacific, 3270–3670 m	Wolff 1962; Brix et al. (2020)
Munnopsidae	*Pirinectes martinezi* Malyutina & Brix, 2020*	BGR CA, CCFZ, 4122 m	Malyutina et al. (2020)
Munnopsidae	*Pirinectes osbornae* Malyutina & Brix, 2020*	French CA, CCFZ, 4946–5019 m	Malyutina et al. (2020)
Munnopsidae	*Rectisura slavai* Malyutina, 2011*	Russian CA, CCFZ, 4742–4821 m	Malyutina (2011)
Munnopsidae	*Storthyngura yuzhmorgeo* Malyutina, 2011*	Russian CA, CCFZ, 4809–4821	Malyutina (2011)
Munnopsidae	*Munneurycope nodifrons* (Hansen, 1916)	North Atlantic, 2702–7000 m	Brix et al. (2020)
Munnopsidae	*Eurycope longiflagrata* Wilson, 1983	NW Atlantic, 2178–2469 m	Brix et al. (2020)
Urstylidae	*Urstylis solicopia* Riehl, Wilson & Malyutina, 2014b*	Russian CA, CCFZ, 4750–5031	Riehl et al. (2014b)

families in epibenthic sledge samples, whereas Nannoniscidae, Macrostylidae and Thambematidae, representing more infaunal taxa, are more prevalent in box corer samples (Figs. 4 and 5, see also Thistle and Wilson 1996; Janssen et al. 2015; De Smet et al. 2017; Yu et al. 2018; Brix et al. 2020; Pasotti et al. 2021). In addition, typically fewer families are found in box corer samples compared to the epibenthic sledge (Fig. 4). On the one hand, this could be related to a gear-dependent bias, in which epibenthic taxa are swept away by a large bow-wave effect when the box corer hits the seafloor (e.g. Lampitt et al. 1986; Pasotti et al. 2021). On the other hand, the patchy distribution of many deep-sea families may play a role, while others are only rarely encountered overall (e.g. families Janirellidae or Katianiridae, cf. Kaiser et al. 2007). As the sample size from the box corer is much larger than that of the epibenthic sledge, many individuals are simply not collected. Thus, since epibenthic sledge and box corer collect species with different sediment associations (more epifaunal vs. more infaunal), together with the above-mentioned sampling specificities of the individual devices, the requirement for simultaneous use of both arises in order to record the diversity of the CCFZ (isopod) fauna more completely.

Remarkably, there is a discrepancy between the family composition of the analysed samples and the number or datasets per family registered in the DeepData database, where most of the records were assigned to the Desmosomatidae, whereas the Munnopsidae are only rarely represented (Figs. 2a and 4). In any case, the database only contains data discriminated to the family level for a relatively small proportion of the isopods (~20%). In addition, the data are geographically very unevenly distributed. By far, the majority of the data comes from the eastern contract areas, such as BGR, whereas hardly any data are available from more westerly located sites (Fig. 2b), which could distort perceived patterns and their interpretation.

Chuar et al. (2020) analysed family-level composition in box corer samples within the OMS contract area and found

Fig. 3 Representative isopod families found in CCFZ collections: **a** Desmosomatidae, *Prochelator* sp. stet.; **b** Mesosignidae, *Mesosignum* sp. stet.; **c** Dendrotionidae, *Dendrotion* sp. stet.; **d** Janirellidae sp. stet.; **e** Haploniscidae sp. stet.; **f** family incertae sedis, *Sugoniscus* cf. *parasitus* Menzies & George, 1972; **g** Munnopsidae, *Eurycope* sp. stet.; **h** Nannoniscidae, *Nannoniscus pedro* Kaiser, Brix & Kihara, 2021; **i** Nannoniscidae, *Ketosoma ruehlemanni* Kaiser & Janssen, 2018; **j** Macrostylidae, *Macrostylis* sp. stet. Images courtesy of A. Janssen, S. Kaiser; B. Wawrzyniak-Wydrowska
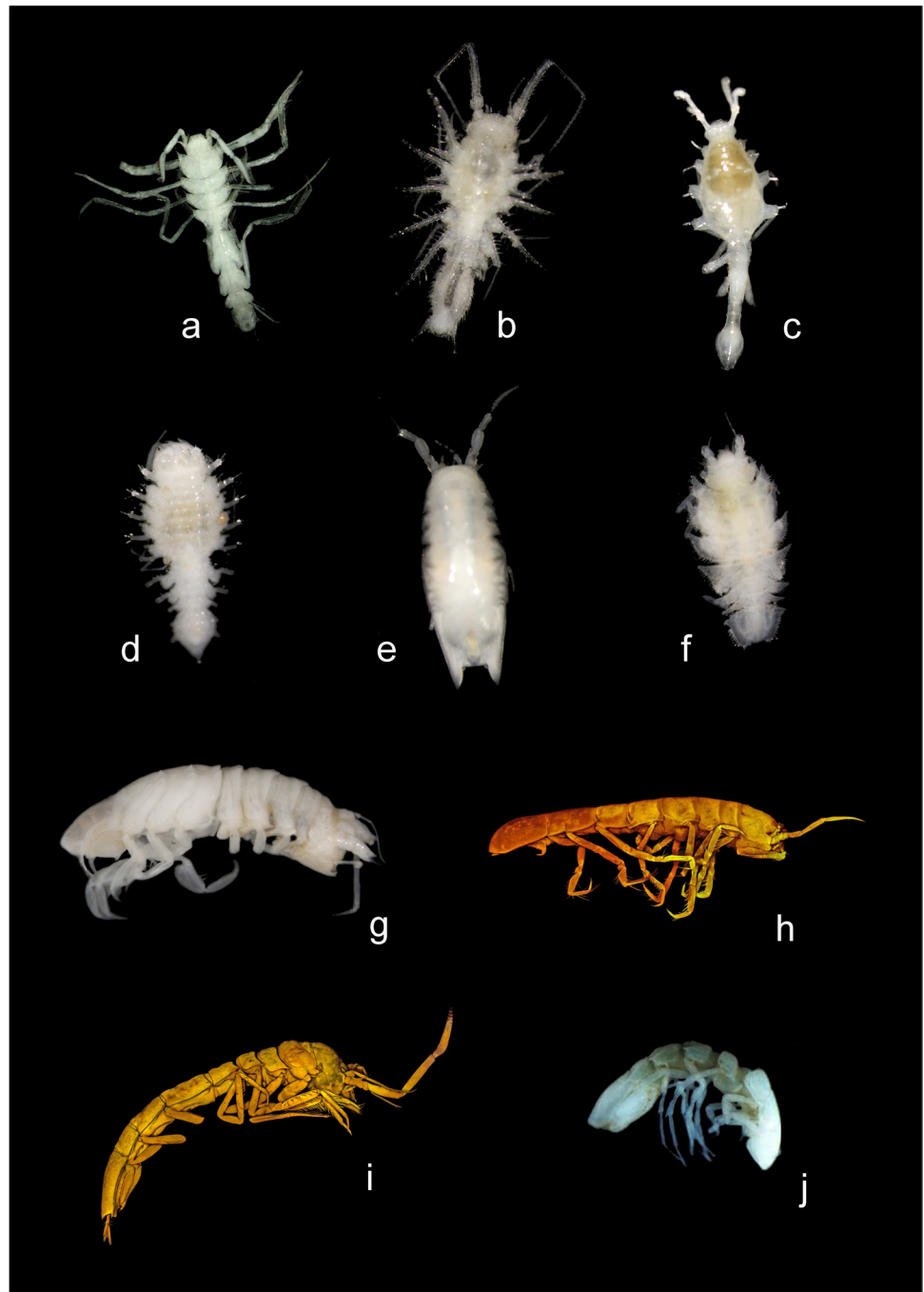
that family composition of isopods differs between stations. Given the small spatial scale of the study, this finding is quite remarkable. We examined the composition of isopod families collected by the epibenthic sledge from a much larger geographic area spanning four contract areas and APEI#3 (Fig. 6). The analysis of these data also revealed a significant variation in family composition (one-way ANOSIM; R: 0.284, $p = 0.0182$, n permutations: 999), but the low global R-value suggests a high faunal overlap between areas. In fact, significant differences could only be demonstrated between the BGR contract area and APEI#3, while there were no differences in family composition between the other areas (Fig. 6). This lack of spatial differentiation at the family level is not uncommon; many isopod families found in the abyss have broad geographic distributions and are found "everywhere", albeit in varying proportions (Brandt et al. 2007; Meyer-Löbbecke et al. 2014; Elsner et al. 2015). In addition, most isopods collected from the abyssal CCFZ consume food items of low nutritional value, so that a distinct spatial structure, such as different distribution patterns

Fig. 4 Relative abundances of isopods in epibenthic sledge vs. box corer samples; samples were collected during the JPIO MiningImpact SO239 expedition (Martínez Arbizu and Haeckel 2015). APEI, Area of Particular Environmental Interest; BGR, eastern German contract area; GSR, Global Sea Mineral Resources NV (Belgian contract area); IFREMER, L'Institut Français de Recherche pour l'Exploitation de la Mer (French contract area); IOM, Interoceanmetal Joint Organization contract area. Corresponding raw data in Tables S3 and S4 in the electronic supplement
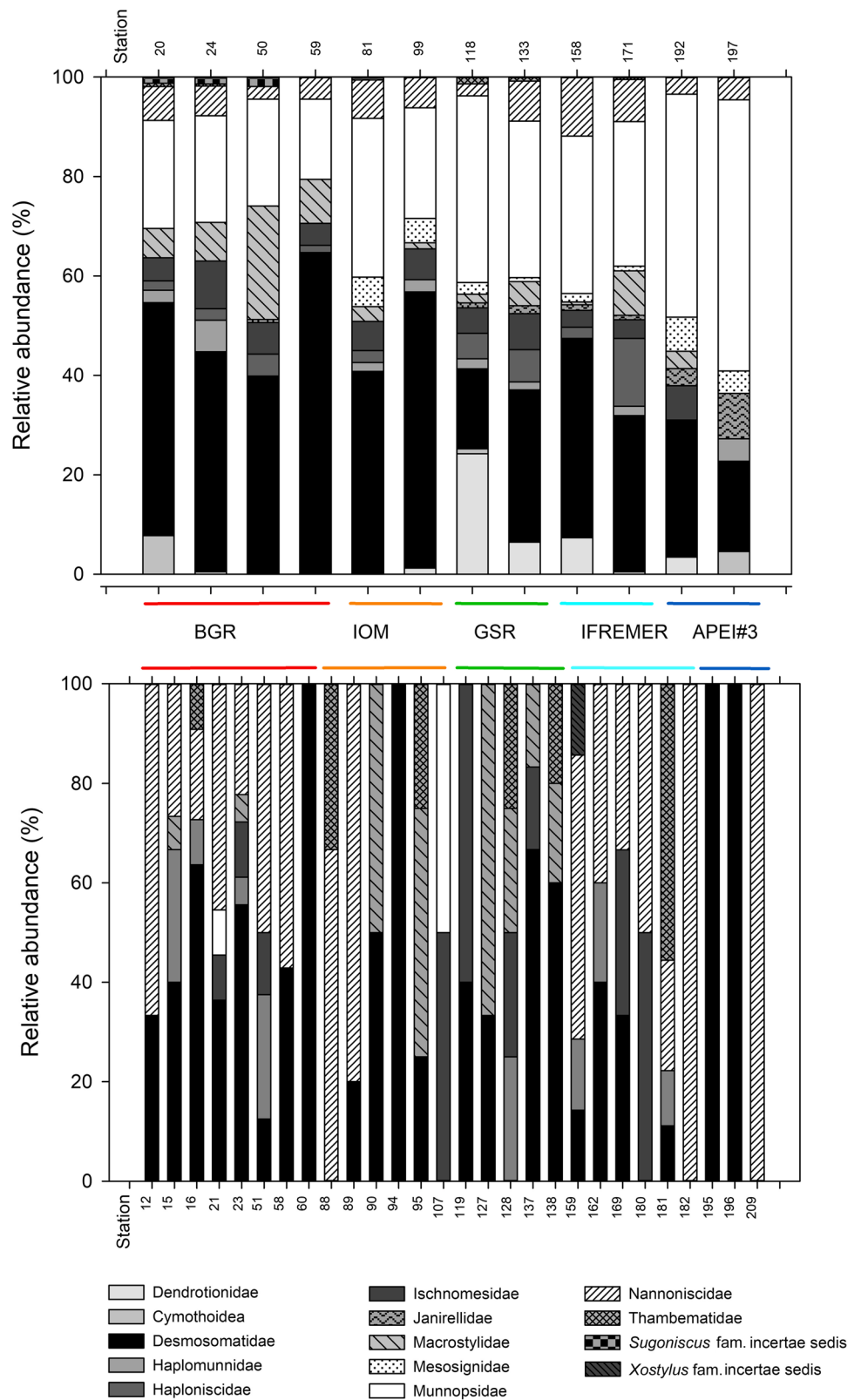
of guilds (e.g. carnivorous vs. deposit feeding families) linked to regionally different inputs of particulate organic carbon (POC) across the CCFZ, as seen in polychaetes (e.g. Bonifacio et al. 2020; Washburn et al. 2021b), was not expected for isopods. However, it is possible that as geographic scale increases, such as when comparing different
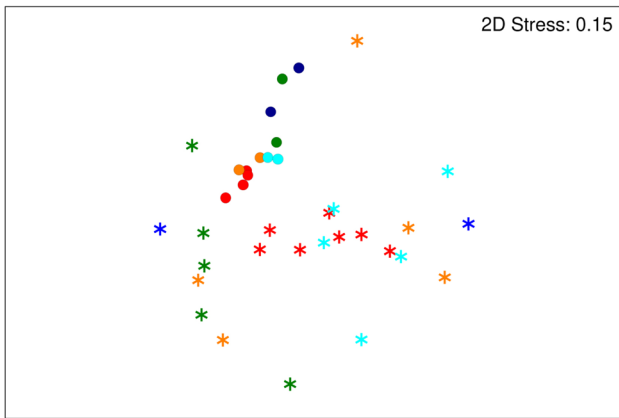
Fig. 5 Nonmetric multidimensional scaling (nMDS) ordination based on a Bray–Curtis resemblance matrix using relative abundances (%), showing differences in isopod family composition between epibenthic sledge (circles) and box corer (asterisks) samples collected during the SO239 expedition. Red: BGR; orange: IOM; green: GSR; turquoise: IFREMER; blue: APEI#3. For interpretation of the references to colour in this figure legend, the reader is referred to the online version of this article
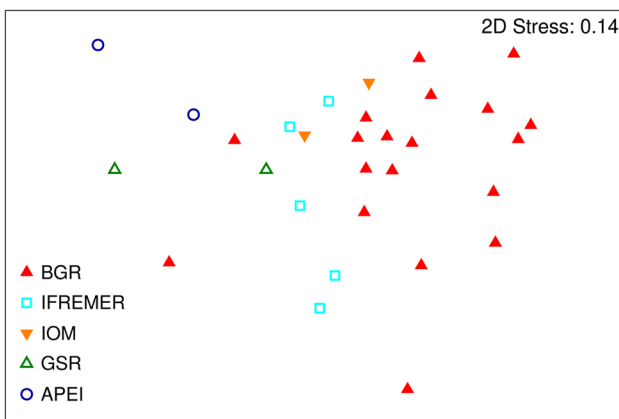
Fig. 6 Nonmetric multidimensional scaling (nMDS) ordination based on a Bray–Curtis resemblance matrix using relative abundances (%), illustrating similarity of isopod family composition between different CCFZ contract areas and APEI#3 (data from SO205, BIONOD, MANGAN 2014 and SO239 expeditions: S. Schnurr, unpubl. data, Kaiser et al. 2015, unpubl. data). Red: BGR; orange: IOM; green: GSR; turquoise: IFREMER; blue: APEI#3. For interpretation of the references to colour in this figure legend, the reader is referred to the online version of this article

ocean basins, we may see a distinct CCFZ community at the family level (Kaiser et al. unpubl. data, see also Singh et al. 2016 for meiofaunal Nematoda).

At enhanced taxonomic resolution, differences between communities became more apparent. Brix et al. (2020) analysed the MOTU composition of four dominant isopod families (Munnopsidae, Desmosomatidae, Macrostylidae and Haploniscidae) in the CCFZ, APEI#3 and the DISCOL

area, with their data representing a subset of our dataset. Based on relative abundance data, they found CCFZ contract areas to be more similar to each other than to APEI#3 or the distantly located DISCOL area (Brix et al. 2020). On the other hand, presence/absence of data revealed high dissimilarity also between CCFZ sites, driven by the high proportion of unique site-endemic species (Brix et al. 2020). Notably, more species were shared between CCFZ contract areas and the DISCOL area more than 5000 km away than with the much closer APEI#3.

The analysis presented here is an extension of datasets from Janssen et al. (2015) and Brix et al. (2020). Overall, there appears to be a decay in isopod similarity with increasing distance, i.e. we found greater similarity between UK-1, OMS, BGR, IOM and GSR contract areas clustering more closely together, while APEI#3 and DISCOL were more dissimilar and the IFREMER contract area taking an intermediate position (Fig. 7a). Low faunal similarity (0–7.7% Bray–Curtis similarity) between APEI#3 and the contract areas indicates that APEI#3 is not fully representative for the isopod biodiversity in the CCFZ—at least based on the current results and for the
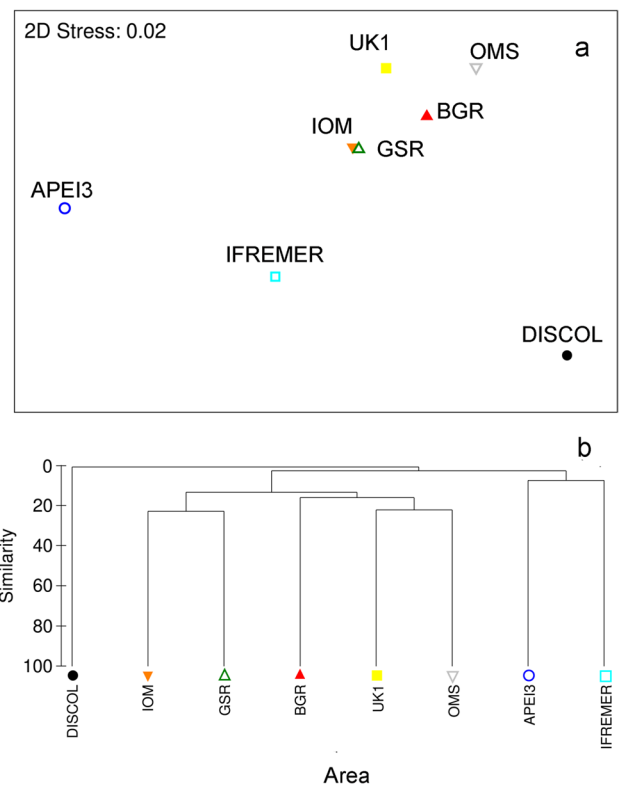
Fig. 7 Nonmetric multidimensional scaling (nMDS) ordination (**a**) and hierarchical cluster analysis (**b**) of transformed (relative abundance) epibenthic sledge data based on a Bray–Curtis similarity matrix for CCFZ isopods at the MOTU level. Red: BGR; yellow: UK-1; grey: OMS; orange: IOM; green: GSR; turquoise: IFREMER; blue: APEI#3; black: DISCOL

contract areas studied. This is consistent with patterns in other taxa suggesting that the faunal composition of APEIs is different from that of CCFZ contract areas (Taboada et al. 2018; Błażewicz et al. 2019; Bonifacio et al. 2020; Brix et al. 2020; Christodoulou et al. 2020; Washburn et al. 2021a, b). This generally casts doubt on the representativeness of the APEIs studied so far as potential faunal sanctuaries for recolonisation. Analyses of the newly defined APEIs will be required to test whether they are more representative of the fauna in the potential mining areas of the CCFZ.

Last but not least, we were able to analyse differences in the MOTU composition between two different areas (PRZ and IRZ) within the BGR contract area. These data allowed us to examine changes in the community over a 3-year period. In addition, valuable data on the representativeness of the PRZ as a protection zone within a contract area could be collected. The results of our time series analysis showed significant differences between IRZ and PRZ (one-way ANOSIM; R: 0.889, $p = 0.029$). However, it also became clear that the communities in each area were not static but varied over the study period (Fig. 8). From this, changes in the sampling strategy can be derived, such as the spatial and temporal intervals of sampling, which will be discussed further below. The strong differences we observed between the IRZ and the PRZ suggest that while the PRZ isopod fauna does partly overlap with the IRZ fauna (i.e. a number of species are shared by both areas), it does not suit its function as a similar control site. It would also not fulfil a complete protective function for the potential recolonisation of the impacted mining site.
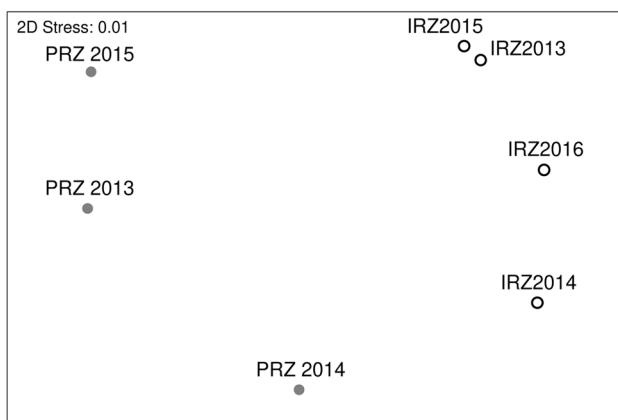
Abundance

Large-scale comparisons of isopod densities (ind. per m^2) obtained with box corer show large differences between and within contract areas (Fig. 9). However, there is a slight decline in densities from more easterly to more westerly located contract areas across the CCFZ (De Smet et al. 2017; Wilson 2017; Chuar et al. 2020; Fig. 9), which appears to be linked to changes in primary productivity (Washburn et al. 2021b). This is the case with other taxa as well (Glover et al. 2002; Wilson 2017; De Smet et al. 2017; Bonifacio et al. 2020; Washburn et al. 2021b). For instance, isopod densities in the OMS contract area in the eastern part of the CCFZ are in the range of 78.91 ± 42.89 (mean $\pm$ SD), compared to 27.02 (mean) ind. per m^2 at DOMES A in the western part of the CCFZ (Wilson 2017; Chuar et al. 2020). PRA sites in the central part of the CCFZ, with 82.25 isopods ind. per m^2 (mean), show values similar to those of the eastern OMS contract area, but significantly higher values than the central Echo 1 (41.07 ind. per m^2) and GSR (15–18 ind. per m^2) areas. This could be associated with a temporarily higher POC flux during the time of sampling (De Smet et al. 2017; Wilson 2017; Chuar et al. 2020; Fig. 9). It should be noted that there is not enough information from the western CCFZ, and more data should be collected in order to adequately assess density differences along latitudinal and longitudinal gradients (see also Washburn et al. 2021b).

In addition to food availability, a number of other environmental parameters could play a role in determining the abundance of isopods in the CCFZ. In contrast to some other taxa (e.g. sessile megafauna, Vanreusel et al. 2016), however, no clear relationship could so far be established
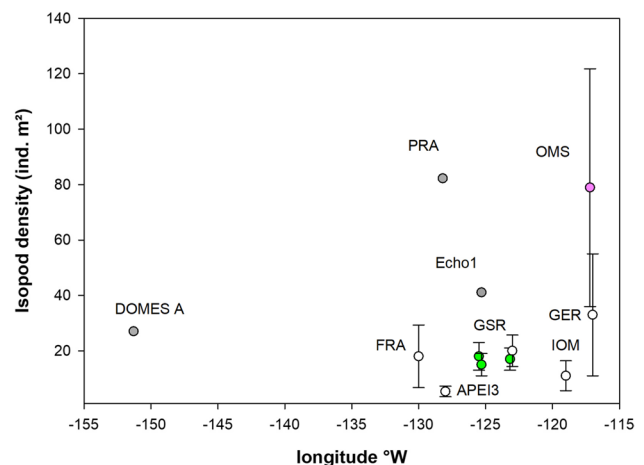

Fig. 8 Nonmetric multidimensional scaling (nMDS) ordination for CCFZ isopod assemblages based on a Bray–Curtis similarity (relative abundance) matrix, reflecting differences in isopod MOTU composition between the BGR Preservation Reference Zone (PRZ) and Impact Reference Zone (IRZ) over a period of 3 years (2013–2016). For PRZ, no sequences are available from 2016

Fig. 9 Comparison of isopod densities (ind. per m.^{2}) between different contract areas and APEI#3 along a longitudinal gradient, as retrieved from box corer samples (green from De Smet et al. 2017; grey: Wilson 2017; pink: Chuar et al. 2020; white: Washburn et al. 2021b)

between isopod and nodule densities (Pasotti et al. 2021; Washburn et al. 2021b). Washburn et al. (2021b) argued that the weak relationship between nodule and isopod densities in their study could be due to the use of regional models for nodule abundance estimation; nodule densities in the CCFZ are quite heterogeneous and can vary greatly within a few tens to hundreds of metres (Peukert et al. 2018). This small-scale variation has not been included in the models and therefore may not correspond to the degree of biological variation (Washburn et al. 2021b). In the study by Pasotti et al. (2021), however, nodule abundance was derived from in situ box corer collections and could therefore be directly related to isopod densities. The lack of a significant relationship between these two variables implies that it is arguably not an artefact.

Depth differences have often proven to be an important factor determining macrofaunal abundances in the deep sea (e.g. Rex et al. 2006), and thus, depth-related factors may also explain the variation in isopod densities in the CCFZ. From the eastern (e.g. BGR, UKSRL, OMS) to the more centrally located contract areas (e.g. IFREMER), depth gradually increases by over 1000 m, and this difference in depth appears, if only slightly, to be reflected in isopod abundances (Washburn et al. 2021b). Yet, because depth changes along a longitudinal gradient as does surface productivity, it can be difficult to disentangle these effects from one another.

Overall, it has become clear that the influence of environmental factors on faunal communities is largely masked by sampling effects. Washburn et al. (2021b), for instance, analysed a large dataset consisting of samples from > 400 box corers across the CCFZ and found that ~36% of the variation in isopod abundance was explained by "random" study-related effects. Thus, differences in sampling efficiency due to the use of different sampling protocols among studies can also have a profound impact on the observed abundance patterns.

Diversity

Patterns of local and regional isopod diversity have been investigated by morphological and molecular means (Thistle and Wilson 1996; Janssen et al. 2015; De Smet et al. 2017; Wilson 2017; Brix et al. 2020; Pasotti et al. 2021; Washburn et al. 2021b). The main objectives of these studies were to compare isopod diversity between different contract areas and APEIs, to identify the primary factors influencing diversity, as well as to assess the representativeness of diversity data at the regional scale (Janssen et al. 2015; De Smet et al. 2017; Brix et al. 2020; Washburn et al. 2021b). As described in the previous section, the comparison of isopod diversity between studies is hampered by differences in existing sampling equipment and protocols (see also Washburn et al. 2021b). Other problems emerged from the

use of different (molecular vs. morphological) approaches; as previously mentioned, morphological assessment tends to underestimate actual species richness (cf. Janssen et al. 2015), while the use of molecular mitochondrial markers typically overestimates species numbers (Song et al. 2008; Dietz et al. 2015; Ribardière et al. 2017). Finally, a meaningful regional comparison can only be made when all species accumulation curves have attained an asymptote (Gotelli and Colwell 2001), which has not yet been achieved for CCFZ isopods in any study (De Smet et al. 2017; Wilson 2017; Brix et al. 2020; Pasotti et al. 2021; Washburn et al. 2021b).

Even within contract areas, local isopod diversity can show large variation. For example, Thistle and Wilson (1996) reported that local richness of isopods from box corer samples in the central part of the CCFZ (Echo 1) varied between 2 and 20 species (mean ± SD; 8.4 ± 5.0 species; 15 box corer deployments). Isopod richness in the close-by GSR area appears to be considerably lower, ranging between 2.00 ± 0.01 and 3.7 ± 0.7 isopod species per box corer (De Smet et al. 2017; Pasotti et al. 2021). Our time series data from the BGR contract area confirm the high spatial but also temporal variation in isopod diversity. That is, we found large differences in diversity between relatively close areas within the BGR contract area (PRZ and IRZ), as well as strong temporal variability within each area (Fig. 10). Differences in sediment parameters and nodule coverage may play a role in explaining spatial diversity variation at the local scale. Yu et al. (2018), analysing macrofauna (incl. isopods) from the contract area of the Korean Republic, for example, found higher diversity to be linked to higher nodule coverage. By contrast, Pasotti et al. (2021) tested the effect of nodule densities on local diversity patterns and did not find a significant relationship. Episodic food input
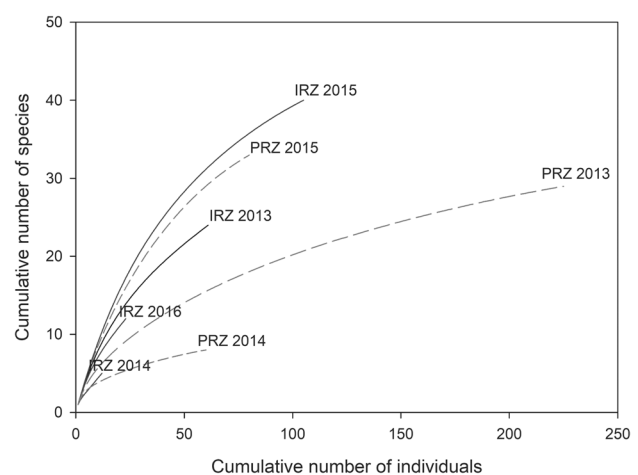

Fig. 10 Individual-based rarefaction curves of cumulative number of CCFZ isopod species (MOTUs), visualising differences in diversity between the Preservation Reference Zone (PRZ) and Impact Reference Zone (IRZ) over time (2013–2016)

from surface waters and changes in hydrographic conditions (e.g. erosive currents, cf. Thistle and Wilson 1987, 1996; Levin et al. 2001) are probably explanatory parameters for temporal differences in diversity. Overall, it has been shown that abyssal areas can be very dynamic on relatively small spatial and temporal scales (Kaiser et al. 2007; Kuhnz et al. 2014; Taylor et al. 2017). However, better understanding of natural spatial and temporal trends is important to distinguish background variability from the impact of deep-sea mining (Amon et al. 2022). One-off or short-term studies are therefore not sufficient to capture the natural variability of abyssal communities (e.g. Kuhnz et al. 2014) and this should be considered in future investigations.

To date, the studies by Janssen et al. (2015), Wilson (2017), Brix et al. (2020) and Washburn et al. (2021b) are the most spatially extensive (i.e. comparing isopod diversity between different nodule areas). Janssen et al. (2015) and Brix et al. (2020) investigated differences in isopod diversity based on epibenthic sledge samples using an integrative (morphological/molecular) approach, while the data in Wilson (2017) and Washburn et al. (2021b) derive from box corers and relate to the morpho-species level. Assessing differences in isopod and polychaete diversity between the eastern BGR and the IFREMER contract area was one focus of the study by Janssen et al. (2015). In total, they could distinguish 95 MOTUs within seven isopod families, whereby significant differences between the BGR and IFREMER contract areas did not become apparent. Brix et al. (2020) looked at only four isopod families but from a much wider geographic area. In their study, a total of 187 species from 22 epibenthic sledge operations were genetically differentiated and morphologically confirmed. As in Janssen et al.'s (2015) study, they did not find much of a difference between contract areas. Janssen et al. (2015) estimated (based on Chao 1, Chao 2 and Jackknife1) that between 55.6 and 62.7% (Chao 1), 56.1 and 71.1% (Chao 2) and 37.9 and 39% (Jackknife1) of the species from the BGR and IFREMER contract areas have not yet been identified (values reflect numbers for isopods and polychaetes combined). Our results of species estimates of the extended isopod dataset using Chao 1 show that only between 23.6 (UK-1) and 70.0% (BGR) of expected species have been collected so far (Fig. 11). In contrast to the two studies mentioned above, we found a clear difference in diversity between different regions, with the BGR, UK-1 and OMS contract areas showing the lowest diversity, compared to the IFREMER contract area yielding highest species diversity, while the remaining had intermediate diversity levels (Figs. 11 and 12). The low observed diversity of OMS and UK-1 is probably due to the lower sample size overall, and some groups, which are usually very species-rich (e.g. Munnopsidae), were underrepresented in the dataset (Table S2 in the online supplement). Considering contract areas only (BGR, UK-1, OMS, IOM, GSR
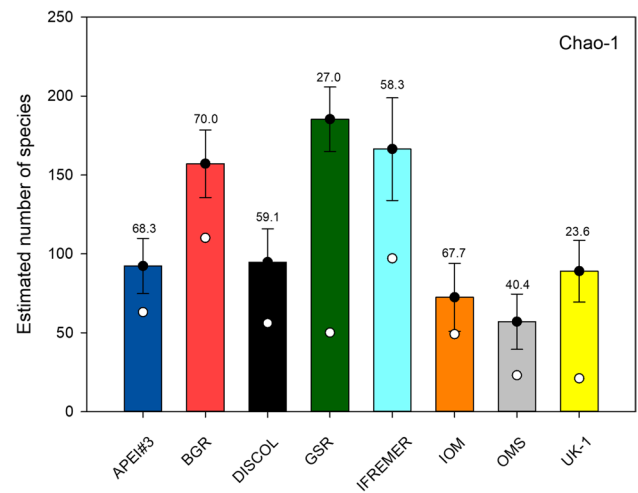
Fig. 11 Comparison of Chao 1 richness estimations ($\pm$ SD) for isopods between different contract areas, APEI#3 and the DISCOL area. Isopod species were defined based on molecular SD methods and partially verified by morphological studies. White dot: number of observed species. Numbers above the bar indicate the estimated proportion of species recovered from the respective area so far
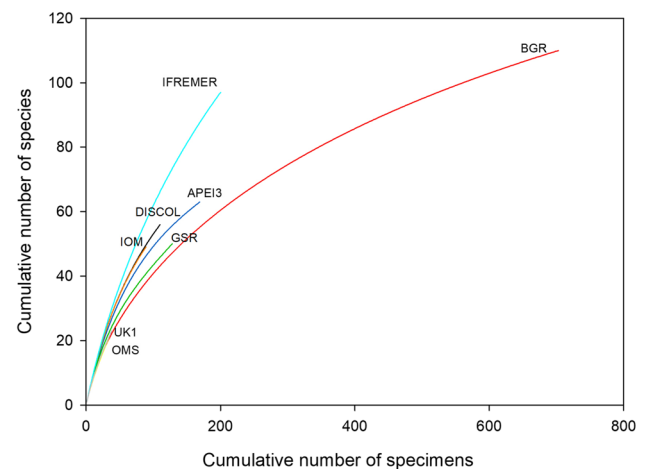
Fig. 12 Individual-based rarefaction curves of cumulative number of CCFZ isopod species (MOTUs) per area collected by an epibenthic sledge; red: BGR; orange: DISCOL: black; IOM; green: GSR; turquoise: IFREMER; OMS: grey; UK-1: yellow; blue: APEI#3. For interpretation of the references to colour in this figure legend, the reader is referred to the online version of this article

and IFREMER), estimates range from 309 to 569 $\pm$ 72 species (Chao 2: 569 $\pm$ 72, Jackknife 1: 394, Jackknife 2: 492, Bootstrap: 309); that is, the estimated percentage of species recovered from the area was between 43.1 (Chao 2) and 79.3% (Bootstrap) of the total observed richness containing 245 MOTUs. In comparison, Brix et al. (2020) estimated (based on Chao 1 and ACE) that between 80.1 and 85.4% of the isopod species have been sampled (Brix et al. 2020, considering BGR, IOM, GSR and IFREMER contract areas

combined). Species accumulation curves do not reach an asymptote in any of the studied regions (Fig. 12), and estimates for species richness exceed the observed values in all cases. This, together with the assumption that richness estimators are very sensitive to small numbers and unique occurrences of species (De Smet et al. 2017; Pasotti et al. 2021), indicates that isopod diversity in the CCFZ is still underestimated.

Wilson (2017) and Washburn et al. (2021b) also found considerable differences in isopod diversity between areas and/or studies. Wilson (2017) examined the diversity of isopods, polychaetes and tanaidaceans in the DOMES, PRA and Echo 1 areas, which show large differences in surface productivity. Notably, his results showed a positive correlation between POC flux and diversity for tanaidaceans and polychaetes, while isopod diversity was negatively correlated with POC input. Despite considering a larger dataset than Wilson's study from a wider productivity spectrum, Washburn et al. (2021b) could not identify any relationships between isopod diversity and productivity. Indeed, differences in isopod diversity could not be associated with any of the parameters studied, suggesting that other factors, not yet assessed, play a more important role (Washburn et al. 2021b). The inconsistency of results between studies clearly warrants further investigation. However, they all agree on one point; significantly more samples are required to representatively record isopod diversity.

Species geographical distribution, range size and potential drivers

In order to assess the fauna's potential to recolonise impacted (mining) areas from pristine, protected areas in or bordering the CCFZ (APEIs) or from within the exploration contract areas themselves (e.g. PRZs), knowledge of species range size and its main drivers is required. This relates to the distances that species travel and whether migrations are directional (cf. Taboada et al. 2018). The use of molecular methods in conjunction with the morphological examination of isopod specimens has helped to achieve great advances in delimiting species more robustly and thus better defining their distributional ranges (Janssen et al. 2015, 2019; Brix et al. 2020; Kaiser et al. 2021). Isopod data from the studies presented here indicate that most species appear to have very restricted distributions, limited to a single contract area or even a single sampling station (e.g. Janssen et al. 2015; Brix et al. 2020; Kaiser et al. 2021). Based on molecular-genetic analyses, the assumed widespread distribution of some morpho-species was refuted, revealing that these instead represent morphologically similar or even identical, but genetically different species (Janssen et al. 2015; Kaiser et al. 2021). Notably, some of them were found in close proximity or even sympatrically (i.e. at the same station;

Janssen et al. 2015; Kaiser et al. 2021). Furthermore, a high proportion of species were represented by only one or two specimens (termed singletons and doubletons, respectively). For example, Janssen et al. (2015) found that 70% of isopod MOTUs were singletons and are therefore defined as rare until further specimens can be encountered. In comparison, the study by Brix et al. (2020) found that the distribution of 77% of species was restricted to one contract area, and around one-third of these species were represented by only one individual. In our study, which contains significantly more data, 76% of the MOTUs have been collected from a single contract area, and 41.3% are singletons (Fig. 13; Table S2). This presumably high level of locally restricted or "endemic" species results in high turnover rates for isopods in the CCFZ (Janssen et al. 2015; Wilson 2017; Brix et al. 2020; Washburn et al. 2021b). However, it is important to note that the lack of knowledge may lead to the declaration of false endemics and that species may actually have a much wider geographic distribution. Nevertheless, a large proportion of the described species also have small geographical distribution areas and are overall scarce or reveal low numbers of individuals. It is thus likely that most of the hitherto undiscovered species may be rare too (Pimm et al. 2014).

The pattern of small range size and suspected rarity has been contrasted by some isopod species being distributed over large geographical areas within the CCFZ and beyond (Janssen et al. 2015; Brix et al. 2020; Kaiser et al. 2021). For instance, Janssen et al. (2015) found two isopod species to be shared between the BGR and IFREMER contract areas about 1300 km apart. Kaiser et al. (2021) found two species within the family Nannoniscidae to be widespread with haplotypes occurring up to 1400 km apart. Brix et al. (2020) recorded 23% of species occurring in two or more contract areas, consistent with our results. Remarkably, there were seven species that were shared between CCFZ contract areas and the DISCOL area, i.e. about 5000 km apart (Fig. 13). The broadest morphologically and genetically confirmed distribution has been proven for *Acanthocope galatheae* with known records from the Atlantic and central Pacific oceans (Bober et al. 2018; Brix et al. 2020; S. Bober pers. communication).

It is not clear which mechanisms underlie the observed distribution patterns, but it does not appear to be unique to isopods or the CCFZ. In deep-sea sediments in general, many species seem to be rare, because they were only found in one or two samples or in relatively small amounts (Grassle and Maciolek 1992; Rex and Etter 2010; McClain 2021). On the other hand, a broad distribution has been demonstrated in a range of deep-sea taxa (e.g. McClain & Hardy 2010; Janssen et al. 2015). It is the low sampling effort and the large distances between the samples hitherto taken from the CCFZ that make the evaluation of "true" geographical ranges and thus extent of rarity problematic (Pimm et al. 2014; Pasotti
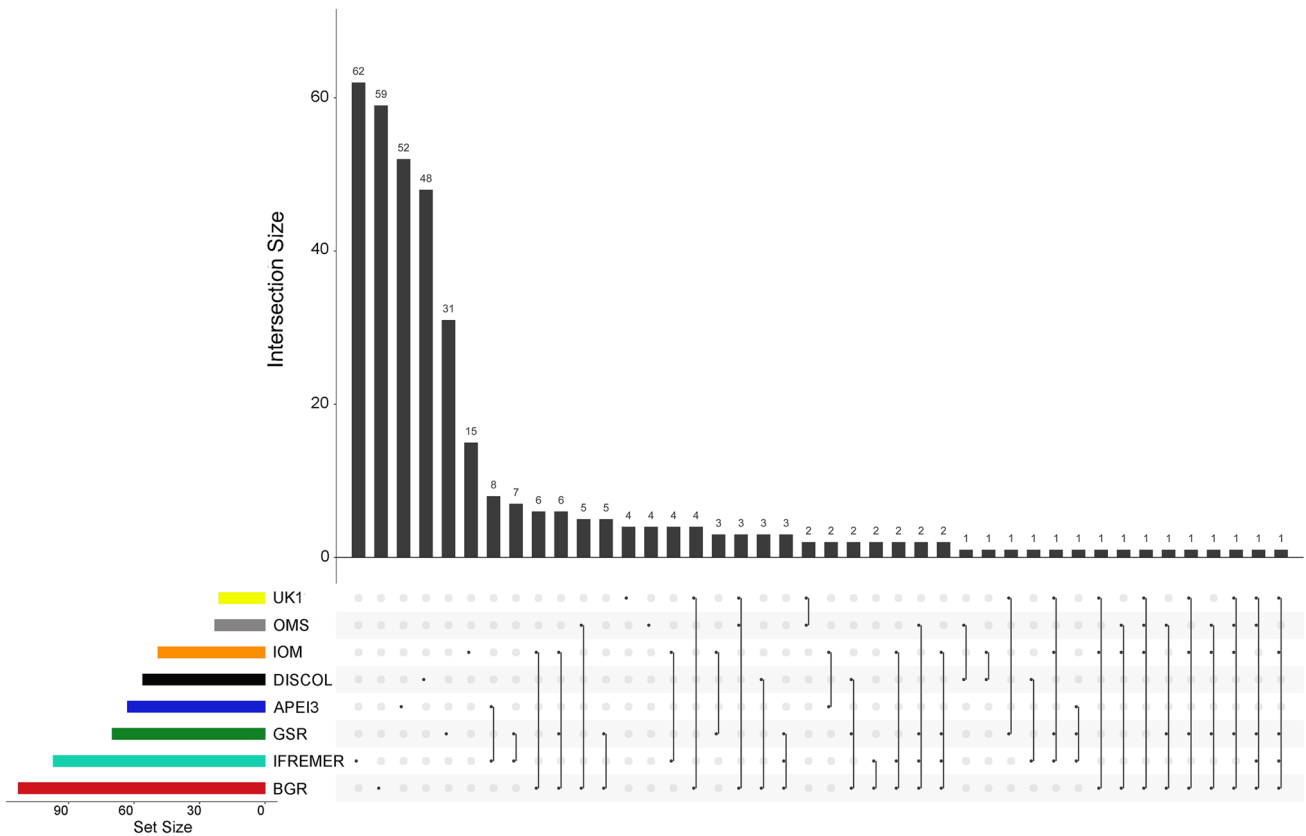
Fig. 13 UpSet plot visualising the number of species (resolved by molecular and partially morphological means) shared between different areas. The panel below shows the various possible combinations of the different areas. Species unique to an area are denoted by a dot (vertical bars 1–6), and the corresponding number above the bars indicates the number of unique species found in that area. Species shared between areas are indicated by dots connected by a vertical line, with the number of species co-occurring in each area shown above the vertical bar in the top panel. Created using the web tool: https://gehlenborglab.shinyapps.io/upsetr/ (Lex et al. 2014)

et al. 2021). The widespread distribution of some species of isopods in the CCFZ, on the other hand, appears to be real.

Due to their brooding way of life, the distribution of isopods is thought to be more limited than, for example, the distribution of broadcasting taxa (cf. Janssen et al. 2015; but see Lester et al. 2007). For CCFZ isopods, a strong influence of adult dispersal capacity on species spatial ranges could be ascertained (Brix et al. 2020). For example, the dispersal distances of the less motile Haploniscidae and Macrostylidae were ~ 1400 km compared to > 5000 km for the Munnopsidae, most of which are good swimmer and some of them have excellent swimming abilities (Brix et al. 2020). Several other factors, such as hydrodynamic or topographic conditions and distance between suitable habitats, have been discussed to play important roles in modulating isopod distributions in the CCFZ (Lester et al. 2007; Janssen et al. 2019; Brix et al. 2020; Kaiser et al. 2021). Near-bottom current velocities are fairly low on average but are considered strong enough to allow isopod species to disperse (Janssen et al. 2019). On the other hand, topographical features such as depressions or seamounts

can hinder but also facilitate current flow, or the presence of strong oceanic fronts can become a barrier between neighbouring populations and thus impair genetic exchange (Taboada et al. 2018; Janssen et al. 2019). The latter could, inter alia, provide one explanation for population and/or species divergence that was found to occur in isopods on fairly small spatial scales (Janssen et al. 2019; Kaiser et al. 2021). Against the background of the assessment of species ranges, we conclude that species with a good ability to spread have a greater potential to recolonise mined areas, provided that environmental conditions of the affected habitats are restored and that populations of these species thrive in nearby protected areas. However, since the vast majority of isopod species are presumably restricted in their distribution, their diversity could be at risk. To what extent this distribution pattern is real, however, urgently requires further research—with more standardised sampling and analysis needed at multiple spatial scales (i.e. relative to key environmental factors from fine scale [tens of m], local scale [tens of km], to regional scale [hundreds of km], see Simon-Lledó et al. 2020).

Bridging the gaps

The CCFZ has become one of the biologically better-known abyssal areas, although the high proportion of new species (> 90%), but also supra-specific taxa that are still being discovered, emphasises the poor knowledge on diversity and distribution ranges at low taxonomic levels that still exists (e.g. Glover et al. 2018). Despite an ever-increasing number of studies on the CCFZ fauna in the past decade, there are still significant gaps in knowledge and data that do not allow us to make reliable predictions of the potential fauna responses to seabed mining (Hunter et al. 2018; Miller et al. 2018; Amon et al. 2022). After polychaetes, isopods are probably the best-studied macrofaunal taxon in the CCFZ, but taxonomic efforts to describe the isopod fauna in this region have been remarkably poor. It is noteworthy that it was not until 2011 that a first description of an isopod species from the area was published (Malyutina 2011). Ongoing efforts have now led to descriptions of 19 species (Malyutina 2011; Kaiser 2014; Riehl et al. 2014b; Kaiser et al. 2018, 2021; Malyutina et al. 2020; Riehl and De Smet 2020), three genera (Riehl et al. 2014b; Kaiser et al. 2018; Malyutina et al. 2020) and one family (Riehl et al. 2014b) new to science (summarised in Table 2). However, diversity estimates suggest that this is only a minor fraction of the isopod species that actually live there (Janssen et al. 2015; Brix et al. 2020, this study).

Overall, our study shows that knowledge on CCFZ isopods is fragmented and limited to certain contract areas, supra-specific taxa or subsets of fauna. This makes a meaningful comparison between studies and datasets difficult (cf. Washburn et al. 2021b; Fig. 2, Tables 1 and 3). Nevertheless, some trends could be observed, for example that

isopod densities appear to be higher in the eastern contract areas than in the more westerly located areas. However, this is not reflected in diversity patterns that show inconsistencies between the studies, and it has not yet been possible to clearly determine, which factors modulate these. There are indications of some widespread species, but the majority of species seems to be locally restricted. A combination of intrinsic (lifestyle) and extrinsic factors and processes, including hydrography, habitat heterogeneity and geographic distance, are likely to play a role in controlling the divergent distribution patterns of isopods. Most importantly, the CCFZ is still severely undersampled and efforts must be made to fill these sampling gaps.

The knowledge gaps identified for the better-known isopods are remarkable in view of the fact that there are a large number of previously neglected groups of organisms, especially among the smaller size fractions that need taxonomic attention (Glover et al. 2016). Furthermore, comparisons with other macrofaunal groups, particularly Polychaeta and Tanaidacea, indicate that one is not an adequate substitute for the other (e.g. Janssen et al. 2015; Wilson 2017; Blazewicz et al. 2019; Washburn et al. 2021b). Therefore, ideally, all of these groups need to be considered to understand how the benthic biodiversity in the CCFZ might be affected by deep-sea mining operations. The intensification of (integrative) taxonomic work, combining morphological, genetic and environmental information, should be promoted to advance knowledge on deep-sea species identities and thus diversity and distributional ranges. Additional samples are just as important as the exchange of samples, experience, know-how and data (accessibility of raw data) between contractors, researchers and research groups to close knowledge gaps and in this way refine our integrated knowledge of

Table 3 Estimating levels of knowledge for a number of indicators key to the assessment of CCFZ isopod biodiversity and distribution

Indicators	Knowledge level	Reference
Undescribed species	> 94%	Brix et al. (2020)
Taxonomic resolution	Heterogenous: (morpho-) species-, MOTU-, family-, order level	Compared with Table 1
Availability of taxonomic keys	For some groups (families, genera), scattered across the literature	e.g. Malyutina & Brandt (2006); Wilson (2008); Brix et al. (2015); Malyutina and Brandt (2018, 2020); Kaiser (2014); Kaiser et al. (2018, 2021)
Taxonomic inter-calibration	Morphological inter-calibration very limited, solely within individual contract areas or projects; comparison between projects only genetically and here only mtDNA (COI, 16S)	Hessler et al. (1979); Hessler and Wilson (1983); Janssen et al. (2015); Wilson (2017); Brix et al. (2020); Riehl & De Smet (2020); Pasotti et al. (2021); Washburn et al. (2021a, b); this study
Sampling	Quantitative, semi-quantitative and qualitative: BC, EBS and ROV	Martinez Arbizu & Haeckel (2015)
Sample coverage	Insufficient, more samples needed, e.g. from APEIs, western and central CCFZ	Brix et al. (2020); Kaiser et al. (2021); Washburn et al. (2021b)
Molecular data	Intermediate: genetic data (COI) from > 10 to 70% of MOTUs being present*	Janssen et al. (2015, 2019); Kaiser et al. (2018, 2021); Brix et al. (2020); this study

*Compared to number of species derived from richness estimators

distribution patterns in the CCFZ and globally. In addition, sampling design and methods should become standardised in terms of gear type, protocol, mesh size, sample processing and taxonomy in order to enable comparability between studies and to increase the efficiency of concerted scientific efforts and data management. The use of different types of gear (e.g. box corer and epibenthic sledge) is still to be encouraged as these complement each other and will ultimately provide a more comprehensive picture of the CCFZ (isopod) fauna.

This results in the following recommendations for baseline studies:

- Concomitant use of box corer and epibenthic sledge is recommended as one is not an adequate substitute for the other, but both contribute to a better assessment of macrofaunal diversity patterns.
- The high spatio-temporal variability observed in our isopod data requires an appropriately adjusted baseline sampling strategy, including sampling across a range of spatial and temporal scales.
- Efforts should be made to implement standardised sampling and sample processing protocols in order to increase the comparability and informative value of the data.
- Family-level identification is not sufficient for comparative or impact analyses—a species or MOTU level identification should be achieved. The latter requires taxonomic expertise, which must be guaranteed.
- Whenever possible, an integrative taxonomic approach should be followed that allows for more robust species identifications. In order to enable both morphological and molecular examination, careful sieving of the benthic material to remove residual sediments and immediate cooling and fixation in high-grade (96%) ethanol are required (Riehl et al. 2014a; Frutos et al. 2022).
- As elsewhere, most species collected from the abyssal CCFZ have not yet been described, which becomes problematic when comparing samples from different contract areas. To enhance taxonomic inter-calibrations, increased accessibility and query of sample collections and data is a mandatory requirement. Data availability via the ISA DeepData database should therefore be promoted, and DNA barcode reference libraries be expanded.
- Thorough taxonomic analysis requires time. This is in conflict with ongoing efforts to accelerate biodiversity assessment given the anticipated start of mining activities in the near future. Hence, new methods should be tested, including eDNA, metabarcoding and -omic approaches, alongside more traditional methods to ensure the reliability of identification and delimitation of species (e.g. Lejzerowicz et al. 2021; Frutos et al. 2022; Kürzel et al. 2022).

- Biodiversity monitoring during exploration and future mining activities in the CCFZ is a long-term activity, spanning several decades of dedicated analysis. Therefore, ideas should be developed to ensure uniform and sustainable taxonomic expertise, for example in the form of workshops offering regular taxonomic inter-calibration exercises or training of the next generation of taxonomists.
- Most of the available faunal data derive from the central and eastern CCFZ, which leads to a somewhat distorted view of species distributions. Further sampling in the more western contract areas, but also between contract areas and certainly in APEIs, is thus needed to better assess species occurrences and their predicted responses to mining disturbances.
- Last but not least, assessing possible impacts of mining activities in the CCFZ requires better information about the species living there (Glover et al. 2018). Therefore, in line with the new ISA Sustainable Seabed Knowledge Initiative (https://www.isa.org.jm/sski), species discovery and description should be fostered in conjunction with the development of identification keys that form the basis for effective conservation management.

Declarations

Conflict of interest   The authors declare no competing interests.

Ethics approval   No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with unregulated invertebrate species.

Sampling and field studies   All necessary permits for sampling and observational field studies have been obtained by the authors from the competent authorities and are mentioned in the "Acknowledgements", if applicable.

Data availability   All faunistic data generated or analysed during this study are included in this published article, whereas COI sequences are deposited in GenBank (see electronic supplementary Table S2). The physical specimens that were not used for taxonomic descriptions are located either at the DZMB in Wilhelmshaven (contact: Pedro Martinez Arbizu) or the Marine Biology Research Group of Ghent University (contact: Ellen Pape). Type material of the described species is either stored at the Senckenberg Museum, Frankfurt (SMF), or the Crustacean Collection of the Museum of Nature (Leibniz Institute for the Analysis of Biodiversity Change, LIB) (formerly Center of Natural History, CeNak) in Hamburg.

Author contribution   SK performed the literature search and wrote the manuscript with the help of all co-authors; MC and SK conducted the data analysis; MC was responsible for coordinating and managing the sequence data; AJ, IM, SMS, TCK and FP performed the molecular laboratory work; AV and PMA conceived and designed the research. All authors read and approved the manuscript.

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