

Test-casing the integration of plankton imaging and genomics data for biodiversity indicator assessments in the Southern North Sea

Christina Pavloudi (1), Willem Boone (2), Luz Amadei Martínez (3), Cymon J Cox (4), Hanneloor Heynderickx (3), Jonas Mortelmans (3), Carlota Muñiz (3), Stelios Ninidakis (5), Ioulia Santi (6) and Klaas Deneudt (3)

(1) European Marine Biological Resource Centre (EMBRC-ERIC), Paris, France, christina.pavloudi@embrc.eu

(2) Flanders Marine Institute [Vlaams Instituut voor de Zee (VLIZ)], Belgium, willem.boone@vliz.be

(3) Flanders Marine Institute [Vlaams Instituut voor de Zee (VLIZ)], Belgium

(4) Centro de Ciências do Mar, Universidade do Algarve, Faro, Portugal

(5) Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Hellenic Centre for Marine Research (HCMR), Heraklion, Greece

(6) European Marine Biological Resource Centre (EMBRC-ERIC), Paris, France & Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Hellenic Centre for Marine Research (HCMR), Heraklion, Greece, ioulia.santi@embrc.eu

Abstract: Planktonic organisms are the most abundant organisms in the oceans and are considered excellent indicators for environmental changes; they are the basis of the food web and, thus, ecosystem functioning. Changes in plankton diversity have been used extensively as an indicator of ecosystem health. Plankton assessment is included in two of the Essential Ocean Variables (EOVs), as well as in the assessment Good Environmental Status under Marine Strategy Framework Directive (MSFD). The aim of this study was to test-case eDNA-based biodiversity observations from a large-scale long-term monitoring network, i.e. the European Marine Omics Biodiversity Observation Network (EMO BON), against image-based observations to identify the potential for eDNA to serve as an input source for EOV assessments or MSFD indicators' calculation. We used phyto- and zooplankton imaging data collected from seventeen stations monitored on a monthly or seasonal basis and eDNA data collected bi-monthly from one EMO BON observatory, in the Belgian part of the North Sea (BPNS). This (proof of concept) study is a practical assessment of the suitability and validity of eDNA data for calculation of plankton-based indicators and EOVs. The validity of the information deriving from both methods allows a holistic understanding of coastal biodiversity status.

Keywords: EOVs, plankton indicators, MSFD, eDNA

1. INTRODUCTION

Planktonic organisms are the most abundant organisms in the oceans and are considered to be excellent indicators for environmental changes (Edwards, 2009); they are the basis of the food web and, thus, ecosystem functioning (Chowdhury *et al.*, 2026). Changes in plankton diversity have been used extensively as an indicator of ecosystem health. Plankton community variance over time and space indicates ecological state, with stable sites showing low variance and disturbed or recovering sites showing high variance; thus, tracking changes in planktonic communities helps identify shifts toward species-poor states or ecological improvement in pelagic communities.

The importance of plankton is also evident from the fact that two of the Essential Ocean Variables (EOVs) (i.e., “Phytoplankton biomass and diversity” and “Zooplankton biomass and diversity”), as well as one Essential Climate Variable (ECV) (i.e., “Biological/Ecosystems: plankton”), are focusing on it. In addition, plankton is incorporated in the assessment of Marine Strategy Framework Directive (MSFD) Good Environmental Status (GES) (e.g. Descriptors 1, 2 and 5). Furthermore, assessments of planktonic groups that perform similar functional roles, called “lifeforms”, are also used to evaluate GES under the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) (Holland *et al.*, 2023).

Conventional techniques to study planktonic organisms are based on imaging, either manually (using microscopy) or in a more automated way (using precision flow imaging microscopy or scanner technology). However, genomics-based techniques, such as DNA metabarcoding, can also be used for biodiversity assessments (Zamora-Terol *et al.*, 2020). Environmental DNA (eDNA) based biodiversity

observations are now being employed on a large scale, within coordinated networks at European level such as the European Marine Omics Biodiversity Observation Network (EMO BON) (Santi *et al.*, 2023) to identify planktonic communities from marine waters. Despite eDNA-based observations currently not being used as data source for MSFD assessments, the coordinated nature of EMO BON offers great potential for their use for large-scale assessments.

The aim of this study is to test-case EMO BON eDNA data against image-based observations to identify the similarities between the two methodological approaches and the potential for eDNA to serve as an input source for EOY assessments or MSFD indicators' calculation.

2. METHODS

In the framework of this study, we used phyto- and zooplankton imaging data collected within the scope of the VLIZ biodiversity observatory, which are already being used for MSFD reporting at national level. The considered time series consists of biodiversity data from seventeen stations which are monitored in the Belgian part of the North Sea (BPNS) on a monthly or seasonal basis (zooplankton: since 2014; phytoplankton: since 2017). Phytoplankton (55-300 µm) was analysed with a FlowCAM, and zooplankton (>200 µm) with a ZooScan. The identification of these images (zooplankton: order level; phytoplankton: genus level) was done using automatic image-recognition analysis, followed by manual validation.

eDNA data were obtained from the BPNS EMO BON observatory where water samples are collected bi-monthly since 2021, filtered sequentially (0.2-3 µm and 3-200 µm) and sequenced using shotgun metagenomics, based on the EMO BON handbook (Santi *et al.*, 2021). The raw data (from both size fractions) are subjected to high-throughput shotgun metagenomics sequencing, and the results are processed using the metaGOflow pipeline (Zafeiropoulos *et al.*, 2023). The processing is carried out through Zorba, the High-Performance Computing (HPC) system of IMBBC (Institute of Marine Biology, Biotechnology and Aquaculture) (Zafeiropoulos *et al.*, 2021), the CETA HPC of CCMAR (Centro de Ciências do Mar), and the CIRRUS-D HPC of INCD (National Computing Distributed Infrastructure of Portugal). EMO BON raw data are briefly described in Pavloudi *et al.* (2025) and Santi *et al.* (2026) and can be found in the European Nucleotide Archive (ENA) (David *et al.*, 2026) under the umbrella project accession [PRJEB51688](#); in particular, the sequence data for BPNS are under the project accession [PRJEB51652](#).

Part of the study's computational analyses was conducted on the European Digital Twin Ocean (EDITO) and made use of tools developed for the Demonstrator Use Cases (DUC) of DTO-BioFlow project and facilitating the calculation of pelagic habitat (PH) indicators. The tool that was used is deployed on EDITO and is available at <https://datalab.dive.edito.eu/launcher/service-playground/ph1> (Boone *et al.*, 2026). This service is based on PH1 calculations, developed by Holland and McQuatters-Gollop (2022), which is an established indicator for assessment of changes in phytoplankton and zooplankton communities (OSPAR Commission, 2023).

The respective DUC for which this tool was developed is focusing on the assessment of pelagic biodiversity and the impact of human activities in marine ecosystems (Lagaisse *et al.*, 2025). This use case is vital for informing environmental policy and management, as it contributes directly to the OSPAR thematic assessments.

3. RESULTS

One of the most used outputs of the PH1 calculation are state-space plots. State-space plots are used to define a regime, i.e. to establish a set of conditions as the basis for making comparisons, visually represented by an envelope; next, a new set of data is mapped onto the same state-space to compare the assessment period with the new condition. The value of the Plankton Index (PI) is then calculated as the proportion of new points that fall between the inner and outer envelopes; a PI value of 1 would indicate no change, and a value of zero would show complete change and a likely permanent deviation from the initial regime. Calculations based on the BPNS imaging data, processed using the PH1 script in EDITO, are provided in Fig. 1.

In this study, we evaluate the integration of eDNA into lifform time series as a complementary data source to imaging. This approach addresses both temporal limitations and methodological differences in detection capacity and taxonomic resolution between datasets. Our results provide insights on plankton functional diversity in the Southern North Sea and showcase the potential of this approach to be replicated in other areas at European level, capitalising on the EMO BON network. The validity of the information deriving from both the eDNA and the imaging approaches allows for a better and holistic understanding of coastal biodiversity and its status.

This (proof of concept) study is a practical assessment of the suitability and validity of eDNA data for calculation of plankton-based indicators and EOVs.

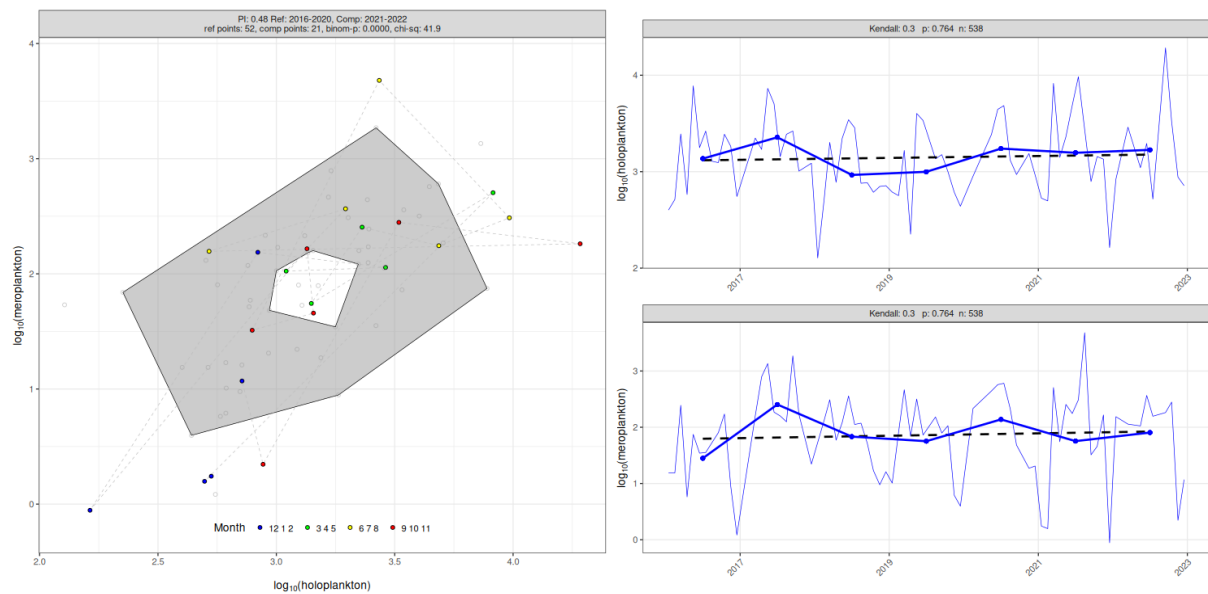


Fig. 1. Holoplankton-Meroplankton comparison using BPNS imaging data and processed using PH1 script in EDITO service.

Acknowledgements

We would like to thank Rune Lagaisse (VLIZ), Felipe Artigas (CNRS ULCO) and Ankita Vaswani (Hereon) for the conceptualizing of the PH1 tool in EDITO. The PH1 calculations are based on the work of Matthew Holland. This study has received funding from [DTO-BioFlow](#) (Grant agreement ID: 101112823) and [MARCO-BOLO](#) (Grant agreement ID: 101082021) Horizon Europe projects. This research was supported in part through computational resources provided by IMBBC (Institute of Marine Biology, Biotechnology and Aquaculture) of the HCMR (Hellenic Centre for Marine Research). Funding for establishing the IMBBC HPC has been received by the MARBIGEN (EU Regpot) project, LifeWatchGreece RI and the CMBR (Centre for the study and sustainable exploitation of Marine Biological Resources) RI. This work used data resources provided by the European Marine Omics Biodiversity Observation Network (EMO BON) project, coordinated by the European Marine Biological Resource Centre - European Research Infrastructure Consortium (EMBRIC-ERIC). This study received Portuguese national funds from FCT - Foundation for Science and Technology through [UID/04326/2025](#), [UID/PRR/04326/2025](#) and [LA/P/0101/2020](#), and from the operational programmes CRESC Algarve 2020 and COMPETE 2020 through contracts EMBRC.PT ALG-01-0145-FEDER-022121 and BIODATA.PT ALG-01-0145-FEDER-022231. This study also received support from National Computing Distributed Infrastructure (INCD) of Portugal.

REFERENCES

- Boone, W., Deneudt, K., and Muñiz, C. (2026). DTO-BioFlow: DUC 3 Assessing pelagic biodiversity and human impact - Changes in Plankton Communities. *Zenodo*. <https://doi.org/10.5281/zenodo.18997115>
- Chowdhury, P., Khan, S.J., Sumon, M.A.A., El-Regal, M.A.A., Tayyatham, K. and Van Doan, H. (2026). The role of planktons in sustaining the function and diversity of marine ecosystems: a systematic review. *Discover Animals*, 3(1), 9.
- David, Y., Alisha, A., Awais, A., Rajkumar, D., Dipayan, G., Muhammad, H., Maira, I., Eugene, I., Vishnukumar, K., Amnon, K. Manish, K., *et al.* (2026). The European Nucleotide Archive in 2025. *Nucleic Acids Research*, 54(D1), D120-D127.
- Edwards, M. (2009). Sea life (pelagic and planktonic ecosystems) as an indicator of climate and global change. In: *Climate Change*, pp. 233-251. Elsevier.
- Holland, M.M., and McQuatters-Gollop, A. (2022). PH1_PLET_tool (Version 2.0) [Computer software]. https://github.com/hollam2/PH1_PLET_tool
- Holland, M., Louchart, A., Artigas, L. F. and McQuatters-Gollop, A. 2023. Changes in Phytoplankton and Zooplankton Communities. In: *OSPAR, 2023: The 2023 Quality Status Report for the Northeast Atlantic*. OSPAR Commission, London.
- Lagaisse, R., Boone, W., Artigas, F., Vaswani, A., and De Carlo, F. (2025). DTO-BioFlow DUC 3 - Integrated Monitoring of Pelagic Biodiversity and Human Impact. *Zenodo*. <https://doi.org/10.5281/zenodo.17491176>
- OSPAR Commission. (2023). Changes in plankton diversity. OSPAR Quality Status Report 2023. Retrieved from <https://oap.ospar.org/en/ospar-assessments/quality-status-reports/qsr-2023/indicator-assessments/changes-plankton-diversity/>
- Pavloudi, C., Santi, I., Azua, I., Baña, Z., Bastianini, M., Belser, C., Bilbao, J., Bitz-Thorsen, J., Broudin, C., Camusat, M., Cancio, I., Caray-Counil, L., Casotti, R., *et al.* (2025). First release of the European marine omics biodiversity observation network (EMO BON) shotgun metagenomics data from water and sediment samples. *Biodiversity Data Journal*, 13, e143585.
- Santi, I., Casotti, R., Comtet, T., Cunliffe, M., Koulouri, Y., Macheriotou, L., Not, F., Obst, M., Pavloudi, C., Romac, S., Thiebaut, E., Vanaverbeke, J. and Pade, N. (2021). European Marine Omics Biodiversity Observation Network (EMO BON) Handbook. (Version 1.0). Paris, France, EMBRC-ERIC, 63pp.
- Santi, I., Beluche, O., Beraud, M., Buttigieg, P.L., Casotti, R., Cox, C.J., Cunliffe, M., Davies, N., de Cerio, O.D., Exter, K., Kervella, A.E., Kotoulas, G., *et al.* (2023). European marine omics biodiversity observation network: a strategic outline for the implementation of omics approaches in ocean observation. *Frontiers in Marine Science*, 10, 1118120.
- Santi, I., Pavloudi, C., Abagnale, M., Azua, I., Baña, Z., Bastianini, M., Belser, C., Berg, K., Bilbao, J., Bird, K., Broudin, C., Camusat, M., Cancio, I., *et al.* (2026) Next release of the European Marine Omics Biodiversity Observation Network (EMO BON) shotgun metagenomic data from water and sediment samples (Release 2). *Biodiversity Data Journal*, 14, e178484.
- Zafeiropoulos, H., Gioti, A., Ninidakis, S., Potirakis, A., Paragkamian, S., Angelova, N., Antoniou, A., Danis, T., Kaitetzidou, E., Kasapidis, P. *et al.* (2021). 0s and 1s in marine molecular research: A regional HPC perspective. *GigaScience*, 10(8), giab053.
- Zafeiropoulos, H., Beracochea, M., Ninidakis, S., Exter, K., Potirakis, A., De Moro, G., Richardson, L., Corre, E., Machado, J., *et al.* (2023). metaGOflow: a workflow for the analysis of marine Genomic Observatories shotgun metagenomics data. *Gigascience*, 12, giad078.
- Zamora-Terol, S., Novotny, A. and Winder, M. (2020). Reconstructing marine plankton food web interactions using DNA metabarcoding. *Molecular ecology*, 29(17), 3380-3395.