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Data Paper

First release of the European marine omics biodiversity observation network (EMO BON) shotgun metagenomics data from water and sediment samples

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Abstract

The European Marine Omics Biodiversity Observation Network (EMO BON) is an initiative of the European Marine Biological Resource Centre (EMBRC) to establish a persistent genomic observatory amongst designated European coastal marine sites, sharing the same protocols for sampling and data curation. Environmental samples are collected from the water column and, at some sites, soft sediments and hard substrates (Autonomous Reef Monitoring Structures - ARMS), together with a set of mandatory and discretionary metadata (including Essential Ocean Variables - EOVs). Samples are collected following standardised protocols at regular and specified intervals and sequenced in large six-monthly batches at a centralised sequencing facility. The use of standard operating procedures (SOPs) during data collection, library preparation and sequencing aims to provide uniformity amongst the data collected from the sites. Coupled with strict adherence to open and FAIR (Findable, Accessible, Interoperable, Reusable) data principles, this ensures maximum comparability amongst samples and enhances reusability and interoperability of the data with other data sources. The observatory network was launched in June 2021, when the first sampling campaign took place.

Introduction

Here we report the first data release from the European Marine Omics Biodiversity Observation Network (EMO BON) (Santi et al. 2023). This release includes data derived from water and sediment samples that were collected between June and September 2021 from the 13 observatories, across European seas and the Red Sea.

Value of the dataset

This dataset includes raw DNA sequences obtained from shotgun metagenomics sequencing of water and sediment samples from 13 selected observatories across Europe and the Red Sea. The raw sequence data are released in the European Nucleotide Archive (ENA) (Yuan et al. 2024) with metadata associated with the sampling event, sample preparation and sequencing procedures and a diverse set of measured environmental parameters available in the associated BioSamples (Courtot et al. 2021), such as temperature, salinity and nutrient concentrations.

This dataset contributes to the ongoing efforts of the Ocean Biodiversity Information System (OBIS), which aims at filling the gaps in our current knowledge on biodiversity of the world's oceans. Processed data will be published in OBIS and in the Global Biodiversity Information Facility (GBIF), using the DNA extension of the Darwin Core format. In addition, processed data, once available in OBIS and GBIF, will be incorporated in the European Marine Observation and Data Network (EMODnet) and the European Digital Twin of the Ocean (European DTO) initiatives.

Methods

Sampling

Sample collection was conducted under the standard operating procedures of the EMO BON Handbook (Santi et al. 2021). Water samples were collected and processed according to the "Water Column Standard Operating Procedures 1 – WaSOP 1 (basic)": subsurface seawater was collected from the water column sampling site of each observatory, pre-filtered (> 200 µm) and concentrated by sequential filtration on polycarbonate (PC) membrane filters of 142 mm in diameter and pore sizes 3 µm and 0.2 µm. This resulted in two different plankton size fractions: 3-200 µm and 0.2-3 µm. After filtration, each filter membrane was cut into two pieces by a sterile scalpel and each filter piece was considered to represent one replicate. In total, four replicates were collected from each sampling, since two separate sequential filtrations were conducted at each sampling site. Subsequenctly, membranes (replicates 1 and 2) were placed in individual containers with the DNA/RNA shield preservative (Zymo Research), flash frozen in liquid nitrogen and stored at -80^{o}C until shipment to the sequencing facility. Filter membranes that were collected for biobanking (replicates 3 and 4) were preserved in cryotubes without the addition of DNA/RNA Shield and stored at -80^{o}C.

Sediment samples were collected and processed, based on all the three proposed protocols. Briefly, observatories NRMCB and RiaFormosa used the 'Soft Substrate Standard Operating Procedures 1 – SoSOP 1 (intertidal sediments)", while OOB and ROSKOGO used the "Soft substrate Standard Operating Procedures 2 – SoSOP 2 (coastal sediments by diving)" and BPNS used "Soft substrate Standard Operating Procedures 3 – SoSOP 3 (coastal sediments by research vessel)". Regardless of the choice of protocol, the steps regarding collection of sediment for microbial community assessment include the use of sediment cores and the subsequent slicing of the top 5 cm layer. As for the water samples, four replicates are collected for the sediment sampling; DNA/RNA shield was added in two of the replicates, which were the ones shipped for sequencing.

Geographic range

The dataset's geographical range includes 14 locations (13 observatories) across eight ecoregions, based on the Marine Ecoregions of the World (MEOW), proposed by Spalding et al. (2007) (Table 1; Fig. 1); details are also provided regarding the locality of the observatories, from the broader (ocean/sea) to the regional and the local scale.

Table 1.

Locality and coordinates of the sampling stations.

Observatory	Coordinates	Marine Ecoregion of the World (MEOW)	Ocean/Sea	Region	Location	Water sampling	Sediment sampling	Number of collected samples	Number of successfully sequenced samples
AAOT	45.31417 N; 12.508333 E	Adriatic Sea	Mediterranean Sea - Eastern Basin	Adriatic Sea	Gulf of Venice	Yes		8	7
BPNS	51.43333 N; 2.808331 E	North Sea	North Atlantic Ocean	North Sea	Belgian part of the North Sea	Yes	Yes	12	11
EMT21	42.20194 N; -8.798500 E	South European Atlantic Shelf	Atlantic Ocean	North Atlantic Ocean	Vigo Seamount	Yes		4	4
ESC68N	68.92589 N; 17.125619 E	Northern Norway and Finnmark	Arctic Ocean	Norwegian Sea	Norwegian part of the Norwegian Sea	Yes		4	4
HCMR-1	35.34662 N; 25.278761 E	Aegean Sea	Mediterranean Sea - Eastern Basin	Aegean Sea	Crete Sea	Yes		12	5

Observatory	Coordinates	Marine Ecoregion of the World (MEOW)	Ocean/Sea	Region	Location	Water sampling	Sediment sampling	Number of collected samples	Number of successfully sequenced samples
IUIEilat	29.50000 N; 34.916667 E	Northern and Central Red Sea	Indian Ocean	Gulf of Eilat	Gulf of Eilat	Yes		4	4
NRMCB	40.80014 N; 14.250000 E	Western Mediterranean	Mediterranean Sea - Western Basin	Tyrrhenian Sea	Naples Gulf	Yes	Yes	18	13
OOB	42.48417 N; 3.135278 E	Western Mediterranean	Mediterranean Sea - Western Basin	Gulf of Lion	Bay of Banyuls-sur-Mer		Yes	3	1
OSD74	41.14653 N; -8.666639 E	South European Atlantic Shelf	Atlantic Ocean	North Atlantic Ocean	Porto Valley	Yes		3	3
PiEGetxo	43.33858 N; -3.014639 E	South European Atlantic Shelf	North Atlantic Ocean	Bay of Biscay	Abra de Bilbao	Yes		6	6
RFormosa	37.00564 N; -7.969250 E	South European Atlantic Shelf	Atlantic Ocean	North Atlantic Ocean	Ria Formosa	Yes	Yes	6	6
ROSKOGO	48.70833 N; -3.866000 E	Celtic Seas	North Atlantic Ocean	English Channel	French part of the English Channel		Yes	2	2
ROSKOGO	48.77167 N; -3.968333 E	Celtic Seas	North Atlantic Ocean	English Channel	French part of the English Channel	Yes		8	6
VB	43.68300 N; 7.317000 E	Western Mediterranean	Mediterranean Sea - Western Basin	Villefranche Bay	Villefranche Bay - Point B	Yes		8	8

DNA extraction, library preparation and sequencing

DNA extraction was performed at Genoscope, which was the chosen centralised facility to minimise biases and follow the same standardised procedures. For DNA extraction of the water column filter samples, the same protocol as described by Alberti et al. (2017) was used. The procedure consisted of a first step of cell disruption by cryogenic grinding of membrane filters followed by chemical lysis and then nucleic acid purification using

NucleoSpin RNA kits, combined with the NucleoSpin RNA/DNA buffer set (Macherey-Nagel, Düren, Germany). For sediment samples, DNA extraction was performed using the commercially available DNeasy PowerSoil Pro Kit (Qiagen) with slight modifications.

Figure 1.

Map of the observatories collecting:

a: sediment samples; doi

b: water samples. doi

Sequencing was also performed at Genoscope. Metagenome libraries were constructed according to the available DNA: 10 to 100 ng of genomic DNA were sonicated to obtain fragments of around 350 bp, using the Covaris E220 instrument (Covaris, Woburn, MA, USA). Fragments were repaired, 3'-adenylated and NEXTflex PCR freebarcodes adapters (Bioo Scientific, Austin, TX, USA) were added using the NEBNext® Ultra II DNALibrary prep kit for Illumina (New England Biolabs, Ipswich, MA, USA). Ligation products were purified by AMPure XP beads 0:8 volume (Beckmann Coulter, Brea, CA, USA). DNA fragments (> 200 bp) were amplified by PCR (2 PCR reactions, 14 cycles) using Illumina adapter-specific primers and NEBNext® Ultra II Q5 Master Mix (NEB). All libraries were subjected to size profile analysis conducted by Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and to qPCR quantification using the KAPA Library Quantification Kit for Illumina Libraries (KapaBiosystems, Wilmington, MA, USA). All metagenomic libraries validated by the quality-control were sequenced using 151-bp pairwise read chemistry on an Illumina NovaSeq6000 sequencer, using S4 Flowcells (Illumina, San Diego, CA, USA). A minimum of 40,000 million useful paired-end reads were obtained per sample. Short Illumina reads were bioinformatically post-processed *sensu* Alberti et al. (2017) to filter out low-quality data. First, low-quality nucleotides (Q < 20) were discarded from both read ends. Then the remaining Illumina sequencing adapters and primer sequences were removed and only reads ≥ 30 nucleotides were retained. These filtering steps were done using in-house-designed software, based on the FastX package (Engelen and Aury 2016). Finally, read pairs mapping to the phage phiX genome were identifed and discarded using SOAP aligner (Li et al. (2008), default parameters) and the Enterobacteria phage PhiX174 reference sequence (GenBank: NC_001422.1).

Biodiversity scope

Target

The target of the dataset was to assess prokaryotic and eukaryotic diversity associated with the collected samples.

Taxonomic range

Archaea, Bacteria, Eukaryota

Data Resources

Details for the samples can be found in Suppl. material 1 and their basic metadata can be found in Suppl. material 2. All the raw sequence files of this study were submitted to ENA (Yuan et al. 2024) with the umbrella study accession number PRJEB51688. The accession numbers of the component projects under the umbrella study are PRJEB51662, PRJEB51661, PRJEB51660, PRJEB51659, PRJEB51658, PRJEB51665, PRJEB51664, PRJEB51656, PRJEB51655, PRJEB51654, PRJEB51653, PRJEB51652 and PRJEB50566. All sampling event and environmental data, linked to the respective accession numbers, are also available to browse and download from EMO BON's data landing page.

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Conflicts of interest

The authors have declared that no competing interests exist.

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Supplementary materials

Suppl. material 1: ENA sample, run, experiment and project accession numbers for the first release of the EMO BON shotgun metagenomics data from water and sediment samples doi

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Suppl. material 2: BioSamples MIxS checklists doi

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