



**Template for the review of the draft standards and guidelines
associated with the draft regulations on exploitation of mineral resources in the Area**

TEMPLATE FOR COMMENTS

<i>Document reviewed</i>	
Title of the draft being reviewed:	Draft Guidelines for the Establishment of Baseline Environmental Data
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<i>General Comments</i>	
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We kindly acknowledge the efforts undertaken by the LTC and its TWG in drafting these guidelines for the establishment of baseline environmental data. Certainly, this has been a difficult task, since many scientific methodologies and technologies have not yet been standardized or have reached the technical readiness level (TRL) required for the application by industry. Moreover, the technology for collecting environmental baseline data is steadily evolving. Nevertheless, collecting detailed baseline data is the essential basis for impact assessments as well as environmental monitoring and management plans and thus needs to be conducted in a comparable and standardized way.

However, the present draft shows too much incoherency and inconsistency in several places in order to provide sufficient guidance and clarity for contractors to ensure a robust, standardized, and comparable collection and assessment of baseline environmental data. For instance, there are significant differences regarding the level of detail provided between the individual sections. Moreover, it remains unclear at times, which guidance pertains to which environmental compartment or aspect. This is particularly problematic in the section on chemical oceanography and (sediment) biogeochemistry, where the grouping of the two disciplines leads to confusion with respect to sampling, sample handling, and data processing.

In its current form, the guidelines create uncertainty, which could lead to inconsistent approaches for the collection and analyses of baseline environmental data, making the comparison of data gathered by different entities (including contractors) incomparable.

Please find our general and specific comments and suggestions below.

Standards for baseline environmental data are needed

Since “the purpose of the acquisition and establishment of baseline data is to enable an assessment of the possible impacts of exploitation activities on the marine environment” as stated in the Background paragraph 2, the document should define binding standards (or obligatory minimum requirements) for environmental baseline data, in addition to only providing guidelines and recommendations. These should comprise those variables that are essential for enabling an assessment of impacts, based on current scientific knowledge, and should be revised regularly. Such standards will also allow comparing environmental data between contract areas and on regional scales (e.g. CCZ, MAR), which is essential for REMPs and integrative assessment of larger scale impacts.

Hence, the environmental baseline standards also need to be standards in the environmental monitoring of mining operations. There is only vague reference to monitoring in the document (e.g., in the “Purpose and Scope” section). What is the regulatory hierarchy of the different standards and guidelines, and also with respect to older ISBA documents?

Structure of the document: Separate sections for Sediment Biogeochemistry and Chemical Oceanography

Biogeochemistry (NOT Biochemistry, which is a different discipline referring to reactions on the cell level and metabolism) and Chemical Oceanography are two specific scientific disciplines that deal with different environmental compartments, the seafloor (porewater and solid phase sediment) and the water column, respectively. While some variables have the same names, the sampling methodology, the relevant spatiotemporal scales, analytical procedures, data analyses and modeling approaches as well as the importance of the

variables are largely different between the respective environmental compartments. Thus, they need to be dealt with in separate sections of the document. In the current version of the document, important details get lost or are unclear, such as which method applies to which environmental compartment. Unfortunately, this problem in the document structure has been adopted from the Recommendations ISBA/25/LTC/6, where the variables necessary to monitor and evaluate the benthic impacts are somewhat distributed and hidden in the chemical oceanography and geology section or are kept as a separate section not connected to any of the disciplines (e.g., sediment community oxygen consumption).

However, the benthic environment of the seabed will experience most of the impacts from mining operations and here strong effects on essential ecosystem functions are expected. Thus, it is essential that the inappropriate structure of the Recommendations ISBA/25/LTC/6 is revised in these Baseline Guidelines.

Context of observation variables and cumulative effects with other stressors

The relevance and importance of requested variables and measurements or timescales of observations is missing throughout the document. Why is it important to determine certain (sets of) variables? Over which timescales should they be assessed? The latter is, for example, also important to account for future baseline shifts due to other anthropogenic stressors, such as climate change, ocean acidification, oxygen minimum zone extension, and pollution. The document, particularly in the chemical oceanography and sediment biogeochemistry sections, often lists variables and methodologies without any or only very little context. It should become clear that requested environmental baseline data serve specific purposes in the context of mining-related activities. If this cannot be included in the main document, it should be contained in a comprehensive appendix of the Guidelines.

Specific Comments

Page	Line	Comment
2	2	The document structure will hardly allow to be extended to other mineral types (massive sulfides and ferromanganese crusts) without becoming very confusing. Separate documents for each of the other two mineral types need to be developed in the future. Hence, we suggest adding “for polymetallic nodule habitats” to the title.
2	24	Chapter V needs to be split up into 2 separate chapters: “Chemical Oceanography” and “Sediment Biogeochemistry” (see detailed explanations above).
4	78-80	Regional Environmental Management Plan (REMP) should be added to the list of EIA, EIS, EMMP, and EMS.
4	82-86	Suggestion to edit paragraph 5 to read: “The primary goal of the acquisition of baseline data is to characterize the existing environment to enable an assessment of the impacts of exploration and exploitation activities on the marine environment prior to those activities taking place. It also defines the methodologies and forms the basis for long-term monitoring of environmental impacts to make sure that those are in line with the environmental impact assessments and environmental monitoring and management plan once exploitation commences.”

4	91-93	The baseline data also need to characterize the water column, not only the seabed. The statement needs to be corrected accordingly. E.g., by referring to the “marine environment” and not only to “sediment properties” and “the Area”.
5	102	“Chemical Oceanography” and “Sediment Biogeochemistry” should be referred to in separate bullet points and the chapter be divided into 2 separate ones.
5	105	Sampling and data acquisition. We recommend the addition of a separate best practice section in the general part.
5	107-109	Replace “representation” by “ <u>sampling effort and replication</u> ”
5	107	Oceanographic conditions are environmental conditions. There is no need to list them separately at this point.
5	109-111	“Without this knowledge, deviations from pre-mining conditions observed during mining operations could only be assigned to exploitation activities.” This is an important statement as it shows how the sampling effort invested during baseline studies is beneficial to the contractors at later stages as it will allow to differentiate between natural and man-made (i.e. contractor-made) changes. Please emphasize even more clearly that required baseline data need to be fit for purpose.
5	115-116	Suggest using the word “compartments” instead of “components” here.
5	118-119	Add: This should be increasingly achieved with stationary robots.
5	127-138	Analyses of biogeography and seasons should not be limited to the water column, but need to integrate the benthic environment as well.
6	147	Please add “(physical oceanography, section IV, <u>chemical oceanography, section V, and geological properties, section VI</u>)”.
6	157	‘AUV’ needs to be spelled out the first time the term is mentioned.
7	167	<u>“over three years”</u>
7	167-169	“Observations in similar seasons or environmental conditions should be carried out over at least three years to assess interannual variability and increase the chance to capture periodic events.” This should cover different seasons to better understand the influence of seasonal variability. As this is likely difficult to achieve for individual contractors, especially for variables determined by station work, cooperative research, and the use of resident robots should be envisaged here.
7	182	Add: ... and bottom 500 m (e.g., at 0-1m , 5m,...)
7	191	“...up to 5 m” Replace with “at least 5 m”
7	196	“...to cover the variability and discriminate between units.” If these „units“ refer to the physiographic units mentioned in Fig.1, please add the term “physiographic” in the sentence above.
8	223-228	The Guidelines need to be revised and updated regularly. How is the “Independent feedback” of experts reported and checked?

9	251-252	It should become a standard (not only a recommendation) that the data of contractors is made publicly available to the scientific community as well as for independent assessments by other groups.
9	253-262	Para. 29 should be moved to section D or a new Best Practices or Methodology sections.
9	253-260	This information is very specific and should, thus, be addressed in the individual sub-sections.
9	263 ff.	<p>Data quality section: Data quality not only includes the quality (i.e., accuracy, precision) of measured variables (physical, chemical, biological, biogeochemical etc.), but also their usefulness for the purpose.</p> <p>For chemical oceanography we suggest the following text:</p> <p>“Good data quality needs to meet two main criteria: (1) data values themselves need to be of high quality, and (2) the quality of data needs to be fit for purpose, i.e., the correct variable are chosen for monitoring, data are obtained at required temporal and spatial resolutions, and the measurement uncertainties are sufficiently small to resolve expected potential changes related to the mining activity.</p> <p>Sampling and analytical methodologies broadly adopted by the ocean research community (best practices) should be followed. Furthermore, database and literature research should be conducted where possible to assess the expected range of concentrations (e.g., nutrients, trace elements), activities (e.g., radiotracers), and fluxes (e.g., organic carbon export fluxes. This will serve as an external quality control check.</p> <p>Furthermore, it should be specified why a measurement is needed to determine the required uncertainty relative to the needs as defined by the application.</p> <p>Data quality metadata are needed to determine the fitness of a data set for use, i.e., are the data fit for purpose (data quality assessment)? Therefore, it needs to be guaranteed that measurements are properly representative of the area of interest, i.e., cover spatial and temporal scales of variability. To reduce the uncertainty of data for a specific location or time, repeated sampling should be carried out (see section 1.3 on sampling strategy).</p>
9	266-272	“A good agreement.....or more samples” This part seems to specifically refer to physical oceanography models and should not form the first, general, paragraph of this section.
10	293	“near to use as possible to their use” needs to be adjusted.
10	300	“Data, sample, and specimen...” Please replace with “Data and metadata, and...”
10	311	“Data and findings should be published in international, peer reviewed and open access scientific journals and presented at international

		<p>scientific conferences to facilitate the dissemination of new information. Publication also enables feedback and approval from multiple independent experts.”</p> <p>Publication in addition to submission to the ISA as reports is indeed beneficial to make sure that data are acquired in agreement with the current state of the art and keep the scientific community updated. We suggest to additionally advise contractors to strengthen outreach activities also beyond the scientific community. It would be beneficial to present information to the general public, as well.</p>
10	317	<p>“Standard metadata (including position, water depth, expedition and station ID, principal investigator) should be recorded following <u>established metadata standards.</u>”</p> <p>Where are these to be found? Is there a source (e.g., website) where this is explained? Please provide a reference and/or link.</p>
10	317	Standard metadata should also include time and date.
11	335	A description of the general methodology is completely missing. This information is currently intermingled in the sample resolution section.
11	346-348	<p>“The sea-water parameters that discrete water masses within which other variables should be measured and determine water column stratification.”</p> <p>Please replace with <u>“These sea-water parameters determine water column stratification and discrete water masses within which other variables should be measured”</u></p>
11	353	Replace “tides cycles” with “ <u>tidal</u> cycles”
12	382-388	<p>In addition to the suggested depths, the bottom water above the seafloor needs to be sampled at higher resolution again (see also paragraph 72) because this is the main zone of impact from mining operations that should, therefore, be thoroughly monitored.</p> <p>As practical consideration with regards to the suggested sampling depths it should be noted that more than one water sampling cast is needed for such sampling scheme because the total number exceeds what can be taken with a standard Rosette system.</p>
12	400-402	<p>“Current measurements should be determined throughout the water column and in addition to the depths noted in section III.A at the following depths, surface, 10m, 25m, ...”.</p> <p>If current measurements are done throughout the water column, what is the need to specify these particular depths?</p>
13	430	The term “remote” is not appropriate for the sensing by ROV, AUV or glider. Suggest to delete.
13	427-428	What are these “appropriate depths” where temporal variability of currents should be studied with floats and drifters? The appropriate depth for assessing the dispersion of operational sediment plumes by currents is > 4000 m, well beyond the reach of floats and drifters.

14	470-473	Compared to the exhaustive detail in the description of biological methods, the description of the characteristics of an appropriate ocean circulation-sediment transport model seems under-detailed.
14	474	It seems odd that graphical representation is specifically mentioned with regards to current velocity data. Is this less applicable to other types of data?
16	561-563	The reference to “numerous methods and configuration standards” is rather unspecific with regards to what is fit for purpose. Suggest to add: “To allow assessment of excess concentration of suspended particulate matter in operational and discharge plumes, optical or acoustic turbidity data need to be converted to suspended particulate matter concentration. To this end, optical or acoustic sensors should be calibrated to the suspended particulate material locally present in the water column. For baseline studies, this should be done by reference to suspended particulate matter concentration determined in water samples taken simultaneously with the turbidity measurements. For monitoring of operational and discharge plumes, the same approach can be used if water samples can be taken directly from the plume. Otherwise, sensors should be calibrated ex-situ in suspensions made with filtered local seawater and added plume source material.”
19	671	“V. Chemical oceanography and biogeochemistry” We strongly advocate for a separation of the chemical oceanography and sediment biogeochemistry chapters (see comments above). We also want to highlight that the title of this section in the table of contents needs correction (see comment above).
19	671 ff.	It needs to be made clear in this section that the water column geochemistry is linked to porewater and sediment geochemistry and vice versa and all three compartments should be studied collectively, particularly, with respect to plume dispersion. It would also be important to link the geochemistry with the bulk sediment composition in the Geology section V and vice versa. In the Geology section V, the part on bulk sediment composition is too unspecific - which elements/minerals should be determined and what are the appropriate methods for these analyses? From a geochemical point of view (but also depending on the region) Mn, Ca, Al would be variables that should be measured as they provide valuable information for monitoring purposes.
19	675-676	“...direct impacts of mining activities on the seafloor as well as indirect impacts from suspended” This should be more precise or at least examples should be provided (e.g., sediment removal (and mixing), pore water release, plume dispersion, sediment compaction).
19	676	“...indirect impacts from suspended sediment plumes...” There should be a distinction between operational and discharge plumes. Moreover, details on the problems associated with the plumes are missing, as well

		as details regarding differences in terms of their (geo)chemical composition.
19	676	<p>We suggest replacing this section with the following text:</p> <p>“Mining-induced impacts in the water column result from the anthropogenic plumes formed by (1) the mining vehicle at the seafloor (i.e., operational plume) and by (2) the release of material after ore processing on board of a mining ship (i.e., discharge plume). Current knowledge suggests the release of the discharge plumes into abyssopelagic zones at similar depths as the operational plume (Drazen et al., 2020). These plumes consist of suspended surface sediment material and crushed ore material (the latter likely dominating the discharge plume) and are likely enriched in potentially toxic substances such as metals, which may occur in particulate or dissolved form. Plume dispersal especially of fine-grained particles and colloids potentially over tens of kilometers (conservative estimation) will transfer mining-induced effects far away from the actual mine site and will affect large areas. Increased concentrations of suspended sediment affect the pelagic ecosystem by clogging respiratory and olfactory surfaces and filter nets of suspension feeders (see Drazen et al., 2020 and references therein) and dilute POC concentrations as the main food source for filter feeders in bathypelagic and abyssopelagic regions by inorganic sediment. Trace metals being essential micronutrients for microbial communities in the ocean, such as Fe, Cu, Co, Zn, Mn, as well as other metal such as Cd, As may become toxic at elevated concentrations, and enhanced metal concentrations in anthropogenic mining plumes may induce potential eco-toxicological effects (also see Section 7.10 Ecotoxicology). Increased metal concentrations arise from (1) the presence of the ore material itself (e.g., Mn oxides) in particulate or colloidal form, (2) the high scavenging potential of these ore particles and newly formed Fe oxyhydroxide particles and colloids, leading to additional enrichment of dissolved trace elements such as Co, Ni, Pb from the water column, and (3) remineralization and desorption/dissolution processes releasing metals from the suspended material.”</p>
19	676	<p>“...indirect impacts from suspended sediment plumes...”</p> <p>Is this still referring to areas where the mining vehicle was operating and has directly disturbed the seafloor? This is confusing also because the plume dispersion and the associated pore water release will directly affect the chemical oceanography. Therefore, we suggest sticking to one denotation throughout the document: with respect to the seafloor, distinguish between “directly impacted” or IRZ, which is the denotation that the contractors use (where mining vehicles operate) and “indirectly impacted” or PIRZ seafloor sites (initially undisturbed seafloor where plume particles re-settle) in the surrounding areas. Where a sediment plume is present, the water column would be “directly impacted”.</p> <p>See EIAs: https://www.isa.org.jm/environmental-impact-assessments</p>

19	680	“different chemical composition to the surrounding water” should be replaced by “...different <u>particle size and</u> chemical composition”.
19	683-685	“Marine biogeochemistry focuses on seafloor processes and functions and combines studies of chemical conversions with the observations of the biological and geological processes involved.” Please replace with “Marine <u>sediment</u> biogeochemistry focuses on seafloor processes and functions and combines studies of <u>biogeochemical</u> conversions with the observations of the biological, <u>geochemical</u> , and geological processes involved.”
19	694	“...(i.e., the distribution, transport, and conversion of the reactants and products of these reactions).” Please replace with “...(i.e., the distribution, transport, and <u>transformation</u> of the reactants and products of these reactions).”
20	702	Pore-water ammonium concentrations are very low, and quantification is challenging. Even with high precision equipment, ammonium concentrations are mostly below LOQ. Therefore, we have advised already in previous versions to exclude the determination of pore-water ammonium.
20	703	Redox conditions are mainly indicated by oxygen and pore-water Mn in these sediments as nitrate is mostly available throughout.
20	704-708	“Oxygen - Oxygen concentrations in the water column provide information on organic matter production in the surface layer and its remineralization during export towards the seafloor and the distribution of oxygen in the sediment and the flux across the sediment-water interface provides a measure of benthic organic matter remineralization and the activity of the benthic community.” Please replace with “Oxygen - Oxygen concentrations in the water column provide information on organic matter production in the surface layer and its remineralization during export towards the <u>seafloor</u> . <u>The</u> distribution of oxygen in the sediment and the flux across the sediment-water interface provides a measure of benthic organic matter remineralization and the activity of the benthic community. <u>Furthermore, the availability/presence of oxygen significantly limits the mobility of most trace/heavy metals.</u> ”
20	708	Add “oxygen penetration depth” as an important part of the oxygen distribution in the sediments and its geochemical control regarding the mobility of potentially toxic substances in the sediments and their possible release during mining activities.
20	723	„Observations in the water column address productivity and export while measurements at the seafloor quantify the amount and quality of the organic material that is available to seafloor and the dynamics of benthic organic matter cycling.“ Please replace with: „Observations in the water column address productivity and export while measurements at the seafloor quantify the amount and quality of the organic material <u>that is available to benthic</u>

		<u>organisms and biogeochemical cycling at the seafloor</u> and the dynamics of benthic organic matter cycling.”
20	728	The determination of naturally occurring radioisotopes serves as baseline data, not the distribution.
20	729	“...direct impacts of mining activities on the sediments....” This should be more precise or at least examples should be provided (e.g., sediment removal (and mixing), etc.)
20	731	“...as well as enabling the assessment of radioisotopes in the nodules once mining commences.” Please replace with “...as well as enabling the assessment of radioisotopes <u>and hence the intensity of natural radioactive radiation</u> in the nodules once mining commences.”
20	730-731	“...enabling the assessment of radioisotopes in the nodules once mining commences.” Please clarify what this means.
20	736-737	“...using the most relevant of the 4 techniques” This sounds like a choice, which it shouldn't be. Some parameters can only be determined by applying the listed techniques. Please clarify.
21	751	Please add to para. 112 information on pore water sampling for nutrient analyses: “Pore water should be extracted using rhizon samplers with a pore size of 0.1 µm (Seeberg-Elverfeldt et al., 2005). Pore water can be extracted on recovery of cores or, with the exception of oxygen measurements, the cores can be stored colder than 4°C (before or after subsampling into sediment horizons) and pore water extracted later. Pore water analysis should be undertaken within 2 hours of when the pore water was extracted from the sediment.”
21	para . 112	As sampling strategies are often specific to the variable under consideration, we suggest that general information is included in the “general methodology” section while more variable-specific information is covered in the respective subsections concerned with these variables. For example, one could include one or two sentences here on the general methodology for sediment core sampling with MUC and PUC (see first sentence of 112), to obtain data for chemical and biogeochemical variables. And include more specific parts in the variable sections (where it should be subdivided for chemical oceanography and biogeochemistry, as completely different approaches).
21	753-758	“The method publications of the Integrated Ocean Drilling Program (IODP), the Global Ocean Ship-based Hydrographic Investigations Program (GO-SHIP), the GEOTRACES initiative, and - the Ocean Best Practices repository hosted by the International Oceanographic Data and Information Exchange (IODE) of the Intergovernmental Oceanographic Commission (IOC) should be consulted for commonly accepted and agreed methods for chemical oceanographical and biogeochemical sampling.” Please replace with <u>“The majority of the requested biogeochemical</u>

		<u><i>variables for chemical oceanography in this document belongs to the Essential Ocean Variables (EOVs) as defined by the Global Ocean Observing System (GOOS). The EOVS specification sheets provide detailed information on sub-variables, derived variables, supporting variables, phenomena to capture, temporal and spatial scales of phenomena, current observing networks, and refer to best practices, guides and background information.</i></u>
21	759	Please state the “appropriate methods”.
21	765-765	“Sediment cores not investigated for porewater can be stored at 4°C or colder (before subsampling into sediment horizons).” This should be specific: for which solid-phase analyses can cores be stored before sub-sampling and for how long?
21	766-769	For some sensitive constituents (e.g., nutrients), pore water analyses should be undertaken on board as soon as possible after pore waters are extracted from the sediment, while other analyses may be performed in the onshore lab on samples transported frozen or cooled and appropriately preserved. Please provide a complete list of variables that have to be analyzed on board and which could be addressed in the shore lab. Combine the second part of the sentence with the sentence in line 762ff ('For some dissolved components...')
21	771	“...overlying the sediment in the core liner should always be sampled as the seawater “endmember” for the pore water...” Please replace with “...overlying the sediment in <u>the liner of the multicorer</u> should always be sampled as the seawater “endmember” for the pore water...”
21-22	782-786	The Global Ocean Observing System (GOOS - www.goosocean.org/) is a sustained collaborative system of ocean observations, encompassing in situ networks, satellite systems, governments, UN agencies and individual scientists and the majority of the variables belong to the Essential Ocean Variables (EOVs) as defined by the Global Ocean Observing System (GOOS)” Add: “Note, however, that variables and the rationale provided by GOOS to date are mostly focusing on the water column while biogeochemical processes at the seafloor are not fully covered by the current set of EOVs.”
22	787	We suggest adding the following text about in-situ observations, which we deem important: “State of the art methodologies for data acquisition for ocean chemical variables will develop more and more towards in situ observational approaches, which offer an expanded scale of observation both in space and time. Currently, high quality measurements of ocean chemistry variables such as pCO ₂ , pH, dissolved inorganic carbon (DIC), and total alkalinity (TA), oxygen (O ₂), trace metal concentrations are mainly conducted on discrete water samples collected from research vessels,

		<p>but sample collection and laboratory-based analysis are laborious, expensive, and provide low temporal and spatial resolution. For the deep sea, further limitations arise from sampling artefacts such as depressurization and temperature change which require in situ measurements (for example pH). The envisaged integrated assessment of sediment biogeochemistry and chemical oceanography at the sediment-water interface (SWI) will also be best approached with a time- and space-resolved in situ observation strategy, which is partly already being addressed in these guidelines (see sections 4 and 6). In the coming years major progress in sensor technology development is expected and suitable measurement and sampling instruments will become increasingly available for mining-related observing demands in the water column and the benthic boundary layer. Considering this, an adaptive strategy of data acquisition for chemical oceanography in these guidelines is necessary. In situ observations include measurement and sampling instruments such as (1) logging biogeochemical, biological and acoustic sensors, (2) optical imaging devices, (3) in situ analyses such as lab on chip techniques conducting miniaturized chemical and biological analyses, benthic chambers to investigate time-resolved biogeochemical activities and fluxes at the sediment-water interface, (4) autonomous samplers to obtain and fix sample material (traps, water samplers), with analyses after retrieval, and (5) passive samplers capturing labile elements, with analyses after retrieval. These instruments can be incorporated in underway (towed) systems or mounted to stationary or mobile (autonomous or tethered) platforms or deployed at the seafloor.”</p>
22	823-826	<p>“Sampling should be collocated wherever possible (section III.C) and follow the nested stratified sampling scheme and the general considerations to cover spatial and temporal variability (section III.A) with further details provided for specific variables in the sections below”</p> <p>Please note that sampling resolution for biogeochemistry observations needs to be covered, too - as pointed out preferably in a specific biogeochemistry chapter separated from chemical oceanography. If these aspects are covered by the general sampling scheme described in section III or in the sections on specific biogeochemical variables, this should be explicitly mentioned here.</p>
22	820-822	<p>“Besides point sampling with CTD, this should include long-term deployments of passive samplers along a vertical gradient from the seabed up to 10 m above the seafloor.”</p> <p>This should be a suggestion.</p>
23	835-838	<p>The sentence “Even with high-precision equipment, quantification of ammonium in deep-sea porewaters is difficult because of very low concentrations and, therefore, in cases where concentrations prove to be close to the detection limit, the determination of pore water ammonium can be excluded until better analytical methods become</p>

		available” should be moved to where ammonia is introduced as one of the main nutrients to be measured.
24	873	Why are ammonium and silica mentioned here again although it was said earlier that they can be excluded from baseline observations (see para. 124)?
24	890	add “Revsbech and Jorgensen, 1986” as a reference in addition to Mewes et al., 2014)
24	895-896	“Larger and temporally more stable optical sensors (macrooptodes) should be used for time series measurements of oxygen in benthic chambers or bottom waters.” Add: “Sensors should be thoroughly calibrated in the lab and recordings obtained in situ should be validated by comparing measurements taken above the sediments with bottom water concentrations determined with the methods mentioned in paragraph 130.”
24	906-908	“Chamber incubations determine total oxygen uptake (TOU) and microprofiler measure diffusive oxygen uptake (DOU). Total oxygen uptake (TOU) is also referred to as sediment community oxygen consumption (SCOC).” Please replace with: “Chamber incubations determine total oxygen uptake (TOU, <u>also referred to as sediment community oxygen consumption, SCOC</u>) and microprofiler measure diffusive oxygen uptake (DOU).”
25	919-920	“based on the at the given” Please revise the wording.
25	925	Please add “see next paragraph” at the end of the last sentence of paragraph 134.
25	946-952	Please add a proper description of the scientific rationale for paragraph 138.
25	952	“The redox zonation in the sediment should also be characterized” Please replace with: “ <u>In combination with other redox-sensitive biogeochemical variables (e.g., nutrients, trace metals), oxygen observations in the sediment should also be used to characterize the redox zonation in the sediment.</u> ”
27	1044	When mentioning the ‘GEOTRACES Cookbook’ first time, the official title of the document should be used (i.e., “Sampling and sample-handling protocols for GEOTRACES cruises”. Subsequently, the abbreviation “GEOTRACES Cookbook” can be used.
28	1049	“...rather than total dissolved concentration”: But total dissolved concentrations are needed as a basis for comparison with existing data.
28	1054	“For physical size speciation of seawater and pore water, potential methods include:” Replace with “For physical size <u>fractionation</u> of seawater and pore water, potential methods include:”
28	1056	replace “0.02 µm (total dissolved)” with “ <u>0.2</u> µm (total dissolved)”

28	1058	Please complete the sentence "...on-board acidification of non-filtered samples (for total dissolvable concentrations)."
28	1076-1078	"Generally, metal concentration data should be determined using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) or Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)." Replace with "Generally, metal concentration data should be determined using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) <i>and</i> Inductively Coupled Plasma-Mass Spectrometry (ICP-MS).
28	1078	For trace metals in seawater and pore water, the use of a SeaFAST pre-concentration and matrix separation device is strongly recommended.
28	1083	Please add "...SLEW-3, <i>GEOTRACES intercalibration standards</i>)..."
29	1106	"The main emphasis of baseline observations should be on a well-replicated characterization of Particulate Inorganic Carbon (PIC), Particulate Organic Matter (POM) and Dissolved Organic Matter (DOM) in the water column and uppermost decimetres of the sediment where biogeochemical conversion rates are highest, and where current knowledge suggests the impacts are most likely to be expected." Please replace with: The main emphasis of baseline observations should be on a well-replicated characterization of Particulate Inorganic Carbon (PIC), Particulate Organic Matter (POM) and Dissolved Organic Matter (DOM) in the water column and uppermost decimetres of the sediment where biogeochemical conversion rates are highest, and where current knowledge suggests the impacts are most likely to be <i>most pronounced</i> ."
29	1110-1112	"For seabed analysis, the distribution of PIC and POM should be determined in subsamples taken from distinct depth layers of retrieved cores while DOM should be analysed in pore-waters extracted from distinct depth layers." Please replace with: "For seabed analysis, <i>the distribution of the amount and characteristics of PIC and POM</i> should be determined in subsamples taken from distinct depth layers of retrieved cores while DOM should be analysed in pore-waters extracted from distinct depth layers."
29	1120-1122	The ratio of DOC to dissolved organic nitrogen (DON, calculated by subtracting the sum of NH ₄ ⁺ , NO ₃ ⁻ , and NO ₂ ⁻ from TDN) provides a first indication of the quality of DOM so the DOM's chemical composition should be characterised. Please replace with: "The ratio of DOC to dissolved organic nitrogen (DON, calculated by subtracting the sum of NH ₄ ⁺ , NO ₃ ⁻ , and NO ₂ ⁻ from TDN) <i>provides a first indication of the DOM's chemical composition and should be used for a general characterisation of DOM quality (i.e., potential availability to organisms as food source)</i> ."
29	1127	This should be "2007" instead of "20007"
30	1136-1139	"For the sediments, observations should be used to determine the quantity and quality of organic matter and its spatiotemporal variability to quantify and explain organic matter remineralization rates, and provides contextual information on metal complexation and

		<p>bioavailability.”</p> <p>Please replace with:</p> <p>“For the sediments, observations should be used to determine the quantity and quality of organic matter and its spatiotemporal variability to quantify and explain organic matter remineralization rates, <u>and should be used in combination with trace metal observations to address metal complexation and bioavailability.</u>”</p>
30	1149-1153	<p>“While sampling techniques based on filtration of water samples collected with water sampling devices such as NISKIN or GO-FLO bottles are limited to relatively small volumes <12l, in situ pumps specifically aimed at collecting larger masses of particles are capable of filtering large volumes (hundreds of litres per hour) required for some investigations (such as activities of specific radioisotopes)”</p> <p>Please replace with: “While sampling techniques based on filtration of water samples collected with water sampling devices such as NISKIN or GO-FLO bottles are limited to relatively small volumes <12 l, <u>in situ pumps, capable of filtering large volumes (hundreds of litres per hour) should be used to collect larger masses of particles as they are required for some investigations (such as activities of specific radioisotopes).</u>”</p>
30	1155-1157	<p>“Filtered seawater from bottles and pumps should be used for particle concentrations, type and quantity, and is suitable for trace metal investigations.”</p> <p>Please replace with: <u>Particles obtained from filtering seawater from bottles and with in-situ pumps should be used for particle concentrations, type and quantity, and is</u> suitable for trace metal investigations.”</p> <p>Comment: Is this true irrespective of the type and material of the bottles and in situ pumps being used?</p>
30	1157-1158	<p>The size spectrum of these particles reflects a mixture of sinking and non-sinking particles.</p> <p>This is difficult to understand. Please consider replacing with: <u>If and how fast particles sink (i.e., their contribution to export fluxes) depends on their individual size, shape, and density'</u></p>
30	1159-1160	<p>“The most direct method of measuring particle flux uses sediment traps, which collect sinking particles at a certain depth over a period of several days to months.”</p> <p>Please replace with: <u>'In addition, direct measurements of particle fluxes should be obtained with sediment traps,</u> which collect sinking particles at a certain depth over a period of several days to months.”</p> <p>We also recommend additional direct observations with state of the art technologies (lowered or moored particle cameras, if possible in</p>

		combination with recovery of intact particles for analyses (e.g., using gel traps).
30	1161	<p>“Quantity, type, and quality of sinking particulate matter should be directly assessed.”</p> <p>Please clarify what 'directly' means in this context means (directly on board the vessel, or directly in situ?). How should this assessment be done? Does this refer to paragraph 171?</p>
31	1182	In addition to the mentioned optical techniques, acoustic techniques are commonly used for assessment of variation in suspended particulate matter concentration. Acoustic frequency dependent sensitivity of sensors to certain particle size ranges should be taken into account.
31	1196	“a particular emphasis”
31	1204-1205	<p>“The Ratio of TOC/TN (C:N ratio) provides a first indication of POM quality”</p> <p>Here it should be emphasized that this often fails in sediments with very low organic matter contents where C:N ratios tend to indicate unreasonably high relative N-amounts. Measurements are still important to be able to characterize conditions in order to later detect changes. However, a proper analysis of POM quality / lability needs observations of other quality proxies’.</p>
31	1215	<p>“In conjunction with measurements of TOC and TN also PIC should be measured with a CNS element analyzer”</p> <p>Please mention how this is being done (subtracting measurements of acidified from measurements of non-acidified samples?).</p>
32	1237-1240	<p>“This information should be combined with observations of organic and inorganic particulate matter export fluxes, oxygen uptake, the carbonate system, nutrients, and trace metals, by means of transport-reaction models to quantitatively assess benthic biogeochemical cycling in organic matter, nutrients, and trace elements.”</p> <p>The importance of models to turn individual biogeochemical observations into knowledge on biogeochemical functioning deserves a separate section and should not be 'hidden' under a specific variable. This is key to validate the sampling strategy in terms of selection of the most important variables, coverage of spatial and seasonal variability, but also an assessment of the quality and consistency of the data obtained. Although this is covered in very general terms in paragraph 30 it should be mentioned more prominently also in the biogeochemistry section - together with relevant references.</p>
34	1324	Please explain the reason for “deviations of <30%” and “a statistical power of at least 0.8” and provide appropriate references.
34	1333	Data sets consist of both the actual measurements and also descriptive data, generally referred to as metadata.

38	1409	Add the following sentence at the beginning of para. 218: “Most of the species living in the deep-sea is not yet described. For benthic macrofauna, one of the fractions used for environmental monitoring, 95% of the species remain to be described and named but also larger animals such as many megafauna species, are currently not possible to identify to species. The taxonomic work to describe the species present in the deep-sea (including morphology and DNA barcodes), is a major part of the effort to assess baseline community structure and connectivity and thus a prerequisite for any future assessment of impact.”
39	1509	Connectivity is not limited to genetic connectivity but include the full range of ecological connectivity. It should also be noted that any fauna depending on or restricted to nodules cannot recover (unless appropriate hard substrate restoration measures are executed), since its habitat is lost by the mining for millions of years.
39	1543	What is meant by “quite homogeneous”?
42	1675	Add “Virus” to microbiota.
43	1691	Add „...ROV, <i>crawler</i> , or towed camera platform...”
44	1741	Video/photo observations of species should be verified by taxonomic and/or genetic analyses of several collected specimens.
44	1746	“Both macrofauna living on nodules and those in the sediment should be <u>collected</u> .” Please replace “collected” with “ <u>sampled</u> ”.
44	1747-1748	“For nodule living fauna, when box cores are recovered, nodules with obvious epifauna should be identified.” Please replace with “For nodule- <u>associated</u> fauna, when box cores are recovered, obvious epifauna <u>attached to the nodule surface</u> should be identified.”
44	1748-1750	“Nodule fauna still attached to the nodules should be imaged live in special small aquaria with cold filtered seawater, fauna removed, snippet sample for DNA taken in a 2 ml tube with cold 96% Ethanol and the animal fixed in a separate tube.” Please replace with “Nodule fauna should be imaged <u>when still attached to the nodules</u> in special small aquaria with cold (<u>4°C</u>) filtered seawater (<u>CFS</u>), fauna removed, snippet sample for DNA <u>analysis</u> taken in a 2 ml tube with cold (<u>-20°C</u>) 96% Ethanol and the <u>organism</u> fixed in a separate tube.”
44	1752-1753	The size and weight of the nodules should be recorded and preserved in formalin or cold ethanol. Please replace with “The size and weight of the nodules should be recorded and <u>nodules should be</u> preserved in formalin, <u>cold (-20°C) ethanol, <i>DESS</i> (Yoder et al. 2006), or frozen (-20°C) without fixative, depending on intended further analyses. Note that in some fixatives, like <i>DESS</i> (Yoder et al. 2006), nodules have been reported to disintegrate.</u> ”

		Reference: Yoder et al. (2006) DESS: a versatile solution for preserving morphology and extractable DNA of nematodes. <i>Nematology</i> 8, 367–376.
44	1754-1756	The sediment should be divided into 0-3cm, 3-5cm and 5-10cm depths and each sieved with cold filtered seawater. Please replace with “The sediment should be divided into 0-3 cm, 3-5 cm, and 5-10 cm <u>layers</u> and each <u>of these</u> sieved with <u>CFS (4°C).</u> ”
44	1756-1758	“The uppermost sample should be sorted immediately, the residue from the deeper slices should be kept in a cold lab in cold filtered seawater until they are processed.” Please replace with “The uppermost sample (<u>0-3 cm</u>) should <u>preferably</u> be sorted immediately and at 4°C, the residue from the deeper slices should be kept in a cold (<u>4°C</u>) lab in <u>CFS</u> until they are processed.”
45	1759-1761	“For morphology and ecological analyses, in the refrigerated laboratory, the 0-3 cm layer of sediment should be sieved with CFS, the residues preserved in 10% buffered formaldehyde.” Please replace with “For <u>morphological</u> and <u>molecular analyses</u> , in the refrigerated (<u>4°C</u>) laboratory, the 0-3 cm layer of sediment should be sieved with CFS, the residues preserved in 10% buffered formaldehyde.”
45	1761-1763	“In the wet lab, the 3-5 cm and 5-10 cm layers of sediments should be sieved with cold filtered seawater, and the residues fixed in 10% buffered formaldehyde or 96% Ethanol.” Please replace with “In the wet lab, the 3-5 cm and 5-10 cm layers of sediments should be sieved with <u>CFS</u> , and the residues fixed in 10% buffered formaldehyde or 96% Ethanol.”
45	1765-1766	“Formaldehyde should not be used for fixing crustacean groups such as isopods; for such taxa preservation in 96% Ethanol is advised.” Please replace with “Formaldehyde should not be used for fixing crustacean groups such as isopods and tanaids; for <u>these</u> taxa preservation in 96% pre-cooled Ethanol is advised.”
45	1766-1768	“Samples should be fixed in formaldehyde solution for at least 24 hours, then as soon as is practicable, all samples should be transferred from formaldehyde solutions into 70-80% EtOH solution.” Please replace with “Samples should be fixed in formaldehyde solution for at least 24 hours, then as soon as is practicable, all samples should be transferred from formaldehyde solutions into 70-80% <u>Ethanol solutions.</u> ”
45	1769-1771	“For molecular, morphology and biodiversity studies, the residues of the upper 0-3 cm layer should be sieved and retained, the sample kept as cold as possible sorting all metazoans into easily identifiable taxonomic groups over an “ice bed.” Please replace with “ <u>For molecular and morphological studies</u> , the residues of the upper 0-3 cm layer should be sieved and retained, the sample kept as cold as possible when sorting all metazoans into easily identifiable taxonomic groups over an “ice bed.”

45	1771-1772	Please add “DESS (<i>Yoder et al. 2006</i>) can be...”
45	1772-1774	“Other layers should be sieved and the residues examined as above or preserved in 96% Ethanol.” Please replace with “ <i>The deeper sediment</i> layers should be sieved and the residues examined as above or preserved in 96% Ethanol.”
45	1774-1775	“Polychaete should be preserved in cold 80% Ethanol, nematodes in DESS (and stored at 4°C), and all other groups in cold 96% Ethanol.” Please replace with “ <i>Polychaetes</i> should be preserved in cold (<i>-20°C</i>) 80% Ethanol, nematodes in DESS (and stored at 4°C), and all other groups in cold (<i>-20°C</i>) 96% Ethanol.”
45	1775-1776	“The ethanol should be changed after 24-48 hours and the samples stored at -20°C.” Please replace with “The ethanol should be changed after 24-48 hours (<i>to prevent dilution</i>), and the samples stored at -20°C.”
45	1784-1786	“Metazoan meiofauna should be sampled using the methodology outlined in ISA Technical Study No. 7: Marine Benthic Nematode Molecular Protocol Handbook (Nematode Barcoding).” Please replace with “ <i>Part of the sampling methodology (i.e., core retrieval from the multicorer, slicing of sediment cores) for Metazoan meiofauna should follow the methodology</i> outlined in ISA Technical Study No. 7: Marine Benthic Nematode Molecular Protocol Handbook (Nematode Barcoding).”
45	1787-1790	“For biodiversity analyses, meiofauna should be restricted to those elements of the sediment fauna commonly recognised as meiofauna, e.g. nematodes, harpacticoid copepods, kinorhyncha, etc. Macrofaunal elements captured in meiofaunal samples can be noted but should not be included in the meiofaunal abundance estimates.” Please replace with “For biodiversity analyses, meiofauna should be restricted to those <i>taxa</i> of the sediment fauna commonly recognized as meiofauna, e.g., nematodes, harpacticoid copepods, <i>Kinorhyncha</i> , etc. Macrofaunal <i>taxa (e.g., polychaets and Tanaidacea)</i> captured in meiofaunal samples can be noted but <i>do not need to be</i> included in the meiofaunal abundance estimates.”
45	1795	Please add: “Metabarcoding can be done on meiofauna extracted from the sediments or on sediment samples as such; the latter would constitute eDNA samples.”
45	1797-1799	“Therefore, different analyses should be prioritised in advance of each deployment with the least disturbed cores assigned to those with the highest priority, with the priority rankings being rotated between deployments.” Please replace with “Therefore, different analyses should be prioritised in advance of each deployment with the least disturbed cores assigned to those with the highest priority (<i>depending e.g., on the data already available</i>), with the priority rankings being rotated between deployments.”

46	1800-1802	<p>“Overlying water of the core for metazoan meiofaunal analyses should be siphoned off over a 32 µm sieve with the use of a 1802 plastic hose, and processed together with the surface sediments.”</p> <p>Please replace with “Overlying water of the core for metazoan meiofaunal analyses should be siphoned off over a 32 µm sieve with the use of a plastic hose, <u>and the sieve residue should be</u> processed together with the surface sediments.”</p>
46	1803-1804	<p>“Typically, the presence of nodules prevents slicing, 1804 in which case the entire unsliced 0-5cm section of the core should be preserved.”</p> <p>Please replace “should” with “<u>may</u>”.</p>
46	1811	<p>“Preservation of meiofaunal samples should be explicitly mentioned.”</p> <p>Please replace with “<u>The temperature and chemical solution (type and concentration) used for the preservation</u> of meiofaunal samples should be explicitly mentioned. <u>The intended analysis determines the type of sample preservation needed.</u>”</p>
46	1822	<p>“At least one core should be subsampled for metabarcoding of small-sized eukaryotes (protists and metazoans)”</p> <p>Please replace with “At least one core should be subsampled for <u>eDNA</u> metabarcoding of small-sized eukaryotes (protists and metazoans).”</p>
46	1823	<p>“From each, three sediment subsamples (approximately 2 ml volume) should be taken using a sterile spoon, placed directly in plastic vials with 5 ml of a suitable soil preservation solution and stored at -20°C.”</p> <p>Please replace with “From each <u>core</u>, three sediment subsamples (approximately 2 ml volume) should be taken using a sterile spoon, placed directly in plastic vials with 5 ml of a suitable soil preservation solution and stored at -20°C.”</p>
46	1826-1827	<p>“Where nodules are encountered, these should be preserved separately for further analyses of crevice fauna.”</p> <p>Please replace with “Where nodules are encountered, these should be preserved separately for further analyses of <u>nodule-associated</u> fauna.”</p>
46	1829-1831	<p>“For metazoan meiofauna, a flotation and centrifugation (4000 rpm) method should be used as it is known to yield up to >80% of the fauna (McIntyre and Warwick, 1984).”</p> <p>Please replace with “For metazoan meiofauna, a flotation and centrifugation (<u>e.g., 1905 rcf method</u>) should be used as it is known to yield up to >80% of the fauna (McIntyre and Warwick, 1984).”</p>
47	1848	<p>“Sieve residues should be stained in Rose Bengal solution (1 g in 1 litre tap water), for example, by placing the sieve containing residue in a dish of stain solution overnight and then washing the residue on the sieve to remove excess stain.”</p> <p>Replace with ““Sieve residues <u>for morphological analyses</u> should be stained in Rose Bengal solution (<u>e.g., 1 g in 1 l</u> tap water), for example, by placing the sieve containing residue in a dish of stain solution overnight and then washing the residue on the sieve to remove excess stain.”</p>

47	betw. para. 270 and 271	<p>Suggestion to add additional paragraph:</p> <p>“Potential target loci for nematode (meta)barcoding and suggested primer sets can be found in ISA Technical Study No. 7: Marine Benthic Nematode Molecular Protocol Handbook (Nematode Barcoding). For other meiofaunal taxa, the relevant peer-reviewed scientific literature needs to be consulted. For metabarcoding, library preparation can, for instance, be completed following the Illumina suggested protocol for the MiSeq platform (https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf) using universal eukaryote primers SSUF04 × SSUR22 targeting the V1-V2 region of the 18S rRNA gene.”</p>
47	1874-1875	<p>“All polymetallic nodules in the sediment should be carefully removed, photographed and examined for the presence of epifauna.”</p> <p>Please replace with “<i>At least part of the</i> polymetallic nodules in the sediment should be carefully removed, photographed and examined for the presence of epifauna.”</p>
47	1877-1879	<p>“For metazoan meiofauna, all the epifaunal organisms should be photographed immediately, carefully removed from the nodule and stored in 90% ethanol for further microscopic and other laboratory analysis.”</p> <p>Please replace with “All the epifaunal organisms attached to the exterior surface of the nodules should be photographed immediately, carefully removed from the nodule and stored in <u>96%</u> ethanol for further microscopic and other laboratory <u>analyses.</u>” (Reason: Most of the nodule epifauna is NOT metazoan meiofauna, but rather macrofauna or protozoan meiofauna (forams).</p>
47	1879-1880	<p>“Each nodule should be then washed separately on a 25µm mesh sieve and stored in 90% ethanol along with the sieved material for further analysis.”</p> <p>Please replace with “<u>The surface of</u> each nodule should then be washed separately on a <u>32µm mesh sieve and the sieved material should be considered part of the ambient sediment fauna.</u>”</p>
47	add after line 1880	<p>Please delete “The soft sediment on the nodule should also be washed separately, preferably on a fine-mesh sieve (20-25 µm) and sieved material should be considered as part of the fauna containing sediment of their respective layers.”</p> <p>Please instead add “<u>The fixative needed to store the nodules in is determined by the intended further analyses. For morphological analyses of nodule crevice meiofauna, nodules can be stored in buffered formaldehyde. For molecular, whether or not combined with morphological analyses, nodules can be stored in DESS at 4°C or frozen (-20°C). Do note that the fixation may have an impact on the physical integrity of the nodules</u>”</p>

47	1883-1885	<p>“In the laboratory, nodules should be examined for crevice fauna, washed, measured (comment: please specify what exactly needs to be measured) and weighed. The clean nodules should be broken down mechanically to sand-sized grains and fixed in 90% ethanol. This will yield the sample to be considered as nodule crevices fauna.”</p> <p>Replace with <u>“For nodule crevice metazoan meiofauna, nodules should be carefully washed to remove adhering sediments, measured and weighed. The clean nodules should be broken down mechanically (if needed) (comment: Crevice fauna is inside the nodules, so how can you inspect crevice fauna without breaking the nodules?) and fixed in e.g., buffered formaldehyde or DESS for morphological and molecular investigations, respectively.”</u></p>
47	1886-1888	<p>“This sample then can be processed using any standard meiofauna extraction procedure. However, it is recommended to use a flotation and centrifugation (4000 rpm) method which is known to yield up to >80% of the fauna (McIntyre and Warwick, 1984).”</p> <p>Replace with “This sample then can be processed using any standard meiofauna extraction procedure. However, it is recommended to use a flotation and centrifugation (e.g., 1905 rcf) method which is known to yield up to >80% of the fauna (McIntyre and Warwick, 1984).</p>
48	1889-1892	<p>“The supernatant has to be then washed onto <u>32 µm</u> mesh size sieve. The sieve residue should be carefully examined under a stereo-microscope (e.g., >40× magnification). All the faunal organisms should be identified to the species level, counted, sorted and stored separately in 90% ethanol so they can later be used for molecular identification.”</p> <p>Replace with “The supernatant has to be then washed onto 20-32 µm mesh size sieve. The sieve residue should be carefully examined under a stereo-microscope (40× magnification). All the faunal organisms should be identified to the <u>lowest taxonomic level possible</u>, counted, sorted and stored separately in <u>DESS at 4°C</u> so they can later be used for molecular identification.”</p>
51	2042-2044	<p>“Infauna samples (a minimum of 10-12 randomly selected sites) or natural isotope abundance for food-web analysis should be sampled at 0-1, 1-2 cm for meiofauna and 0-1, 1-5 and 5-10 cm for macrofauna.”</p> <p>Replace with “Infauna samples (a minimum of 10-12 randomly selected sites) for natural <u>carbon and nitrogen</u> isotope abundance should be collected for food-web structure analysis. Sediments should be sampled at <u>0-5 cm (bulk, multiple corers) for meiofauna (in at least 3 replicate MUC corer per site) and 0-1, 1-5 and 5-10 cm for macrofauna (for identification analysis). For macrofauna at least 5 individuals per family/species per location should be analyzed therefore when individuals would be too small to give a reliable isotopic signal or not abundant enough to be analyzed as single species/family, several individuals should be pooled together in one single replicate species/family/taxon sample.</u> (Comment: We are not sure if you will have enough individuals (biomass) for stable isotope analysis if you slice the sediment. In this case, it might be better to take bulk samples)</p>

51	2049-2053	<p>“Meiofauna for stable isotope analysis should be sampled using megacores or multicorers and sampled from the 0-1cm and 1-2cm layers. They should be sieved over a 32 or a 63 μm sieve using cold (0-2°C) filtered seawater. Macrofauna should be collected using a 0.25m² box-corer and sampled at 0-1, 1-5 and 5-10 cm sediment depth and the sediment slices sieved on a 300 μm sieve using cold-filtered seawater.”</p> <p>Replace with “Meiofauna for stable isotope analysis should be sampled using megacores or multicores and from the <u>0.5 cm sediment layer. The sediments need to be stored frozen (at least -20°C) without any preservative till further analysis in the home laboratory.</u> They should be sieved over a <u>32 μm sieve</u> using <u>CFS (0-2°C).</u> Macrofauna should be collected using a 0.25 m² box corer and sampled at e.g., <u>0-3, 3-5, and 5-10 cm</u> sediment depth and the sediment slices sieved on a 300 μm sieve using <u>CFS. Alternatively or additionally, the epibenthic sledqe can be used to collect macrofauna samples for stable isotope analysis.</u></p>
52	2063-2068	<p>“Meiofauna and macrofauna sieve residues should be placed in a plastic bag and flash-frozen in liquid Nitrogen, and subsequently stored at -20°C. Alcohol-based fixatives should never be used when fixing samples for stable isotopes.” Replace with “<u>Sediments for meiofauna stable isotope analysis need to be stored at -20°C. Macrofauna</u> sieve residues should be placed in a well-sealed container and <u>(flash-frozen in liquid nitrogen)</u> and subsequently stored at -20°C. Alcohol-based fixative should never be used when fixing samples for stable isotopes <u>and formaldehyde-based fixative should be avoided. If formalin needs to be used, it should be used across the entire study and in the same concentration to allow for comparison since after 6 months the effect of a specific formalin solution seems to stabilize its depletion effect on the $\delta^{13}\text{C}$ values across the same species/taxa (Edwards et al. 2002, Lau et al. 2012, Pasotti et al. 2015 and references therein).</u></p> <p>References: Edwards et al. (2002) Short- and long-term effects of fixation and preservation on stable isotope values ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$) of fluid-preserved museum specimens. Copeia 4, 1106-1112. https://www.jstor.org/stable/1448532 Lau et al. (2012) Preservation effects on C/N ratios and stable isotope signatures of freshwater and benthic macroinvertebrates. Limnology and Oceanography 10(2), 75-89. https://doi.org/10.4319/lom.2012.10.75 Pasotti et al. (2015) Benthic trophic interactions in an Antarctic shallow water ecosystem affected by recent glacier retreat. PLOS ONE 10(11), e0141742. https://doi.org/10.1371/journal.pone.0141742</p>
52	2070	<p>Please add “<u>Given their small size, typically a high minimal number of meiofauna (and often macrofauna) individuals per taxon is required for reliable signals of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (depending on the GS-IRMS lower sensibility at the selected Analysis Facilities; minimum values can vary as</u></p>

		<u>greatly as 5-20µg C and 1-4 µg N). In general, a subsample of megafaunal organisms will suffice for stable isotope analysis, whereas for meio- and macrofauna entire individuals or even samples consisting of multiple individuals will be needed.</u>
52	2070-2071	<p>“Fauna should be washed of attached organic debris in cold, filtered seawater and placed in pre-weighed tin or silver (if calcareous) isotope analysis cups. Target tissues (e.g., body wall, muscle, ophiuroid arm) from megafauna should be removed in the laboratory, taking care to minimize tissue warming, and placed on foil.”</p> <p>Please replace with “Fauna should be washed of attached organic debris in <u>CFS</u> and placed in pre-weighed tin or silver (if calcareous, <u>to add HCl</u>) isotope analysis cups. Target tissues (e.g., body wall, muscle, ophiuroid arm) from megafauna should be removed in the laboratory, taking care to minimize tissue warming, and placed on <u>aluminum</u> foil.”</p>
52	2082-2084	<p>“To quantify the dominant food-types for the fauna, sediment trap POM samples and sediment samples (section V.H) should also be prepared for stable isotope analyses and their isotope signatures corrected if samples have been preserved in formaldehyde solution.”</p> <p>Replace with “To quantify the dominant food-<u>items</u> for the fauna, sediment trap POM samples and sediment samples (section V.H) should also be prepared for stable <u>carbon and nitrogen</u> isotope analyses and their isotope signatures corrected if samples have been preserved in formaldehyde solution.”</p>
53	2110	Please delete “or 63 µm”
53	2123	Please replace “(13C, 15N)” with “(<u>δ¹³C, δ¹⁵N</u>)”
53	2126	Please add “(see above)” at the end of the sentence “for formalin preservation (<u>see above</u>).”
53	2130-2132	<p>“The parameters that should be recorded for natural isotope analysis are species lists, delta 13C signatures, delta 15N signatures, and means together with number of samples and appropriate error estimates”</p> <p>Please replace with “The parameters that should be recorded for natural isotope analysis are species lists, <u>δ¹³C and δ¹⁵N signatures, biomass (in terms of µg C and N), and means together with number of samples and appropriate error estimates.</u>”</p>
53	2139	Please replace “(13C, 15N)” with “(<u>δ¹³C, δ¹⁵N</u>)”
57	2279	<p>Suggested addition: “The abundance data reported should be checked for quality by referring to (and comparing with) published (or otherwise available) information provided by studies carried out in the same habitat/area.”</p> <p>Comment: The quality of faunistic data submitted should be addressed, similarly to the quality of abiotic data mentioned earlier.</p> <p>In some instances faunistic data submitted to ISA are widely diverging from what could be expected, for example, because of an inappropriate gear and methodology used (e.g. for the meiofauna, subsampling of a box</p>

		corer after the overlying water has been removed!). Submission of such data should be treated as non-compliance.
57	2311	Please spell out "RDP" before using this abbreviation for the first time.
58	2329-2334	Please provide references for the mentioned numbers of 25 nodules and 3 box cores.
58	2356	Please add "or BOLD (Barcode Of Life Database, https://www.boldsystems.org/)."
59	2372	The bibliography should be carefully checked as it is incomplete; references listed in the text are missing.