Reply to Danovaro et al.   https://www.nature.com/articles/s41559-019-1091-z#citeas

Ecological variables for deep-ocean monitoring must include microbiota and meiofauna for effective conservation.

Jeroen Ingels*, Ann Vanreusel, Ellen Pape, Francesca Pasotti, Pedro Martinez Arbizu, Lara Macheriotou, Martin Vinther Sorensen, Virginia Edgcomb, Jyotsna Sharma, Nuria Sanchez, William B. Homoky, Daniel Leduc, Andrew J. Gooday, Jan Pawlowski, John Dolan, Michaela Schratzberger, Sabine Gollner, Alexandra Schoenle, Hartmut Arndt, Clare Woulds, Daniela Zeppilli

Recently, Danovaro et al. [1] prioritized deep-sea essential ecological variables (DEEVs), based on opinions from 112 deep-sea experts worldwide, to support development of a global deep-ocean monitoring and conservation strategy. While a set of universally important DEEVs is necessary to ensure appropriate monitoring, we challenge the conclusion that macro- and megafauna should be prioritized over microscopic organisms, notably eukaryotic and prokaryotic microbes, and meiofauna.

Status assessments of deep-sea habitats, and indeed any habitat, need to capture the most ecologically significant aspects, given current scientific understanding and existing methodologies. Danovaro et al.'s [1] claim that there is consensus among experts that deep-ocean monitoring should prioritize large organisms (macro- and megafauna) is partly a result of skewed expertise among survey respondents, 55% of whom were macrofauna and megafauna experts. With the addition of expertise in fish and large vertebrates, the proportion of large-animal specialists increases to approximately 75% of respondents [1], leaving small, yet crucial, organisms, and associated processes, chronically underrepresented.

The fact that nearly 20% of all respondents to the Danovaro et al. [1] survey were the authors themselves, and that the DEEV priority list was based on <10% of elicitations (112, including authors, out of 1,155 invitations), renders the results of this study questionable. They do not adequately represent the global deep-ocean scientific and stakeholder communities, despite efforts to include a variety of disciplines. Equally worrying is the lack of transparency and criteria for distinguishing between the "most significant" and "minor" respondent comments and particularly the omission of the latter, owing to perceived "lack of expertise", or "scant knowledge". Such selectivity likely introduced bias to the interpretation of survey results, which ultimately informed the selection of DEEVs.

It is unclear why macrofauna and megafauna were prioritized. Worth values and 'quasi' standard errors ('quasi' was not defined) substantially overlap (and the size of the error bars is remarkably consistent across variables within each DEEV component, cf. Fig. 2 in [1]), suggesting that meiofauna (organisms between 20 and 1000 µm, but boundaries vary) are valued as important as megafauna. No statistically justified explanation is given for separating priority categories between meiofauna (medium) and megafauna (high). Furthermore, the numbers reported in Danovaro et al. [1] do not correspond with previously published studies in favor of large organisms. The claim that 50-90% of large-sized species (e.g. deep-water corals and sharks) remain undiscovered is unsupported. Over three quarters (77%) of fish species and 62-79 % of hexacorals and octocorals are known to science, and 39% of all deep-sea species are yet to be discovered [2]. However, 88% of nematode species, the most abundant metazoan meiofauna phylum, remain undocumented [2].

Danovaro et al. [1] share our concerns regarding the importance of microorganisms by expressing surprise at the low to medium ranking of their biodiversity. The authors overturn the 'expert elicitation' results, highlighting that microbial heterotrophic and chemoautotrophic carbon production and elemental cycling are essential ecological variables to understand key processes that sustain the functioning of deep-sea food

webs and biogeochemical cycles. Selectively overturning survey results, however, undermines the objectivity and validity of the study. The importance of meiofauna and microorganisms (bacteria, archaea, unicellular eukaryotes) in deep-sea ecosystems has repeatedly been highlighted [3-9], challenging the assumption that prioritization of larger organisms can meet deep-ocean monitoring requirements. Further conjectures [1] ascribe deep-sea energy transfer, carbon flow, and biomass mainly to large-sized animals (p 185), despite acknowledging the increasing importance of generally smaller fauna at greater depth. This point cannot be understated: we know that deep-sea microbial biomass is estimated between 10 and 30% of Earth's living biomass [5], and that meiofauna dominates metazoan biomass below 3000m [10]. Moreover, smaller organisms grow and reproduce much faster than larger organisms [11,12], and deep-sea benthic metabolism is largely driven by microorganisms [9,13].

In developing conservation and monitoring goals, comparisons between marine and terrestrial ecosystems must be approached in the knowledge that, in many respects, they are fundamentally different. This applies particularly in deep-sea settings, where larger fauna are sparse and small organisms dominate biomass and diversity and are largely responsible for maintaining ecosystem function [14,15]. While conservation of more charismatic and larger organisms may pave the way for habitat protection, monitoring early change by establishing indicators or sentinels at the base of food webs and ecosystem functions, rather than among its end members, allows more efficient monitoring and timely conservation responses.

In valuing deep-sea ecosystem components for monitoring and conservation purposes, the complementarity of different monitoring tools, meaningful biological ecosystem elements and metrics of ecosystem health (e.g. EU Marine Strategy Framework Directive 2008/56/EC & Decision 2017/848) must be taken into consideration. These elements respond differently to environmental change and stressors, and only an analysis of a representative set of ecological variables can produce a robust environmental assessment. Singling out certain components is not sufficient to make informed decisions. Instead, we would advocate an ecosystem-based approach that involves 1) research to support operational monitoring (incl. indicator development), 2) research to identify conservation priorities, and 3) research to generate the ecosystem understanding that supports both. Evidence-based conservation is imperative [16] and so should be the monitoring tools implemented to support it. It should also be noted that although related, conservation and monitoring are intrinsically different. While conservation targets protection and measures to mitigate species, habitat, or the loss of ecosystem functions and services, monitoring involves the observation of patterns and processes over time, and allows detection of change using representative and sensitive system indicators.

Meiofauna are well documented as sentinels for monitoring change in ecosystems worldwide [17,18], including the deep sea [19]. Their potential contribution to an effective and comprehensive deep-ocean monitoring strategy, however, is under-represented in Danovaro et al [1], particularly in light of rapidly advancing technologies such as quantitative or digital PCR, high-throughput sequencing (HTS) [20-22], and new imaging techniques [23]. These widely available and rapid sequencing methods for prokaryotes and protozoans, in particular, offer a rapid way to assess diversity and function, and monitor change [e.g. 24], and the same will apply for meiofauna in the near future [25]. The technology readiness level of these techniques are valued as low in [1], where acoustic, sonar, and imaging techniques dominate the actions proposed to monitor the most important DEEVs. However, current advances already allow for small-organism monitoring and longer-term robust approaches should incorporate important current developments.

Protists are hardly mentioned by Danovaro et al. [1], even though they play diverse roles in many marine ecosystems [12,26] by influencing deep-sea food webs and carbon nutrient pools directly and indirectly through ecological and trophic interactions [27,28]. Although small, naked protists (e.g. ciliates, flagellates, amoebae) and monothalamous foraminifera are difficult to study in deep-sea samples [29], new sequencing

techniques are revealing an extraordinary diversity of novel lineages [30], which is valuable for deep-sea monitoring.

Globally, the deep ocean urgently needs sustainable conservation. Undervaluing the contribution of microscopic organisms to biodiversity and ecosystem functioning, and their efficiency in indicating and monitoring early change using latest technological developments, would hamper effective deep-sea management. Identifying variables for long-term, deep-sea monitoring must be driven by expert advice that encompasses balanced input from the broadest possible community of researchers and stakeholders. Without this, we cannot generate the knowledge necessary to adequately understand and protect the largest ecosystem on Earth.

Author information

Affiliations

Coastal and Marine Laboratory, Florida State University, St Teresa, FL, USA
Jeroen Ingels
Marine Biology Department, Ghent University, Gent, Belgium
A. Vanreusel, E. Pape, F. Pasotti, L. Macheriotou
Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark
Martin Vinther Sorensen
Geology & Geophysics Department, Woods Hole Oceanographic Institution, Woodshole, MA, USA
Virginia Edgcomb
University of Texas, San Antonio, TX, USA
Jyotsna Sharma
Universidad Complutense de Madrid, Madrid, Spain
Nuria Sanchez
School of Earth and Environment, University of Leeds, Leeds, UK
William B. Homoky
National Institute of Water and Atmospheric Research, Wellington, New Zealand
Daniel Leduc
National Oceanography Center, Southampton, UK
Andrew J. Gooday
Department of Genetics and Evolution, University of Geneva, Geneva, Austria
Jan Pawlowski
Laboratoire d'Océanographie de Villefranche, CNRS & University Paris VI, Villefranche-Sur-Mer, France
John Dolan
Centre for Environment, Fisheries and Aquaculture Science, Lowestoft, UK
Michaela Schratzberger
Department of Ocean Systems, Royal Netherlands Institute for Sea Research, Texel, Netherlands
Sabine Gollner
Biocenter, Institute for Zoology, General Ecology, University of Cologne, Cologne, Germany
Alexandra Schoenle, Hartmut Arndt

School of Geography, University of Leeds, Leeds, UK
Clare Woulds
Institut Français de Recherche pour l'Exploitation de la MER, Plouzané, France
Daniela Zeppilli

Contributions
J.I., D.Z., A.V. initiated the argument. All authors contributed to the development of the argument and its final form. J.I. led on the writing, and all others made contributions to the writing. J.I. produced the final agreed edited version.

*Corresponding author
J. Ingels, jingels@fsu.edu, +1.850.697.4092

Ethics declarations
Competing interests: The authors declare no competing interests

References

1. Danovaro, R.; Fanelli, E.; Aguzzi, J.; Billett, D.; Carugati, L.; Corinaldesi, C.; Dell'Anno, A.; Gjerde, K.; Jamieson, A.J.; Kark, S., et al. Ecological variables for developing a global deep-ocean monitoring and conservation strategy. *Nature Ecology & Evolution* **2020**, *4*, 181-192, doi:10.1038/s41559-019-1091-z.
2. Appeltans, W.; Ahyong, Shane T.; Anderson, G.; Angel, Martin V.; Artois, T.; Bailly, N.; Bamber, R.; Barber, A.; Bartsch, I.; Berta, A., et al. The Magnitude of Global Marine Species Diversity. *Current Biology* **2012**, *22*, 2189-2202, doi:http://dx.doi.org/10.1016/j.cub.2012.09.036.
3. Sogin, M.L.; Morrison, H.G.; Huber, J.A.; Welch, D.M.; Huse, S.M.; Neal, P.R.; Arrieta, J.M.; Herndl, G.J. Microbial diversity in the deep sea and the underexplored "rare biosphere". *Proceedings of the National Academy of Sciences* **2006**, *103*, 12115-12120.
4. Zeppilli, D.; Leduc, D.; Fontanier, C.; Fontaneto, D.; Fuchs, S.; Gooday, A.J.; Goineau, A.; Ingels, J.; Ivanenko, V.N.; Kristensen, R.M., et al. Characteristics of meiofauna in extreme marine ecosystems: a review. *Marine Biodiversity* **2018**, *48*, 35-71, doi:10.1007/s12526-017-0815-z.
5. Corinaldesi, C. New perspectives in benthic deep-sea microbial ecology. *Frontiers in Marine Science* **2015**, *2*, doi:10.3389/fmars.2015.00017.
6. Boeuf, D.; Edwards, B.R.; Eppley, J.M.; Hu, S.K.; Poff, K.E.; Romano, A.E.; Caron, D.A.; Karl, D.M.; DeLong, E.F. Biological composition and microbial dynamics of sinking particulate organic matter at abyssal depths in the oligotrophic open ocean. *Proceedings of the National Academy of Sciences* **2019**, *116*, 11824-11832, doi:10.1073/pnas.1903080116.
7. López-García, P.; Rodríguez-Valera, F.; Pedrós-Alió, C.; Moreira, D. Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton. *Nature* **2001**, *409*, 603-607, doi:10.1038/35054537.
8. Schoenle, A.; Nitsche, F.; Werner, J.; Arndt, H. Deep-sea ciliates: Recorded diversity and experimental studies on pressure tolerance. *Deep Sea Research Part I: Oceanographic Research Papers* **2017**, *128*, 55-66, doi:https://doi.org/10.1016/j.dsr.2017.08.015.
9. Turley, C. Bacteria in the cold deep-sea benthic boundary layer and sediment—water interface of the NE Atlantic. *FEMS Microbiology Ecology* **2000**, *33*, 89-99, doi:10.1111/j.1574-6941.2000.tb00731.x.
10. Wei, C.-L.; Rowe, G.T.; Escobar-Briones, E.; Boetius, A.; Soltwedel, T.; Caley, M.J.; Soliman, Y.; Huettmann, F.; Qu, F.; Yu, Z., et al. Global Patterns and Predictions of Seafloor Biomass Using Random Forests. *Plos One* **2010**, *5*, e15323.

11. Giere, O. *Meiobenthology: the Microscopic Motile Fauna of Aquatic Sediments*, 2nd edition ed.; Springer-Verlag: Berlin, 2009; pp. 527.
12. Fenchel, T. *Ecology of Protozoa: The biology of free-living phagotropic protists*; Springer-Verlag: 2013.
13. Glud, R.N. Oxygen dynamics of marine sediments. *Marine Biology Research* **2008**, *4*, 243-289.
14. Nascimento, F.J.A.; Naslund, J.; Elmgren, R. Meiofauna enhances organic matter mineralization in soft sediment ecosystems. *Limnology and Oceanography* **2012**, *57*, 338-346, doi:10.4319/lo.2012.57.1.0338.
15. Bonaglia, S.; Nascimento, F.J.A.; Bartoli, M.; Klawonn, I.; Brüchert, V. Meiofauna increases bacterial denitrification in marine sediments. *Nat Commun* **2014**, *5*, 5133, doi:10.1038/ncomms6133.
16. Sutherland, W.J.; Pullin, A.S.; Dolman, P.M.; Knight, T.M. The need for evidence-based conservation. *Trends in Ecology & Evolution* **2004**, *19*, 305-308, doi:https://doi.org/10.1016/j.tree.2004.03.018.
17. Balsamo, M.; Semprucci, F.; Frontalini, F.; Coccioni, R. Meiofauna as a tool for marine ecosystem biomonitoring. In *Marine Ecosystems*, Cruzado, A., Ed. InTech Publisher: 2012; pp. 77-104.
18. Schratzberger, M.; Ingels, J. Meiofauna matters: The roles of meiofauna in benthic ecosystems. *Journal of Experimental Marine Biology and Ecology* **2018**, *502*, 12-25, doi:10.1016/j.jembe.2017.01.007.
19. Zeppilli, D.; Sarrazin, J.; Leduc, D.; Arbizu, P.M.; Fontaneto, D.; Fontanier, C.; Gooday, A.J.; Kristensen, R.M.; Ivanenko, V.N.; Sørensen, M.V., et al. Is the meiofauna a good indicator for climate change and anthropogenic impacts? *Marine Biodiversity* **2015**, *45*, 505-535, doi:10.1007/s12526-015-0359-z.
20. Carugati, L.; Corinaldesi, C.; Dell'Anno, A.; Danovaro, R. Metagenetic tools for the census of marine meiofaunal biodiversity: An overview. *Marine Genomics* **2015**, *24*, 11-20, doi:https://doi.org/10.1016/j.margen.2015.04.010.
21. Danovaro, R.; Carugati, L.; Berzano, M.; Cahill, A.E.; Carvalho, S.; Chenuil, A.; Corinaldesi, C.; Cristina, S.; David, R.; Dell'Anno, A., et al. Implementing and Innovating Marine Monitoring Approaches for Assessing Marine Environmental Status. *Frontiers in Marine Science* **2016**, *3*, doi:10.3389/fmars.2016.00213.
22. Dell'Anno, A.; Carugati, L.; Corinaldesi, C.; Riccioni, G.; Danovaro, R. Unveiling the Biodiversity of Deep-Sea Nematodes through Metabarcoding: Are We Ready to Bypass the Classical Taxonomy? *PLOS ONE* **2015**, *10*, e0144928, doi:10.1371/journal.pone.0144928.
23. Kitahashi, T.; Watanabe, H.K.; Tsuchiya, M.; Yamamoto, H.; Yamamoto, H. A new method for acquiring images of meiobenthic images using the FlowCAM. *MethodsX* **2018**, *5*, 1330-1335, doi:https://doi.org/10.1016/j.mex.2018.10.012.
24. Pawlowski, J.; Esling, P.; Lejzerowicz, F.; Cedhagen, T.; Wilding, T.A. Environmental monitoring through protist next-generation sequencing metabarcoding: assessing the impact of fish farming on benthic foraminifera communities. *Molecular Ecology Resources* **2014**, *14*, 1129-1140, doi:10.1111/1755-0998.12261.
25. Bik, H.M.; Porazinska, D.L.; Creer, S.; Caporaso, J.G.; Knight, R.; Thomas, W.K. Sequencing our way towards understanding global eukaryotic biodiversity. *Trends in Ecology & Evolution* **2012**, *27*, 233-243, doi:http://dx.doi.org/10.1016/j.tree.2011.11.010.
26. Fenchel, T. The ecology of marine microbenthos IV. Structure and function of the benthic ecosystem, its chemical and physical factors and the microfauna commuities with special reference to the ciliated protozoa. *Ophelia* **1969**, *6*, 1-182, doi:10.1080/00785326.1969.10409647.
27. Worden, A.Z.; Follows, M.J.; Giovannoni, S.J.; Wilken, S.; Zimmerman, A.E.; Keeling, P.J. Rethinking the marine carbon cycle: factoring in the multifarious lifestyles of microbes. *Science* **2015**, *347*, 1257594.
28. Gooday, A.J.; Schoenle, A.; Dolan, J.R.; Arndt, H. Protist diversity and function in the dark ocean – Challenging the paradigms of deep-sea ecology with special emphasis on foraminiferans and naked protists. *European Journal of Protistology* **2020**, *75*, 125721, doi:https://doi.org/10.1016/j.ejop.2020.125721.
29. Schoenle, A.; Jeuck, A.; Nitsche, F.; Venter, P.; Prause, D.; Arndt, H. Methodological studies on estimates of abundance and diversity of heterotrophic flagellates from the deep-sea floor. *Journal of Marine Science and Engineering* **2016**, *4*, 22.
30. Lecroq, B.; Lejzerowicz, F.; Bachar, D.; Christen, R.; Esling, P.; Baerlocher, L.; Østerås, M.; Farinelli, L.; Pawlowski, J. Ultra-deep sequencing of foraminiferal microbarcodes unveils hidden richness of early monothalamous lineages in deep-sea sediments. *Proceedings of the National Academy of Sciences* **2011**, *108*, 13177-13182, doi:10.1073/pnas.1018426108.