

Jennifer Le ORCID iD: 0000-0002-6004-8941

Manuscript title: Scientific and budgetary tradeoffs between morphological and molecular methods for deep-sea biodiversity assessment

Author names: Jennifer T. Le^{1*}, Lisa A. Levin¹, Franck Lejzerowicz², Tristan Cordier³, Andrew J. Gooday^{4,5}, Jan Pawlowski^{3,6,7}

Author affiliations: ¹Center for Marine Biodiversity and Conservation, Scripps Institution of Oceanography, University of California San Diego, La Jolla, USA; ²Jacobs School of Engineering, University of California San Diego, La Jolla, USA; ³Department of Genetics & Evolution, University of Geneva, Geneva, Switzerland; ⁴National Oceanography Centre, Southampton, UK; ⁵Life Sciences Department, Natural History Museum, Cromwell Road, London SW7 5BD, UK; ⁶Institute of Oceanology, Polish Academy of Sciences, Sopot, Poland;

⁷ID-Gene Ecodiagnostics, Campus Biotech Innovation Park, Geneva, Switzerland

*Corresponding author email and address: jtl025@ucsd.edu; 1315 East West Highway, Silver Spring, MD 20910

Acknowledgements: The authors declare no conflicts of interest. This work was supported by the Swiss Network for International Studies award (20170024), the Swiss National Science Foundation (316030_150817, 31003A_159709), the University of California San Diego Frontiers of Innovation Scholars Program (g001), and the JM Kaplan Fund. The authors thank A. Vanreusel, C. Neira, H. Bik, C. Cetean, A.K. Enge, F. Frontalini, F. Jorissen, M. Kaminski, M. Nardelli, and J. Schönfeld for input regarding methodologies; G. Gilron and S. Smith for their feedback as editors of this Special Issue

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/ieam.4466.

This article is protected by copyright. All rights reserved.

as well as two anonymous reviewers for their insightful comments; and D. Squires, R. Carson, G. Rouse, and E. Allen for manuscript advice.

Data availability statement: Data and script are available by request to the corresponding author.

Editor's note: This article is part of the special series entitled: "Implications of Deep-sea Mining on Marine Ecosystems." The series comprises the current state of the science regarding deep-sea ocean ecosystems and the likely ecological footprints, risks, and consequences of deep-sea mining. There is a focus on: impact assessment; policy solutions, and practices to aid in the implementation of industry guidance prepared by the International Seabed Authority and other authorities, new monitoring and assessment methods, best management practices, and emerging scientific research related to deep-sea ecosystems.

Abstract: Deep-sea biodiversity, a source of critical ecological functions and ecosystem services, is increasingly subject to the threat of disturbance from existing practices (e.g., fishing, waste disposal, oil and gas extraction) as well as emerging industries such as deep-seabed mining. Current scientific tools may not be adequate for monitoring and assessing subsequent changes to biodiversity. In this paper, we evaluate the scientific and budgetary trade-offs associated with morphology-based taxonomy and metabarcoding approaches to biodiversity surveys in the context of nascent deep-seabed mining for polymetallic nodules in the Clarion-Clipperton Zone, the area of most intense interest. For the dominant taxa of benthic meiofauna, we discuss the types of information produced by these methods, and use cost-effectiveness analysis to compare their abilities to yield biological and ecological data for use in environmental assessment and

management. Based on our evaluation, morphology-based taxonomy is less cost-effective than metabarcoding but offers scientific advantages, such as the generation of density, biomass, and size structure data. Approaches that combine the two methods during the environmental assessment phase of commercial activities may facilitate future biodiversity monitoring and assessment for deep-seabed mining and for other activities in remote deep-sea habitats, for which taxonomic data and expertise are limited.

Keywords: biodiversity, taxonomy, metabarcoding, deep-seabed mining, environmental assessment

INTRODUCTION

Deep-seabed mining (DSM), which could begin commercial production in the coming decades, has raised concerns about potential biodiversity loss (Niner et al., 2018). DSM is expected to alter targeted habitats through physical disturbance and substrate removal as well as sediment resuspension and deposition (Weaver et al., 2018; Christiansen et al., 2019). The Clarion-Clipperton Zone (CCZ), an area that spans 4.5 million km² between Mexico and Hawaii, hosts 16 mineral exploration contract areas (3900-5500 m water depth; each up to 75,000 km² of which up to 50% will be mined) with high densities of polymetallic nodules (ISA, 2010) and biodiversity that shows limited ability to recover from disturbance within several decades (Simon-Lledó et al., 2019; Vonnahme et al., 2020). The International Seabed Authority (ISA), the governing body for international mineral resources, has been mandated to manage the mineral resources of the Area (i.e., international seabed beyond national jurisdiction) while also ensuring the “protection of the marine environment from harmful effects” and “prevention of damage to the flora and fauna of the marine environment” (UNCLOS,

1982). As such, the ISA is developing regulations for mining and has issued guidance related to environmental baseline studies (ISA, 2020). These highlight the need to: (1) establish biodiversity baselines for targeted habitats; (2) predict environmental effects on biodiversity and establish environmental management practices to minimize biodiversity loss (such as set-aside areas); and (3) monitor changes in biodiversity due to mining activities and validate predicted effects.

Current biodiversity monitoring generally employs morphology-based taxonomy (MBT) (e.g., Schönfeld et al., 2012), which can be time-consuming and is limited to taxa that exhibit distinguishing morphology and for which taxonomic expertise is available. These limitations can hamper scaling up spatial and temporal resolution of monitoring and limit rapid adaptive management measures. Molecular tools, like metabarcoding of environmental DNA (eDNA), can provide an alternative to monitor and assess benthic diversity (Porter & Hajibabaei, 2018). eDNA methods based on water samples have the benefit of being non-destructive and less invasive than traditional trawl or net tow approaches; however, this is not the case for biodiversity assessments of sediment infauna, as both MBT and eDNA require coring. Updated ISA exploration guidance for environmental impact assessments (EIAs) includes the collection of eDNA samples to generate molecular data for whole biological communities (ISA, 2020).

In this paper, we compare the scientific and budgetary trade-offs between MBT and metabarcoding (i.e., sequencing of specific gene markers in an environmental sample) for DSM-related benthic diversity monitoring and assessment of small eukaryotes. As a case study, we target the CCZ, a region where commercial interest in polymetallic nodules is particularly intense. Biodiversity in the CCZ largely comprises of

small, rare, patchily-distributed metazoan meiofauna and foraminifera (Lins et al., 2016; Gooday & Goineau, 2019). Metabarcoding is based on small sediment samples, and therefore particularly well-suited to assessing the biodiversity of these small-sized organisms. Decision networks are constructed for each approach to highlight how choices within each affect scientific outcomes and budgetary costs. The cost-effectiveness of each methodology, as well as a combined approach, is assessed.

METHODS

For each methodology (i.e., MBT and metabarcoding), we surveyed deep-sea experts who have worked extensively in the CCZ and published protocols to determine workflows from sample collection to data generation (Bourlat et al., 2016; Fonesca & Lallias, 2016). Steps that require a decision were identified to evaluate the effect of each option on scientific outcomes and budgetary costs. In most cases, scientific questions and desired outcomes (e.g., targeted taxa) dictate how choices are made, creating a range of appropriate protocols, so only general steps are listed in the results.

Deep-sea experts also provided an exhaustive list of consumables and estimates of the work hours required to generate taxonomic data as model parameters for the cost-effectiveness analysis (CEA) (Garber & Phelps, 1997). In economics, the long run refers to a period of time in which all inputs are variable whereas in the short run, at least one variable is fixed. We model the short run because DSM has yet to begin commercially but also provide long-run considerations in the discussion. Model input is defined as total short-run variable cost (TC) (Equations 1-2; metabarcoding is denoted with “meta”):

$$TC_{MBT} = (consumable\ costs_{MBT} + labor\ costs_{MBT}) \times number\ of\ samples_{MBT}$$

(Equation 1)

$$TC_{meta} = (\text{consumable costs}_{meta} \times \text{number of samples}_{meta}) + \text{labor costs}_{meta} \\ + (\text{sequencing costs} \div \text{number of samples}_{meta})$$

(Equation 2)

For each direct consumable, prices (nominal 2016 USD) from common U.S. suppliers (i.e., Fisher Scientific, VWR, Qiagen) were averaged and multiplied by quantity required. Indirect labor costs were developed from best estimates of work hours (i.e., time spent actively transforming sediment samples into taxonomic data) and a 2016 U.S. PhD student salary of \$29,500. We assume that laboratories have basic amenities and access to people who are trained to do these tasks, i.e., identifying target taxa and preparing sediment samples for sequencing. Other non-human capital costs, such as specialized equipment and bioinformatics pipelines, are held fixed in the short run.

To adequately compare the methodologies, we analyzed sampling design and scientific findings of published meiofaunal studies that characterize CCZ benthic diversity using either MBT or metabarcoding (Table 1). Model output was defined as the number of identified operational species, a proxy for actual species because sampled deep-sea organisms are often new to science (i.e., not yet described taxonomically) (Rosli et al., 2018), and this is especially true for the CCZ (Lejzerowicz et al., 2021). Probability distributions were assigned to each variable (Table 2) and a Monte Carlo simulation (100,000 iterations) was conducted to estimate the median cost to identify an operational species and efficiency (i.e., output divided by input).

RESULTS AND DISCUSSION

Scientific trade-offs between morphology-based taxonomy and metabarcoding

Scientific trade-offs between MBT and metabarcoding are summarized in Table 3 (described in detail in Note S1). MBT can readily provide absolute abundance, morphological features, body size, and biomass, which have been used to assess ecosystem health (Siddig et al., 2016), whereas metabarcoding cannot on a practical level. Although metabarcoding can provide some of this information through conducting species-specific calibration studies (e.g., Lacoursiere-Roussel, Cote, Leclerc, & Bernatchez, 2016), the large number of unknown species in the CCZ make that impossible at this time (Lejzerowicz et al., 2021). Whether these types of data are required to understand DSM impacts in international waters will depend in part on the environmental goals and objectives set by the ISA (Tunncliffe et al., 2020). If these data types are considered essential to baseline data collection and monitoring, then MBT will be necessary. If these data types are not considered essential, then a molecular approach may be sufficient for most purposes once robust databases are established. CCZ eukaryotic diversity dominance by small, rare taxa (Gooday & Goineau, 2019) may favor a molecular approach. Additionally, molecular approaches may be preferred to shorten management response times that may determine the extent of environmental damage that ensues, such as identifying environmental thresholds and triggers, and the potential for CCZ ecosystem recovery, which may be negligible over human timescales (Stratman et al., 2018).

There are additional challenges unique to deep-sea biodiversity surveys (whether for basic science, environmental management of mining, oil and gas extraction, or bottom fisheries) that include particular logistical and interpretative considerations. The study of deep-sea biodiversity in abyssal zones, such as the CCZ, is made more challenging by vast areas, remote

locations (and thus extended travel times), requirement for advanced technologies, and high cost of study, together yielding a lack of biodiversity data and a largely undescribed fauna comprised of small and rare taxa. Within the CCZ claim areas that span a longitudinal distance of almost 4500 km (larger than the continental U.S.), data limitation leads to high levels of uncertainty about the diversity, composition, and functions of benthic fauna (Sinniger et al., 2016; Shulze et al., 2017). Deep-sea samples often yield species new to science that are often represented by only a few individuals, which can make accurate estimates of biodiversity difficult (Bonifacio et al., 2020). The scarcity of data demands a precautionary approach, wherein adequate measures are taken in order to manage risk (Rio Declaration, 1992; ISA, 2018). It also underlines the importance of collecting robust baseline data that are critical for protection of the marine environment (Durden et al., 2018), as well as exhaustive monitoring, especially during pilot mining and at the early stages of commercial exploitation.

One challenge facing both MBT and molecular approaches in addressing these data gaps is developing an adequate sampling design. Patchy biodiversity distributions make it difficult to identify appropriate spatial and temporal scales scientists need to sample for accurate characterization (Lejzerowicz et al., 2015). Whereas coastal and shallow-water processes are better constrained with more empirical data, e.g., allowing for accurate modeling and simulations (e.g., Shen et al., 2018; Petersen & Herkul, 2019), it can be challenging to design biodiversity surveys that take into account the extended lifespans, heterogeneity experienced by small-bodied taxa, extent and duration of disturbance characteristic of DSM, diversity of microhabitats, and other factors. In the CCZ, there can be unexpectedly high spatial heterogeneity (Goineau & Gooday, 2019; Simon-Lledó et al., 2019). Additionally, adequate temporal resolution of sampling for

accurate characterization of deep-sea biodiversity is unknown (Francesca et al., 2021). Both MBT and metabarcoding often provide one snapshot in time and, given time constraints associated with MBT, a comprehensive monitoring program might incorporate both methodologies by employing metabarcoding at shorter time intervals and MBT at longer.

Another consideration is that the ISA has yet to adopt a standard methodology for baseline data collection in nodule provinces for the 18 total mining exploration claims from 17 different contractors. Differing methodologies, of which there can be many, can create challenges when trying to compare data across contract areas. This is especially relevant to the CCZ environmental management plan that considers environmental protection and regulation on a regional scale rather than a contract-by-contract basis (ISA, 2011), similar to the FAO regional fisheries management organizations. ISA data collection guidelines are also pivotal because national regulations must be at least as stringent as international regulations by precedent.

Budgetary trade-offs between morphology-based taxonomy and metabarcoding

Choices within the decision networks (Figure 1) are discussed in terms of how they affect short-run total variable costs and include those related to collecting samples and processing samples. In the long run, as more samples are taken, metabarcoding processing costs tend to decrease e.g., splitting costs among more samples and producing reagents in bulk. Additionally, as more samples are taken, the probability of detecting previously identified species using either method increases, which will decrease the cost of subsequent processing.

Sampling costs. Deep-sea samples, whether sediment, water, or individual organisms, are relatively expensive to collect because they typically require research expeditions on global-class vessels equipped with specialized instruments for positioning, bathymetric mapping, and sampling. One day of ship time on a U.S. global class vessel can range from 42,000-48,000 USD, which includes food, fuel, accommodations, crew, and two technicians (Elizabeth Brenner, Scripps Institution of Oceanography, personal communication). It takes approximately 6 days to sail from the nearest continental U.S. port in San Diego, CA to the eastern edge of the CCZ at full speed without stopping, resulting in over half a million dollars in transit time roundtrip. This cost estimate does not include the use of remotely-operated vehicles (ROVs) that can collect sediment samples and conduct visual surveys and are commonly used in the CCZ. The U.S. National Deep Submergence Facility (NDSF) operates ROV *Jason* at a day-rate of 23,000 USD (NDSF, 2018). Other common sampling equipment includes box corers, multi-corers, epibenthic sleds, and CTD rosettes, all of which can be deployed without special vehicles and are cheaper to use. These required costs make the deep sea accessible only to wealthy nations with advanced fleets, in contrast to coastal and shallow water environments that can be more easily and inexpensively accessed. As a result, time at-sea for baseline surveys is budgeted to produce the most useful outcomes, often resulting in interdisciplinary collaborations among scientists.

Sampling details for studies used in this paper to evaluate methodologies are summarized in Table 1. Sediment samples were taken from the CCZ with multi-corers, box corers, or an epibenthic sled in one case; the same sampling devices can be used for both approaches. The total number of samples (counting one sediment core vertical

fraction as one sample) used for analysis ranged from 5-41 and 15-42 per cruise for MBT and metabarcoding, respectively. Sampling costs are the largest contributing factor to the cost of generating biodiversity data, larger than processing costs, which poses a challenge specific to deep-sea studies. However, on-site sampling costs between the two analytical approaches within the same system should not differ significantly. The different methods used on shore in the laboratory to process deep-sea sediment samples for MBT and metabarcoding are therefore the major factor.

Sample processing costs for morphology-based taxonomy. Most MBT workflow decisions (Figure 1) depend upon target taxa and size category, which will determine, for example, the appropriate sieve mesh size. Consumables required for MBT are common in most labs and are readily available (Table S1); costs range from approximately \$13.10 to \$16.29 USD per sample. Labor costs range from approximately \$153 to \$2,876 per sediment core slice, comprising the majority of MBT costs and varying greatly with target taxa, their abundance, and the sieve mesh-size fraction (Table S2). The higher costs are associated with the inclusion of monothalamids (i.e., single-chambered foraminifera that are abundant, diverse, and largely undescribed in the CCZ) (Goineau & Gooday, 2019; Gooday & Goineau, 2019). A comprehensive CCZ biodiversity assessment should not ignore monothalamids, but it may be more practical to confine monitoring to multi-chambered foraminifera, which are taxonomically better known, less diverse, and easier to study. Consultancy companies can rapidly sort and identify foraminifera, often for less than \$100 USD per sample (Claudia Cetean, CGG Robertson and Fabrizio Frontalini, University of Urbino; personal communication), although these analyses are less detailed than full-scale scientific studies, e.g., they have lower resolution. However, ISA

guidelines will determine whether additional information from scientific analyses is necessary. These consumable costs are likely applicable to other systems, but labor costs will differ widely given the abundance of organisms within a sediment core and which taxa are present. Relative to coastal and shallow water systems, there exists much less taxonomic expertise for the fauna of the deep ocean, especially for small class sizes. Dominance by small and rare taxa (Gooday & Goineau, 2019), for example, makes it more difficult to obtain baseline biodiversity data using MBT. Applying a more ‘commercial’ approach to analyzing samples from abyssal sites may help to alleviate this problem, although it could also take longer than in shallow-water settings because the lower faunal densities will require a greater volume of sediment to be sorted in order to recover sufficient numbers of specimens.

Sample processing costs for metabarcoding. The options for processing marine sediments for metabarcoding are reviewed elsewhere (e.g., Ruppert et al. 2020; refer to Note S2 for more detail). Consumable costs for this processing range from approximately \$15 to \$55 USD (Table S3). Labor costs are relatively low, as samples are often processed in large batches, (e.g., in a 96-well plate), and sequencing does not require ‘active’ work hours (Table S4). A metabarcoding approach can also be used in parallel with other ‘-omics’ methods (e.g., metatranscriptomics), if relevant to study objectives (Franzosa et al., 2015). These costs are likely similar when working with marine sediments in any system. One deep-sea consideration is that low bottom temperatures and other environmental parameters lead to high eDNA preservation in deep-sea sediments (Collins et al., 2018; Laroche et al., 2020a). Additionally, eDNA transport and transformation during its descent and settlement are active areas of research, and future

findings could impact how metabarcoding data are interpreted. Scaling up metabarcoding, or other molecular analyses, of CCZ sediments is likely more amenable, given current budgetary and labor restriction, and the ISA is pursuing this channel (ISA, 2020). Although interpretation of molecular data may not be the same as coastal and shallow water systems, genetic sequences provide an immutable trait of an organism that can be compared across space and time.

Cost-effectiveness analysis. Median cost to identify an operational species (without a formal description) was \$32.54 (standard deviation (SD) \$37.55) for MBT and \$3.32 (SD \$1.36) for metabarcoding. Expected mean efficiency (i.e., output divided by input), for metabarcoding (i.e., 0.316) was one order of magnitude greater than MBT (cf. 0.042). MBT labor costs drive the majority of MBT total cost. One advantage of metabarcoding is that samples can be prepared and processed together, which results in labor cost savings. Technology that could reduce labor costs, such as artificial intelligence to identify taxa from photographs or genetic data, could increase the cost-effectiveness of both MBT and metabarcoding (Cordier et al., 2019). While it is likely that metabarcoding will be generally more cost-effective than MBT, the difference may be exacerbated in the CCZ by the large numbers of small, rare, undescribed species (Lejzerowicz et al., 2021). However, the additional knowledge gained from MBT in areas that are severely under-sampled provides a great benefit to science and research, especially if commercial mining is to begin and baseline data are required. MBT may also be the only way to evaluate the diversity of certain foraminifera, notably members of the Komokiacea and other taxa that accumulate stercomata (Gooday et al., 2021). These groups are very common in abyssal samples but it has not yet been possible to amplify

their DNA (Lecroq et al., 2009). Many are also macrofaunal in size and will not be adequately represented in small eDNA samples.

There is some overlap between cost and efficiency ranges of the two approaches as illustrated in Figure 2. However, there was only a 0.003 probability that metabarcoding was more expensive per operational species than MBT. For the same cost, metabarcoding yields more than 13 times more identified operational species. Number of operational species may be a concept fundamental to putting the environmental management of mining on abyssal plains into effect because of rare species, patchiness, and limited sampling (Francesca et al., 2021). Measurements, such as the Chao 1 estimator (Chao, 1984) and rarefaction curves, can help gauge whether species in an area have been adequately sampled by estimating expected species richness of a community. These approaches could be used to determine whether enough biodiversity data have been collected, e.g., an asymptotic rarefaction curve as an indicator of adequate sampling.

The disparity between MBT and metabarcoding costs is great where there is a need to identify all morphospecies, i.e., species grouped together based upon morphology, for comprehensive biodiversity assessment. In relatively well-studied shelf and upper bathyal environments, monitoring methods have been developed based on representative taxa that reflect ecological responses (Jorissen et al., 2018). It may be possible to extend this strategy to the CCZ, e.g., by targeting indicator species that form habitat structures or those involved in essential ecological processes and ecosystem services (Le et al., 2017; ISA, 2020). Identification of these indicator species could substantially reduce labor costs for both methods.

Combined approaches. Without corresponding morphological information, molecular methods may not be sufficient to establish adequate environmental baselines in the CCZ. A large initial investment of resources is required to establish reference databases using linked MBT and genetic barcodes (Glover et al., 2016), and MBT could be phased out or reduced later (Vivien et al., 2020). The cost for the initial combined approach is the cost of employing both approaches for each sample, although there may be additional costs associated with reconciling data not discussed here, and constrained by the minimum number of operational species identified using each approach:

$$TC_{combined} = (consumable\ costs_{MBT+meta} \times number\ of\ samples) + [(labor\ costs_{MBT} \times number\ of\ samples) + labor\ costs_{meta}] + (sequencing\ costs \div number\ of\ samples) \text{ (Equation 3)}$$

Equation 3 above yields an expected mean efficiency of 0.033 and median cost per operational species of \$41.89 (SD \$46.68). This cost is comparable to that of MBT alone, but a combined approach provides the additional benefit of creating a comprehensive database of taxonomic information that can be used across contract areas over time, to ensure adequate comparisons. The trade-offs between number of operational species identified and additional scientific information can be represented by the following:

$$TC_{combined} = \rho TC_{MBT} + (1 - \rho) TC_{meta} \text{ (Equation 4)}$$

Where ρ is a weight determined by stakeholders (e.g., the ISA, mining industry, scientists) based upon available resources, technology, and information needs. This may

also change over time because as more operational species are characterized by morphology, it becomes less difficult to identify species genetically.

CONCLUSIONS

As two methods for biodiversity assessment, MBT and metabarcoding each have specific scientific and budgetary advantages. The comparable costs and additional benefits derived from combining them can justify short-term investment in biodiversity assessment as an attractive, long-term option for the mining industry, and regulators such as the ISA, to more easily and unambiguously identify species, and compare results from mineral exploration claim areas across space and time. Robust biodiversity monitoring and assessment, which will likely include both MBT and metabarcoding in the initial phases, are essential in order to conserve the integrity of deep-sea habitats targeted for DSM by providing information about potential functions and ecosystem services that these habitats provide (Thurber et al., 2014; Le et al., 2017). There is growing recognition of the value of combined MBT and molecular approaches by the ISA Legal and Technical Commission (ISA, 2020), providing a timely opportunity to incorporate these scientific tools into environmental assessment and management frameworks (Tunncliffe et al. 2020). Exhaustive data collected during pilot DSM could result in modifications to guidelines. This may lead to a cautious and phased approach in which the scale of commercial DSM is increased gradually so that resulting changes to the environment can be thoroughly documented using both MBT and metabarcoding. Scaling up these two methods requires communication among stakeholders, standardization of data collection, and data accessibility, but will yield potential gains in meeting environmental requirements with transparency and relatively low uncertainty. This discussion is

applicable to biodiversity characterizations of other deep-sea ecosystems subject to resource extraction, and to other marine, aquatic, and terrestrial systems that are either: (1) under-sampled and data-limited; (2) not easily accessible; or (3) dominated by meiofaunal-size taxa.

REFERENCES

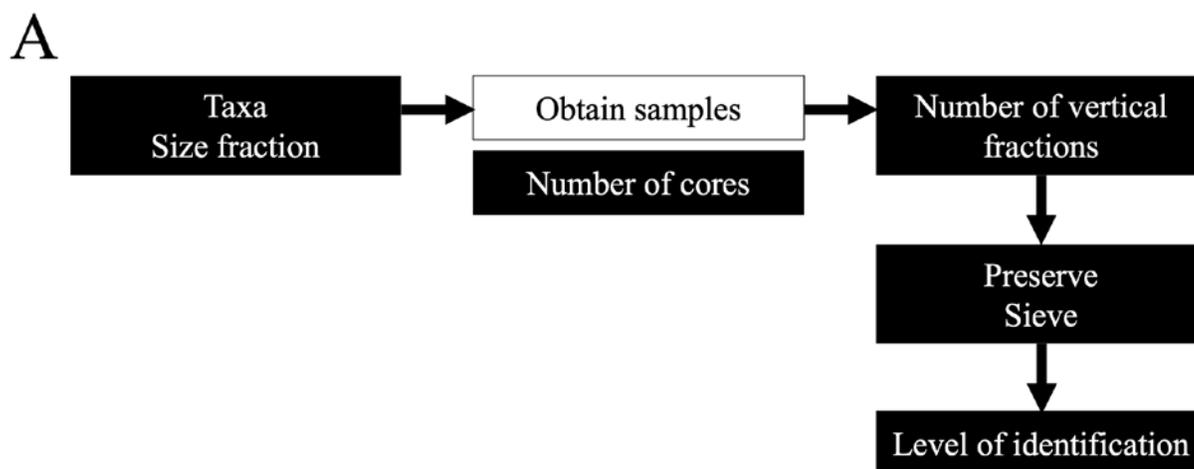
- Bonifacio, P., Arbizu, P.M., and L. Menot. 2020. Alpha and beta diversity patterns of polychaete assemblages across the nodule province of the eastern Clarion-Clipperton Fracture Zone (equatorial Pacific). *Biogeosciences*, 17: 865-886. <https://doi.org/10.5194/bg-17-865-2020>
- Bourlat, S.J. 2016. Preparation of Amplicon Libraries for Metabarcoding of Marine Eukaryotes Using Illumina MiSeq: The Dual-PCR Method. In: Bourlat S. (eds) *Marine Genomics. Methods in Molecular Biology 1452*. Humana Press, New York, NY.
- Chao, A. 1984. Nonparametric Estimation of the Number of Classes in a Population. *Scandinavian Journal of Statistics* 11(4): 265-270.
- Christiansen, B., A. Denda, and S. Christiansen. 2019. Potential effects of deep seabed mining on pelagic and benthopelagic biota. *Marine Policy*. DOI: 10.1016/j.marpol.2019.02.014.
- Collins, R.A., Wangenstein, O. S., O’Gorman, E.J., Mariani, S., Sims, D.W., and M.J. Genner. 2018. Persistence of environmental DNA in marine systems. *Communications Biology*. 1: 185. <https://doi.org/10.1038/s42003-018-0192-6>
- Cordier, T., Lanzén, A., Apothéloz-Perret-Gentil, L., Stoeck, T., and J. Pawlowski. 2019. Embracing Environmental Genomics and Machine Learning for Routine Biomonitoring. *Trends Microbiol.* 27(5):387-397.
- Durden, J.M., Lallier, L.E., Murphy, K., Jaeckel, A., Gjerde, K., and D.O.B. Jones. 2018. Environmental Impact Assessment process for deep-sea mining in ‘the Area’. *Marine Policy*. 87:194-202. <https://doi.org/10.1016/j.marpol.2017.10.013>.
- Fonseca V.G., and D. Lallias. 2016. Metabarcoding Marine Sediments: Preparation of Amplicon Libraries. In: Bourlat S. (eds) *Marine Genomics. Methods in Molecular Biology 1452*. Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-3774-5_12.
- Francesca, P., Mevenkamp, L., Pape, E., Blazewicz, M., Bonafacio, P., Riehl, T., De Smet, B., Lefaible, N., Lins, L., and A. Vanreusel. 2021. A local scale analysis of polymetallic nodules influence on the Clarion-Clipperton Fracture Zone macrobenthos. *Deep Sea Research Part I*, 168: 103449. <https://doi.org/10.1016/j.dsr.2020.103449>
- Franzosa, E. A., T. Hsu, A. Sirota-Madi, A. Shafquat, G. Abu-Ali, X. C. Morgan, and C. Huttenhower. 2015. Sequencing and beyond: Integrating molecular “omics” for microbial community profiling. *Nature Reviews Microbiology* 13:360–372.
- Garber, A. M., and C. E. Phelps. 1997. Economic foundations of cost-effectiveness analysis. *Journal of Health Economics* 16:1–31. DOI: 10.1016/S0167-6296(96)00506-1.
- Glover, A. G., T. G. Dahlgren, H. Wiklund, I. Mohrbeck, and C. R. Smith. 2016. An end-to-end DNA taxonomy methodology for benthic biodiversity survey in the Clarion-Clipperton Zone, central Pacific abyss. *Journal of Marine Science and Engineering* 4. DOI: 10.3390/jmse4010002.
- Goineau, A., and A. J. Gooday. 2017. Novel benthic foraminifera are abundant and diverse in an area of the abyssal equatorial Pacific licensed for polymetallic nodule exploration. *Scientific Reports* 7:45288. DOI: 10.1038/srep45288.
- Goineau, A. & A.J. Gooday. 2019. Diversity and spatial patterns of foraminiferal assemblages in the eastern Clarion–Clipperton zone (abyssal eastern equatorial Pacific). *Deep-Sea Research I*: 149. DOI: <https://doi.org/10.1016/j.dsr.2019.04.014>
- Gooday, A. J., and A. Goineau. 2019. The contribution of fine sieve fractions (63-150 µm) to foraminiferal abundance and diversity in an area of the eastern Pacific Ocean licensed for polymetallic nodule exploration. *Frontiers in Marine Science* 6. DOI: 10.3389/fmars.2019.00114.

- Gooday, A.J., F. Lejzerowicz, A. Goineau, M. Holzmann, O. Kamenskaya, H. Kitazato, S.-C. Lim, J. Pawlowski, T. Radziejewska, Z. Stachowska and B. Wawrzyniak-Wydrowska. 2021. The biodiversity and distribution of abyssal benthic foraminifera and their possible ecological roles: a synthesis across the Clarion-Clipperton Zone. *Frontiers in Marine Science*. DOI: 10.3389/fmars.2021.634726
- Hauquier, F., L. Macheriotou, T. N. Bezerra, G. Egho, P. Martínez Arbizu, and A. Vanreusel. 2019. Distribution of free-living marine nematodes in the Clarion–Clipperton Zone: implications for future deep-sea mining scenarios. *Biogeosciences* 16: 3475-3489. DOI: 10.5194/bg-16-3475-2019.
- International Seabed Authority, 2010. A geological model of polymetallic nodules deposits in the Clarion-Clipperton Fracture Zone. ISA Technical Study No. 6. Kingston, Jamaica.
- International Seabed Authority. 2011. Environmental Management Plan for the Clarion-Clipperton Fracture Zone. ISBA/17/LTC/7.
- International Seabed Authority. 2019. Draft regulations on exploitation of mineral resources in the Area. ISBA/25/C/WP.1. Kingston, Jamaica.
- International Seabed Authority, 2020. Recommendations for the guidance of contractors for the assessment of the possible environmental impacts arising from exploration for marine minerals in the Area. ISBA/25/LTC/6/Rev.1. Kingston, Jamaica.
- Jorissen, J., Nardelli, M.P., Almogi-Labin, A., Barras, C., Bergamin, L., Bicchi, E., El Kateb, A., Ferraro, L., McGann, M., Morigi, C., Romano, E., Sabbatini, A., Schweizer, M., Spezzaferri, S. 2018. Developing Foram-AMBI for biomonitoring in the Mediterranean: Species assignments to ecological categories. *Marine Micropaleontology* 140: 33-45.
- Lacoursiere-Roussel, A., Cote, G., Leclerc, V., and L. Bernatchez. 2016. Quantifying relative fish abundance with eDNA: A promising tool for fisheries management. *Journal of Applied Ecology*. 53: 1148-1157. DOI: 10.1111/1365-2664.12598.
- Lambshhead, P. J. D., C. J. Brown, T. J. Ferrero, L. E. Hawkins, C. R. Smith, and N. J. Mitchell. 2003. Biodiversity of nematode assemblages from the region of the Clarion-Clipperton Fracture Zone, an area of commercial mining interest. *BMC Ecology* 3:1–12. DOI: 10.1186/1472-6785-3-1.
- Laroche, O., Kersten, O., Smith, C.R., and E. Goetze. 2020a. From Sea Surface to Seafloor: A Benthic Allochthonous eDNA Survey for the Abyssal Ocean. *Frontiers in Marine Science*. 8. <https://doi.org/10.3389/fmars.2020.00682>
- Laroche, O., Kersten, O., Smith, C.R., and E. Goetze. 2020b. Environmental DNA surveys detect distinct metazoan communities across abyssal plains and seamounts in the western Clarion Clipperton Zone. *Molecular Ecology*. <https://doi.org/10.1111/mec.15484>.
- Le, J.T., L.A. Levin, and R.T. Carson. 2017. Incorporating ecosystem services into environmental management of deep-seabed mining. *Deep-Sea Research II*. 137:486-503. <http://dx.doi.org/10.1016/j.dsr2.2016.08.007>.
- Lecroq, B., A.J. Gooday, T. Cedhagen, T., A. Sabbatini, and J. Pawlowski. 2009. Molecular analyses reveal high levels of eukaryotic richness associated with enigmatic deep-sea protists (Komokiacea). *Marine Biodiversity* 39, 45–55.
- Lejzerowicz, F., P. Esling, and J. Pawlowski. 2014. Patchiness of deep-sea benthic Foraminifera across the Southern Ocean: Insights from high-throughput DNA sequencing. *Deep-Sea Research Part II* 108:17–26. <http://dx.doi.org/10.1016/j.dsr2.2014.07.018>
- Lejzerowicz, F., P. Esling, L. Pillet, T. A. Wilding, K. D. Black, and J. Pawlowski. 2015. High-throughput sequencing and morphology perform equally well for benthic monitoring of marine ecosystems. *Scientific Reports* 5:13932. DOI: 10.1038/srep13932.
- Lejzerowicz, F., Gooday A.J., Barranechea Angeles, I., Cordier, T., Morard, R., Apothéloz-Perret-Gentil, L., Lins, L., Menot, L., Brandt, A., Levin, L.A., Martinez-Arbizu, P., Smith, C.R. and J. Pawlowski. 2021. Eukaryotic biodiversity and spatial patterns in the Clarion-Clipperton Zone and other abyssal areas: insights from sediment DNA and RNA metabarcoding. *Frontiers of Marine Science*. DOI: 10.3389/fmars.2021.671033

- Lins, L., F. Leliaert, T. Riehl, S. P. Ramalho, E. A. Cordova, A. M. Esteves, and A. Vanreusel. 2016. Species variability and connectivity in the deep sea: evaluating effects of spatial heterogeneity and hydrodynamics. *Biogeosciences Discussions* 134. DOI: 10.5194/bg-2016-134.
- Miljutin, D. M., M. A. Miljutina, P. M. Arbizu, and J. Galéron. 2011. Deep-sea nematode assemblage has not recovered 26 years after experimental mining of polymetallic nodules (Clarion-Clipperton Fracture Zone, Tropical Eastern Pacific). *Deep-Sea Research Part I* 58:885–897. DOI: 10.1016/j.dsr.2011.06.003.
- Miljutina, M.A., D.M. Miljutin, R. Mahatma, et al. 2010. *Marine Biodiversity* 40:1. <https://doi.org/10.1007/s12526-009-0029-0>
- National Deep Submergence Facility. 2018. Retrieved from <http://www.whoi.edu/main/ndsf>
- Niner, H., J.A. Ardron, E.G. Escobar, M. Gianni, A. Jaeckel, D.O.B. Jones, L. Levin, C.R. Smith, T. Thiele, P.J. Turner, C.L. Van Dover, L. Watling, and K.M. Gjerde. 2018. Deep-sea mining with no net loss of biodiversity – An impossible aim. *Frontiers in Marine Science* 5:53. DOI: 10.3389/fmars.2018.00053.
- Nozawa, F., Kitazato, H. Tsuchiya, M. and Gooday, A.J., 2006. ‘Live’ benthic foraminifera at an abyssal site in the equatorial Pacific nodule province: abundance, diversity and taxonomic composition. *Deep-Sea Research I*, 51, 1406-1422.
- Pape, E., T. N. Bezerra, F. Hauquier, and A. Vanreusel. 2017. Limited spatial and temporal variability in meiofauna and nematode communities at distant but environmentally similar sites in an area of interest for deep-sea mining. *Frontiers in Marine Science* 4:205. DOI: 10.3389/fmars.2017.00205.
- Petersen, A., and K. Herkul. 2019. Mapping benthic biodiversity using georeferenced environmental data and predictive modeling. *Marine Biodiversity*, 49, pages131–146. <https://doi.org/10.1007/s12526-017-0765-5>
- Porter, T. M., and M. Hajibabaei. 2018. Scaling up: A guide to high-throughput genomic approaches for biodiversity analysis. *Molecular Ecology* 27:313–338. DOI: 10.1111/mec.14478.
- Rio Declaration. 1992. A/CONF.151/26. Available online at: http://www.unesco.org/education/pdf/RIO_E.PDF
- Rosli, N., D. Leduc, A. A. Rowden, and P. K. Probert. 2018. Review of recent trends in ecological studies of deep-sea meiofauna, with focus on patterns and processes at small to regional spatial scales. *Marine Biodiversity* 48:13–34. DOI: 10.1007/s12526-017-0801-5.
- Ruppert, K.M., Kline, R.J., Rahman, M.S., 2019. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. *Global Ecology and Conservation* 17: e00547. DOI: <https://doi.org/10.1016/j.gecco.2019.e00547>
- Schönfeld, J., E. Alve, E. Geslin, F. Jorissen, S. Korsun, S. Spezzaferri, and FOBIMO group. 2012. The FOBIMO (Foraminiferal Bio-Monitoring) initiative – Towards a standardized protocol for soft-bottom benthic foraminiferal monitoring studies. *Marine Micropalaeontology*. 94-95, 1-13. <https://doi.org/10.1016/j.marmicro.2012.06.001>
- Shen, C., Testa, J.M., Li, M., Cai, W., Waldbusser, G.G., Ni, W., Kemp, W.M., Cornwell, J., Chen, B., Brodeur, J., and J. Su. 2018. Controls on Carbonate System Dynamics in a Coastal Plain Estuary: A Modeling Study. *JGR Biogeosciences*, 124(1): 61-78. <https://doi.org/10.1029/2018JG004802>
- Shulze, C. N., B. Maillot, C. R. Smith, and M. J. Church. 2017. Polymetallic nodules, sediments, and deep waters in the equatorial North Pacific exhibit highly diverse and distinct bacterial, archaeal, and microeukaryotic communities. *Microbiology Open* 6:e428. DOI: 10.1002/mbo3.428.
- Siddig, A. A. H., A. M. Ellison, A. Ochs, C. Villar-Leeman, and M. K. Lau. 2016. How do ecologists select and use indicator species to monitor ecological change? Insights from 14 years of publication in *Ecological Indicators*. *Ecological Indicators* 60:223–230. DOI: 10.1016/j.ecolind.2015.06.036.
- Simon-Lledó, E., B. J. Bett, V. A. I. Huvenne, T. Schoening, N. M. A. Benoist, R. M. Jeffreys, J. M. Durden, and D. O. B. Jones. 2019. Megafaunal variation in the abyssal landscape of the Clarion Clipperton Zone. *Progress in Oceanography* 170:119–133. DOI: 10.1016/j.pocean.2018.11.003
- Sinniger, F., J. Pawlowski, S. Harii, A. J. Gooday, H. Yamamoto, P. Chevaldonné, T. Cedhagen, G. Carvalho, and S. Creer. 2016. Worldwide Analysis of Sedimentary DNA Reveals Major Gaps in Taxonomic Knowledge of Deep-Sea Benthos. *Frontiers in Marine Science* 3:92. DOI: 10.3389/fmars.2016.00092.

- Stratmann, T., Lins, L., Purser, A., Marcon, Y., Rodrigues, C. F., Ravara, A., Cunha, M. R., Simon-Lledó, E., Jones, D. O. B., Sweetman, A. K., Köser, K., and van Oevelen, D.: Abyssal plain faunal carbon flows remain depressed 26 years after a simulated deep-sea mining disturbance, *Biogeosciences*, 15, 4131–4145, <https://doi.org/10.5194/bg-15-4131-2018>, 2018.
- Thurber, A.R., Sweetman, A.K., Narayanaswamy, B.E., and D.O.B. Jones. 2014. Ecosystem function and services provided by the deep sea. *Biogeosciences*, 11:3941-3963. DOI: 10.5194/bg-11-3941-2014
- Tunnickliffe, V., A. Metaxas, J. Le, E. Ramirez-Llodra, and L.A. Levin. 2020. Strategic environmental goals and objectives: setting the basis for environmental regulation of deep seabed mining. *Marine Policy*. <https://doi.org/10.1016/j.marpol.2018.11.010>.
- United Nations General Assembly, Convention on the Law of the Sea, Article 145, 10 December 1982, available at: <http://www.refworld.org/docid/3dd8fd1b4.html>
- Vivien, R., Apothéloz-Perret-Gentil, L., Pawlowski, J., Werner, I., Lafont, M., and B.J.D. Ferrari. 2020. High-throughput DNA barcoding of oligochaetes for abundance-based indices to assess the biological quality of sediments in streams and lakes. *Sci Rep.* 10(1):2041.
- Vonnahme, T.R, M. Molari, F. Janssen, F. Wenzhofer, M. Haeckel, J. Titschack, A. Boetius. 2020. Effects of a deep-sea mining experiment on seafloor microbial communities and functions after 26 years. *Science Advances* 6, no. 18, eaaz5922. DOI: 10.1126/sciadv.aaz5922
- Weaver P.P.E., D.S.M. Billett, and C.L. Van Dover. 2018. Environmental Risks of Deep-sea Mining. In: Salomon M., Markus T. (eds) *Handbook on Marine Environment Protection*. Springer, Cham. https://doi.org/10.1007/978-3-319-60156-4_11

LIST OF FIGURES



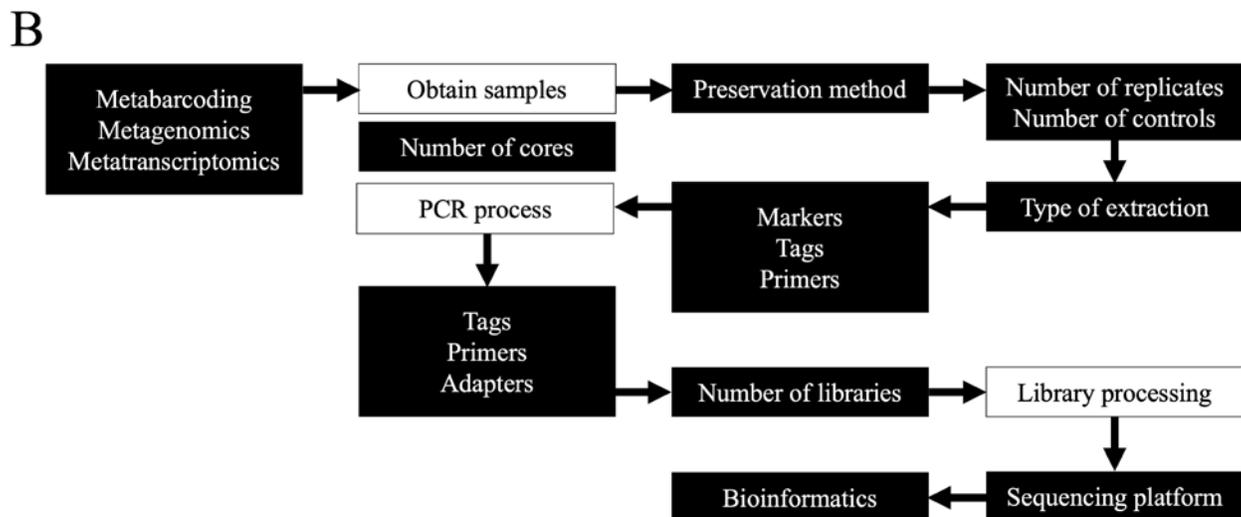


Figure 1. Decision network throughout a: (A) morphology-based taxonomy workflow; and (B) metabarcoding workflow. Decision nodes are black and steps that do not require a decision are unfilled.

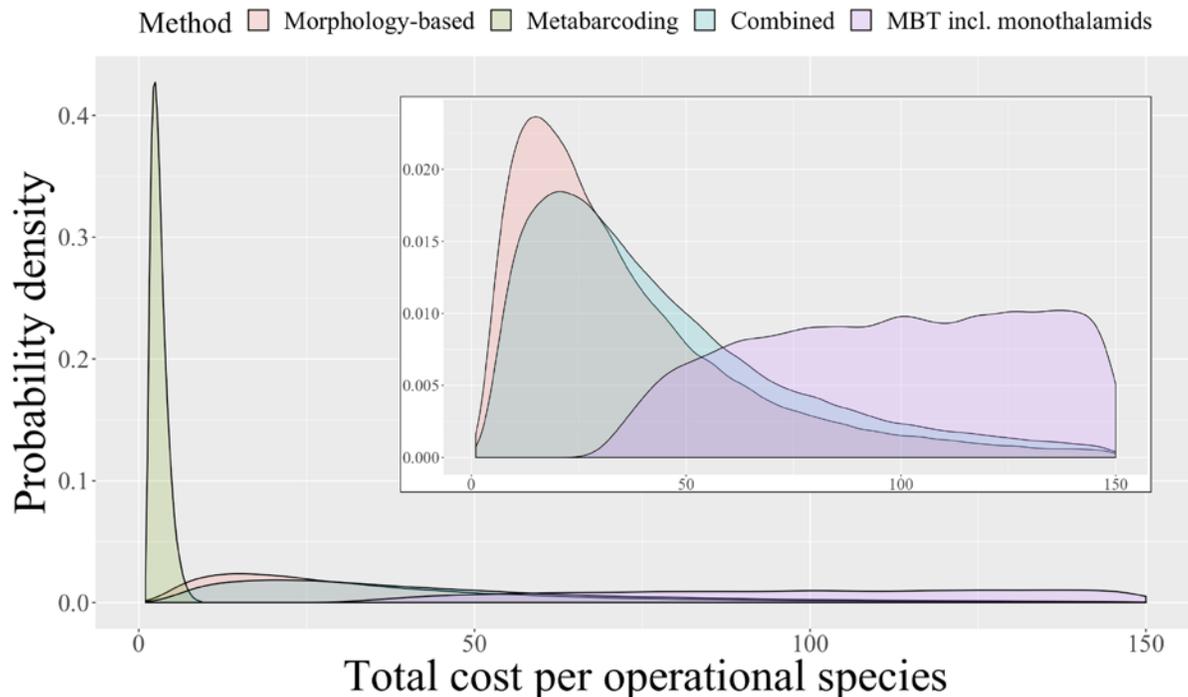


Figure 2. Truncated probability densities of expected total cost per operational species (in USD) of morphology-based taxonomy [pink], metabarcoding [green], a combined

approach [blue], and morphology-based taxonomy (MBT) including monothalamids [purple]. Metabarcoding is removed from the right inset to better illustrate comparisons among the other methodologies.

Table 1. Summary of relevant results and sampling regimes of published papers* aimed at characterizing a component of meiofaunal biodiversity in the Clarion-Clipperton Zone.

Sampling component	Morphology-based taxonomy	Metabarcoding
Number of sites visited	1-6	1-8
Deployments per site	1-11	1-3
Cores used per deployment	1-3	1-3
Sub-samples per core	1-3	1-3
Number of horizontal layers	1-5	1-3
Total number of samples	5-36	20-42
Operational species identified	53-325 (462 including monothalamids)	211-451

*Goineau and Gooday, 2017; Goineau & Gooday, 2019; Hauquier et al., 2019; Lamshead et al., 2003; Laroche et al., 2020b; Lejerowicz et al., 2014; Miljutin et al., 2011; Miljutina et al., 2010; Nozawa et al., 2006; Pape et al., 2017.

Table 2. Variables used in the cost-effectiveness model, their low and high estimates, and probability distributions chosen to incorporate uncertainty. A uniform distribution assigns the same probability to every value within its given range. A triangular distribution assigns the highest probability to the mean of the low and high estimates. MBT refers to morphology-based taxonomy.

Variable description	Low estimate (USD)	High estimate (USD)	Probability distribution
MBT consumables	\$13.10 (without staining)	\$16.29	Uniform
MBT labor	\$153.14	\$606.90	Triangular
Metabarcoding consumables	\$15.33 (in-house chemicals)	\$54.69	Triangular

Metabarcoding sequencing (MiSeq)	\$900 per run	\$1800 per run	Triangular
Metabarcoding labor	\$208.45	\$208.45	Constant

Table 3. Comparative suitability of morphology-based taxonomy and metabarcoding for gaining information relevant to deep-sea biodiversity monitoring and assessment.

Information category	Morphology-Based Taxonomy	Metabarcoding
Small-sized taxa	Possible, but difficult	Better suited
Rare or cryptic species	Poorly suited	Better suited
Abundance (density)	Absolute	Relative
Morphological data	Can provide	Cannot provide
Demographic information (e.g. biomass, size, age, sex)	Can provide	Cannot provide
Species identification	Identified by experts	Barcode matched to database requiring prior identification
Time to results	Weeks to months*	Days to weeks
Expertise required	Months to years of training depending on taxa	Weeks of training for a technician
Biases	Expert subjectivity	Analytical uncertainty

* Time to results could be reduced substantially (perhaps to 1-2 days per sample) if a 'commercial' approach to sample analysis was adopted.