

ECOTOXICOLOGIA

AULA PRÁTICA 10

random] plaseid

The synthesis and
structure of DNA
is a complex process
involving many
different molecules
and enzymes. The
primary structure
of DNA is a double
helix, with the two
strands of the
helix held together
by hydrogen bonds
between the bases.
The bases are
paired according to
the rules of
complementary
base pairing: Adenine
pairs with Thymine,
and Guanine pairs
with Cytosine.

Chemical DNA
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and Guanine pairs
with Cytosine.

Within DNA
chromosomes, the
genes are organized
into a specific
order. This order
is determined by
the sequence of
the DNA bases. The
sequence of the
bases in a gene
determines the
sequence of the
amino acids in
the protein that
the gene encodes.
The sequence of
the amino acids
in a protein
determines the
structure and
function of the
protein.

The most published reports of A-DNA have been
based on X-ray diffraction studies of DNA fibers.
The A-DNA structure is a compact, wide, short
helix, with the two strands of the helix held
together by hydrogen bonds between the bases.
The bases are paired according to the rules of
complementary base pairing: Adenine pairs with
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These results are more typical of conformational
states of DNA. In fact, the A-DNA form is observed
in DNA fibers, and it is also observed in DNA
crystals. The A-DNA form is also observed in
DNA fibers that are treated with certain chemical
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PLANEAMENTO DAS AULAS

AULA 1 (02.03) – INÍCIO DOS TESTES DE ECOTOXICOLOGIA

AULA 2 (09.03) – EFEITOS DO BEZAFIBRATO NA GERMINAÇÃO DOS ORGANISMOS TESTE (TAXAS DE INIBIÇÃO E CONSTANTES DE INIBIÇÃO IC50)

AULA 3 (16.03) – MARCADORES BIOFÍSICOS DE TOXICIDADE I

AULA 4 (23.03) – MARCADORES BIOFÍSICOS DE TOXICIDADE II

AULA 5 (06.04) – ANÁLISE ESTATÍSTICA MULTIVARIADA E ÍNDICES FOTOQUÍMICOS (TEÓRICO-PRÁTICA)

AULA 5 (13.04) - MARCADORES BIOQUÍMICOS DE TOXICIDADE I – PIGMENTOS VEGETAIS

AULA 6 (20.04) – MARCADORES BIOQUÍMICOS DE TOXICIDADE II – PIGMENTOS VEGETAIS II

AULA 7 (27.04) - MARCADORES BIOQUÍMICOS DE TOXICIDADE III – DANO MEMBRANAR

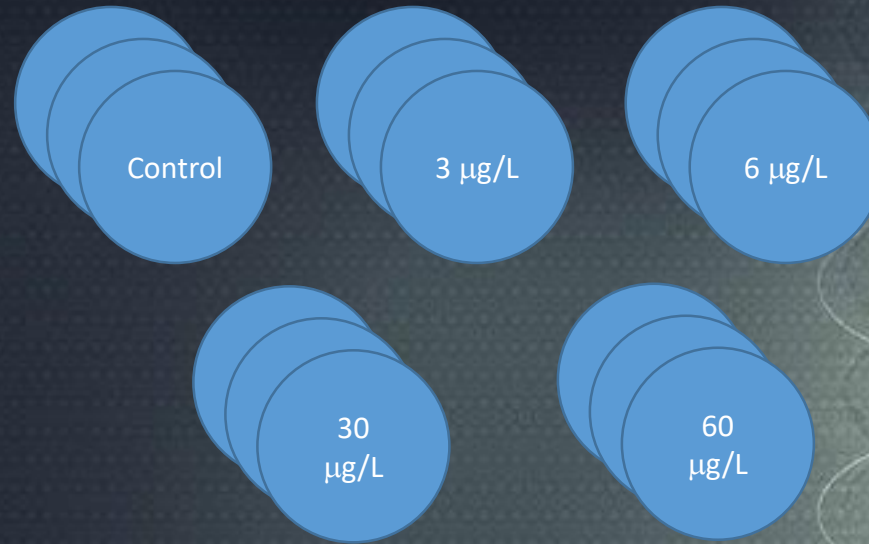
AULA 8 (04.05) – MARCADORES BIOQUÍMICOS DE TOXICIDADE IV – DANO MEMBRANAR

AULA 9 (11.05) – OCEAN METAGENOMICS

AULA 10 (18.05) - NANOTOXICOLOGIA

random][pLasatd

DELINEAMENTO EXPERIMENTAL



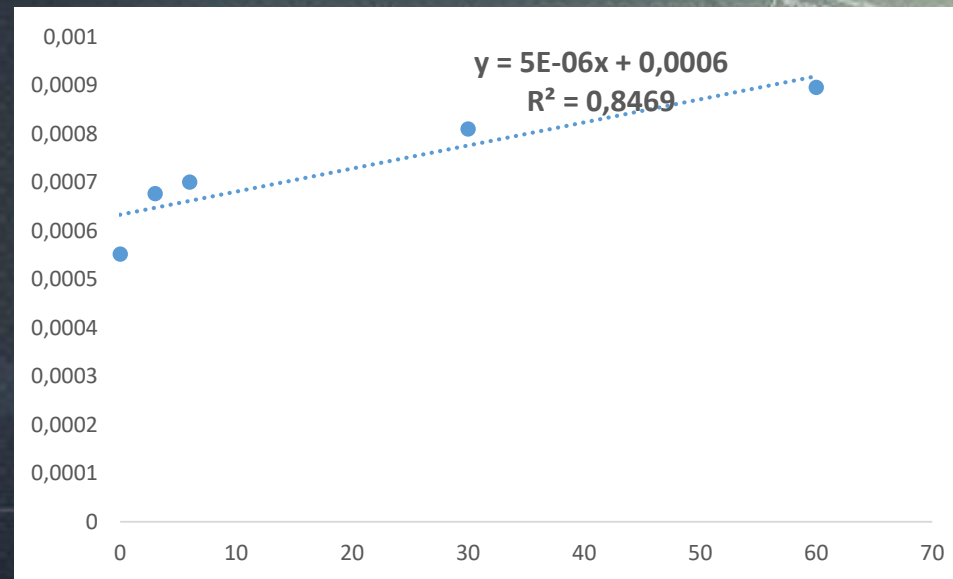
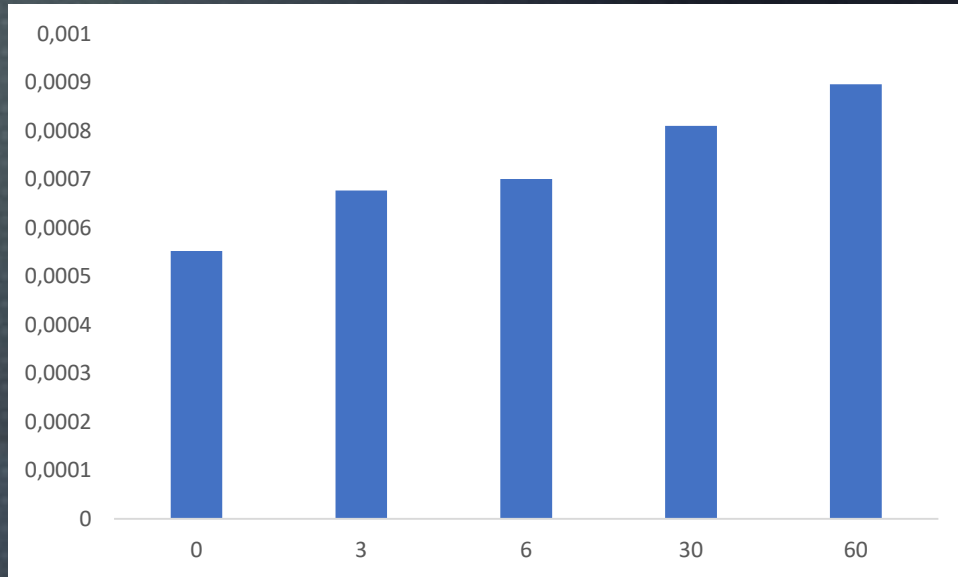
random][pLasatd

Chemically, DNA consists of two complementary strands of deoxyribose sugar-phosphate backbones, with nitrogenous bases of adenine, thymine, guanine, and cytosine. The two strands are held together by hydrogen bonds between the bases. Adenine pairs with thymine, and guanine pairs with cytosine. The sequence of these base pairs is the genetic code, which encodes the instructions for building proteins. The information is passed on to daughter cells during cell division.

When cells divide, DNA is replicated. In eukaryotes, DNA is packaged into chromosomes. Each chromosome consists of a single long DNA molecule. The DNA is wrapped around histone proteins to form nucleosomes. The DNA is then further condensed into higher-order structures, such as the mitotic spindle. The DNA is then distributed to daughter cells during cell division.

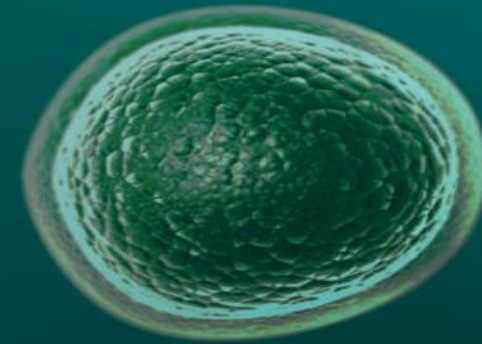


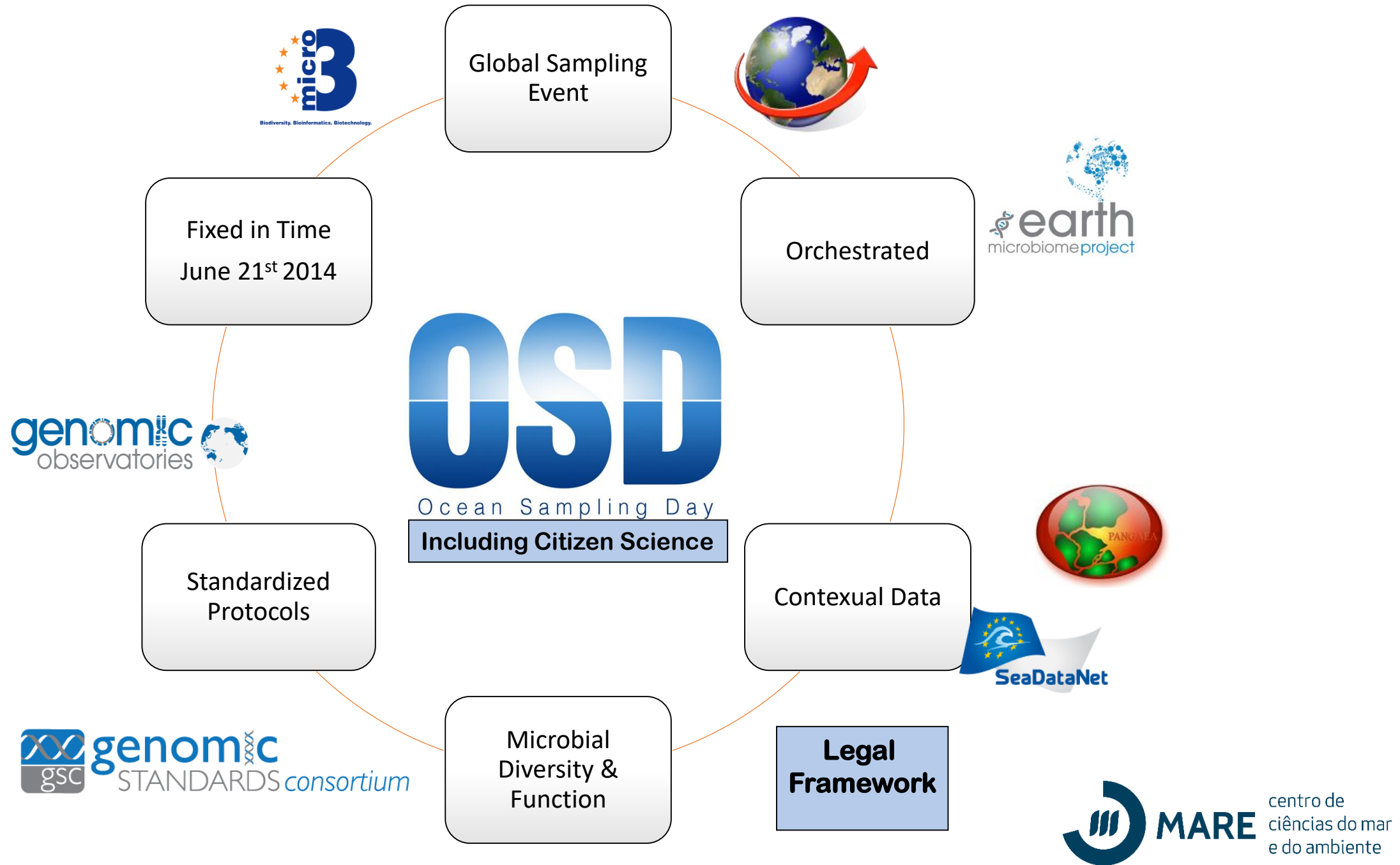
LIPID PEROXIDATION



Projecto MicroB3

Ocean Sampling Day







Sampling from environment



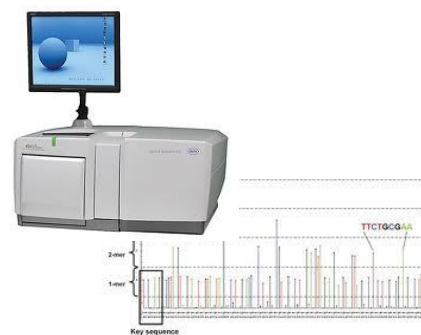
Filtering step

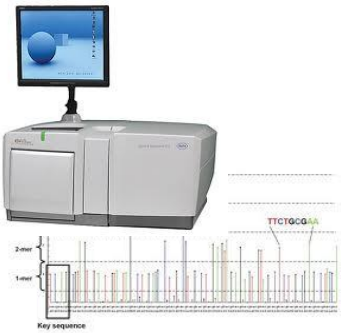


Extraction of DNA



Sequencing

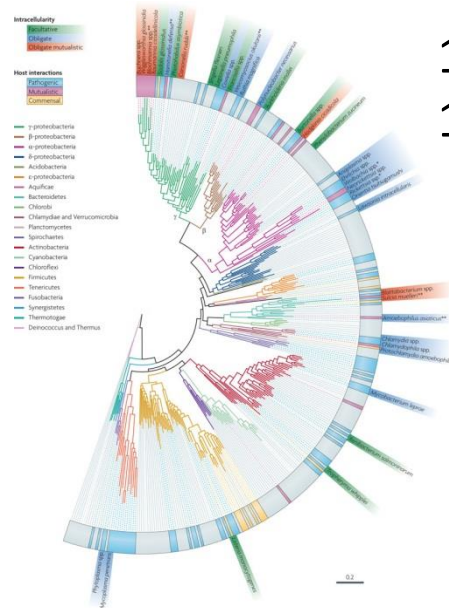




Quality control



Taxonomic analysis

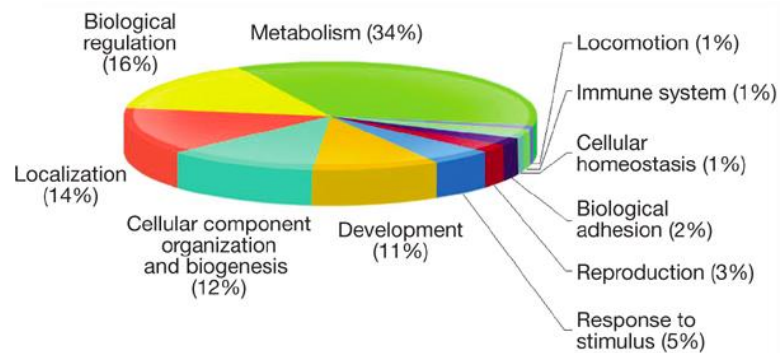


16S rRNA

18S rRNA

ITS

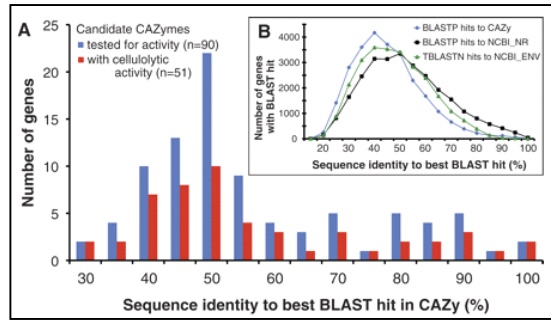
etc



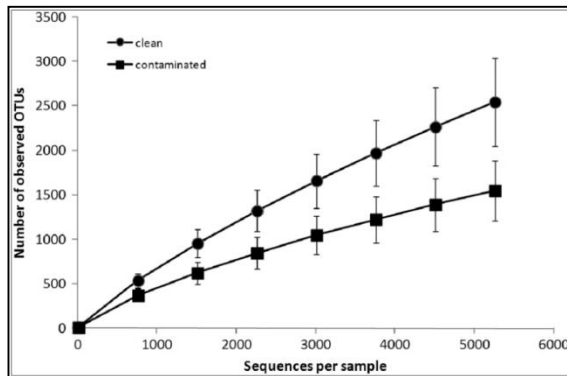
Functional analysis

Identification and characterisation of protein coding sequences

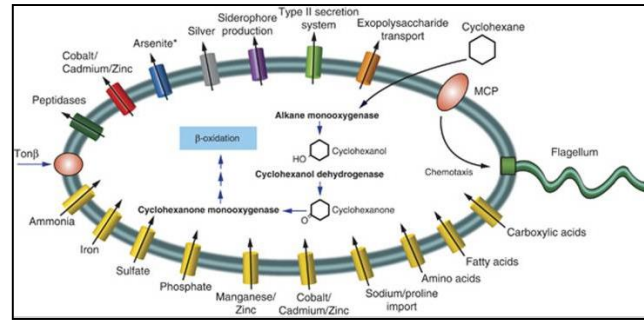
Applications of metagenomics



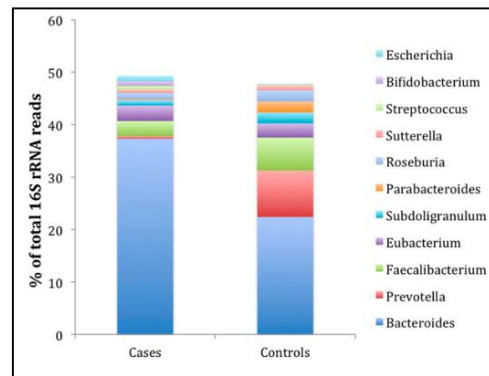
Bioprospecting for novel sequences



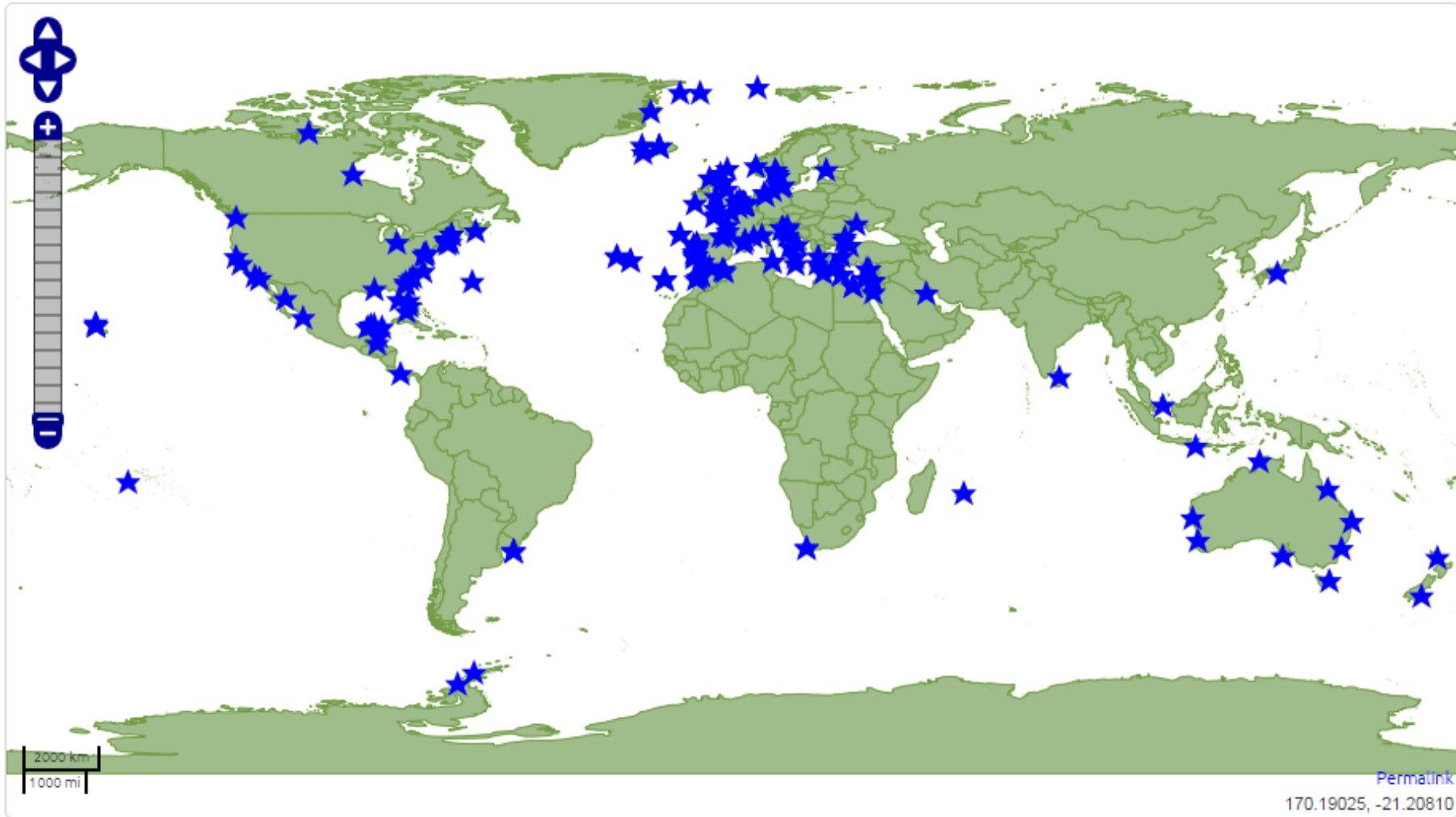
Diversity analysis



Reconstruction of community pathways



Comparing populations/activities from different sites or states



**191 OSD Sites amostrados em simultâneo!
(2015) + 300 Sites MyOSD**



OSD 2014 Main Event: Geographical distribution

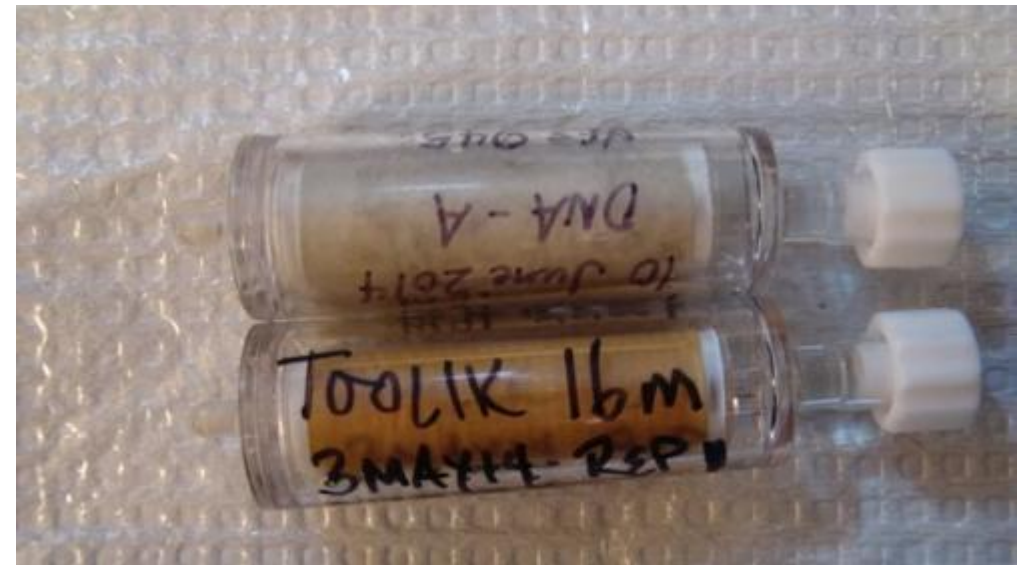
▶ Participating OSD sites ranged from:

- subtropical waters in Hawaii to extreme environments such as the Fram Strait in the Arctic Ocean

▶ All seven major oceanic divisions (Pacific-, Atlantic-, Indian-, Antarctic- and Arctic Ocean) and continents are covered:

- Majority in Europe (105) and North America (34)
- Vast majority in Northern Hemisphere (171)
- 37 sites in the Mediterranean and the Black Sea (OSD-Med Sub-network)
- Best coastal coverage along the western coast of Portugal with 13 sites

> 1000 filtros Sterivex (2014)



> 1,5 Toneladas
de gelo seco (2014)



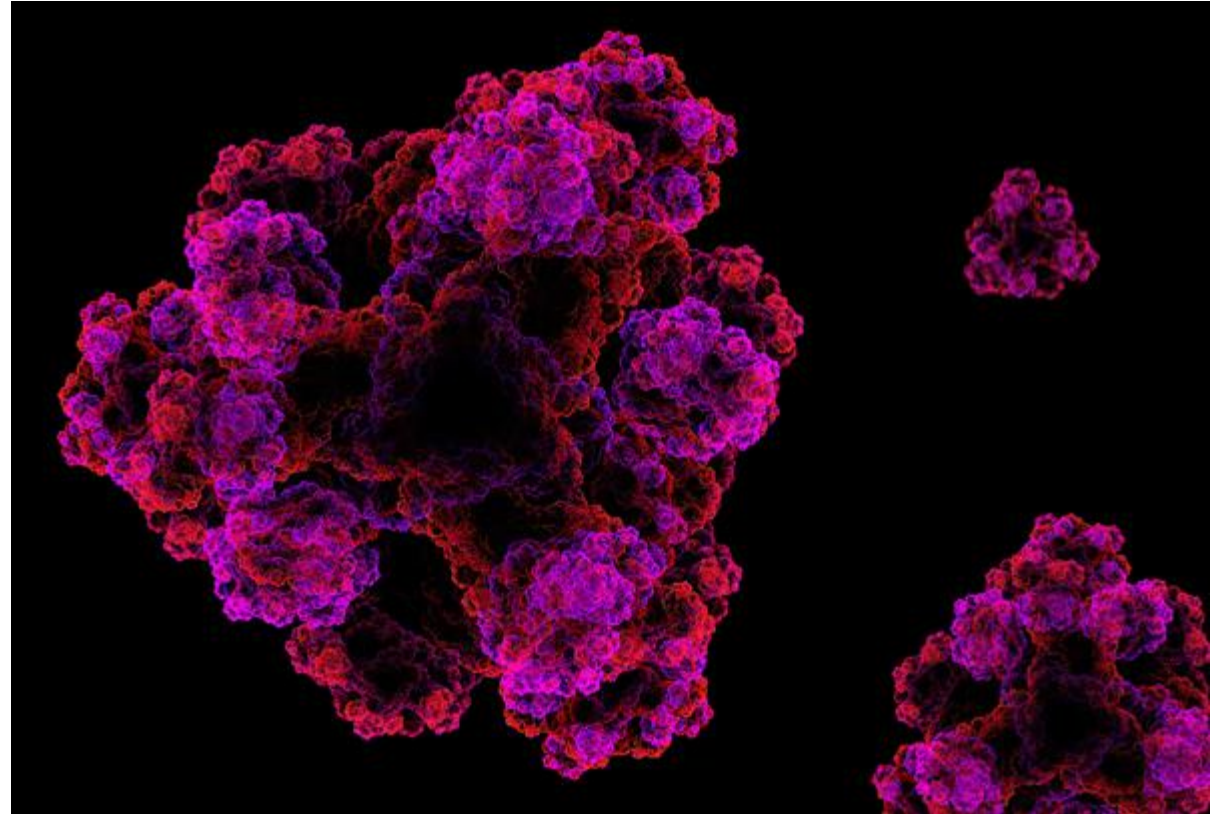
~150 h de trabalho
~ 325 extracções
(2014)



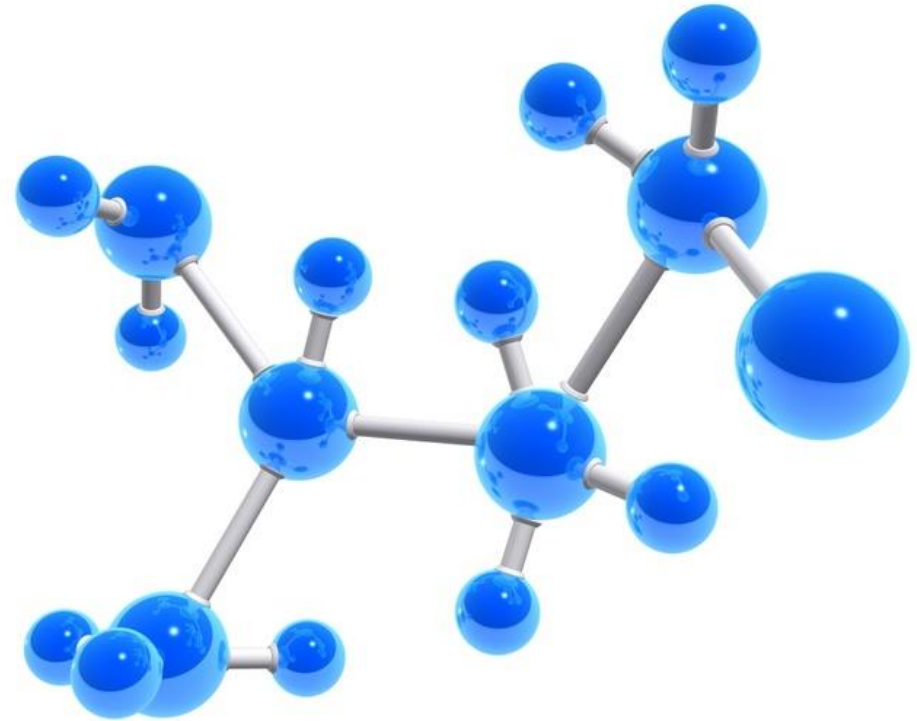
22 616 729 sequências 16S
5 089 384 sequências 18S
(2014)

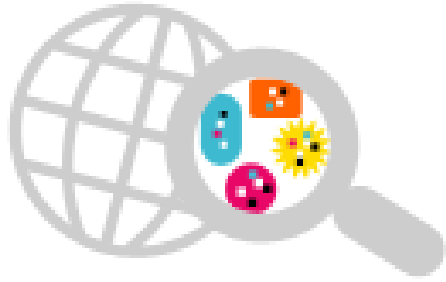


15 007 Proteínas
Identificadas (2014)



21 938 730 Péptidos
Identificados (2014)





EBI Metagenomics

<http://www.ebi.ac.uk/metagenomics>

A **free** resource for the analysis, archiving & browsing of metagenomic study data

Easy submission



Powerful analysis



Data archiving







EBI Metagenomics taxonomy visualizations

Overview Quality control **Taxonomy analysis** Functional analysis Download

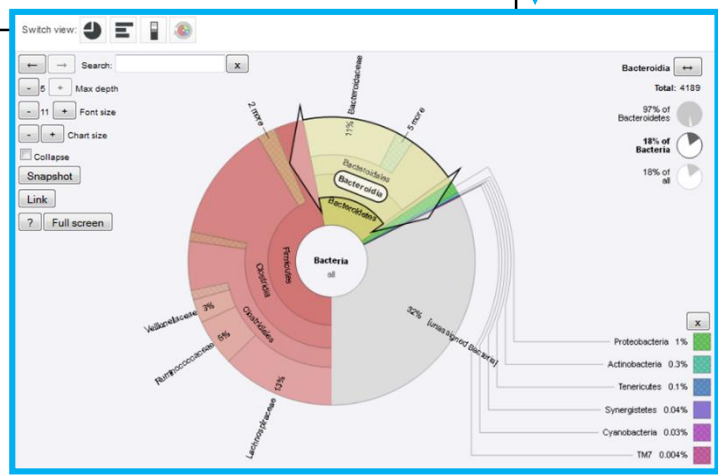
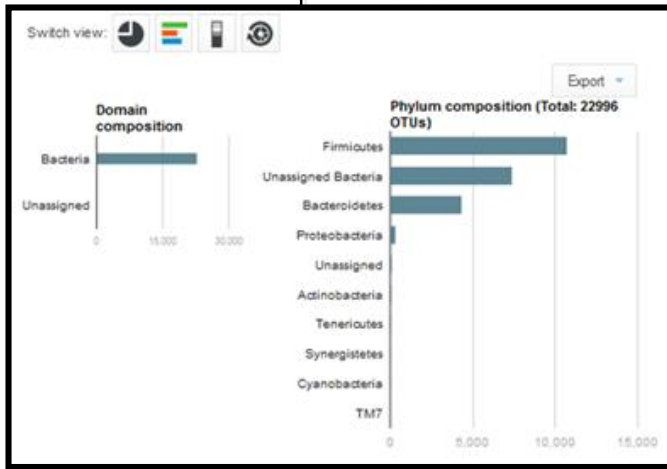
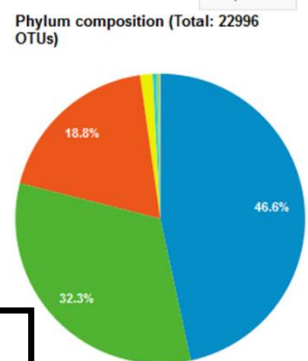
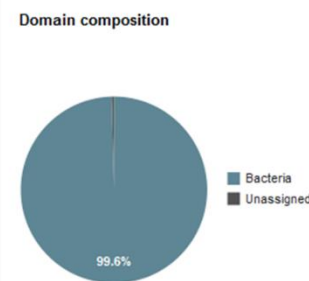
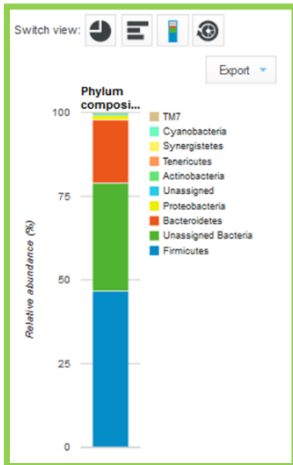
These are the results from the taxonomic analysis steps of our pipeline. You can switch between different views of the data using the menu of icons below (pie, bar, stacked and interactive krona charts). If you wish to download the full set of results, all files are listed under the "Download" tab.

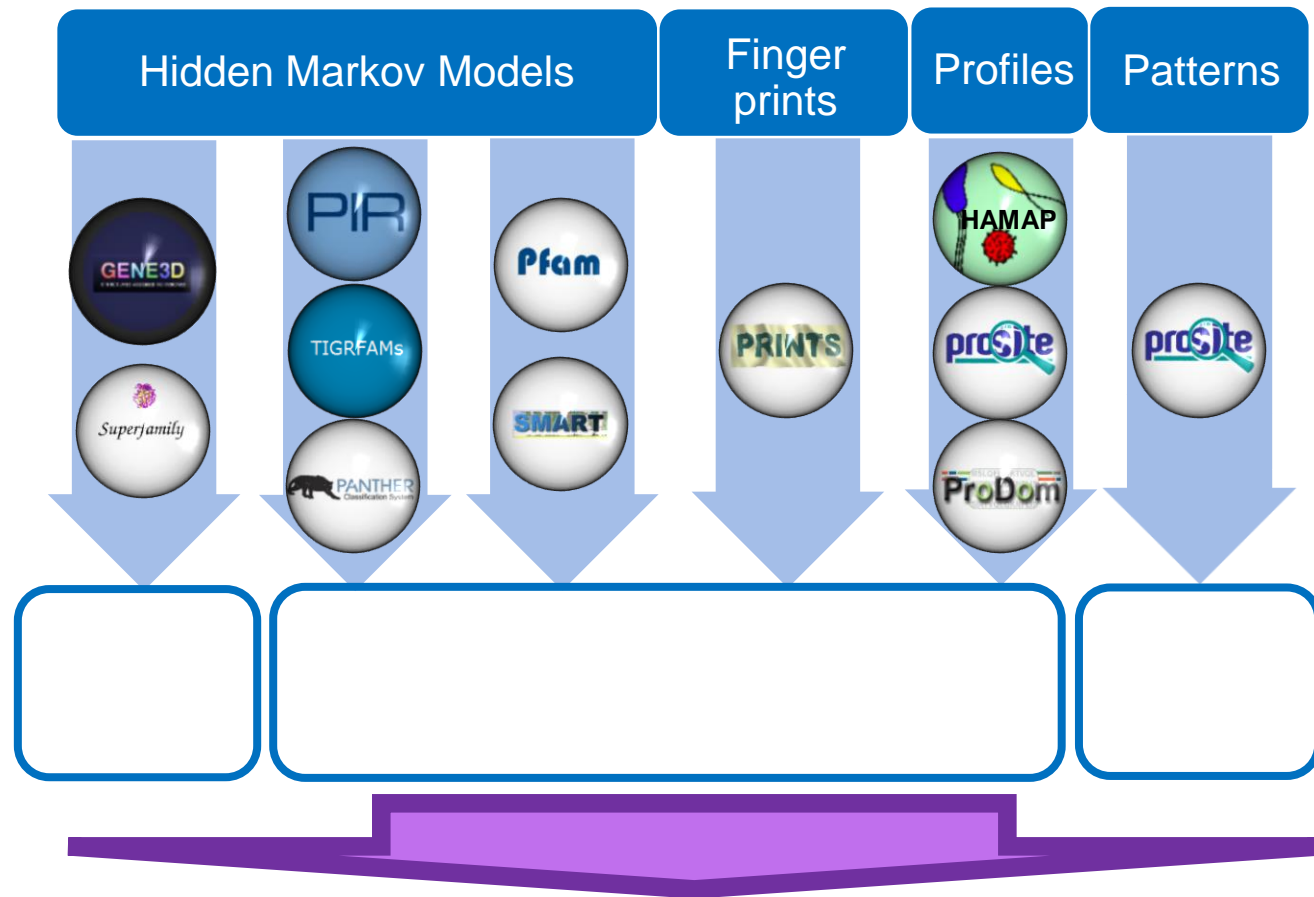
Top taxonomy Hits

Switch view:    

Filter table:

	Phylum	Domain	Unique OTUs	%
1	Firmicutes	Bacteria	10722	46.63
2	Unassigned Bacteria	Bacteria	7430	32.31
3	Bacteroidetes	Bacteria	4329	18.83
4	Proteobacteria	Bacteria	320	1.39
5	Unassigned	Unassigned	90	0.39
6	Actinobacteria	Bacteria	62	0.27
7	Tenericutes	Bacteria	25	0.11
8	Synergistetes	Bacteria	9	0.04
9	Cyanobacteria	Bacteria	8	0.03
10	TM7	Bacteria	1	0





 **Interpro**
Protein sequence analysis & classification

The aim of InterPro

```
>Seq1
MNGTEGPNFYVPFSNKTGVVRSPEAPQYLLAEP
WQFSMLAAYMFLILVLFPIINFLTYVTVQHKKLR
TPLNYILLNLAVADLFMVFGGFTTLYTSLHGYFVF
GPTGCNLEGGFFATLGGGEIALWSLVVLAIERVYVVC
KPMNSNFRFGENHAIMGVAFTW
```



Family

Malate dehydrogenase, type 1, bacterial (IPR023958)

Short name: *Malate_DH_type1_bac*

Family relationships

Malate dehydrogenase, type 1
 ↳ Malate dehydrogenase, type 1, bacterial

Description

This enzyme catalyzes the reversible ox...

GO terms

Biological Process: GO:0006099 tricarbo...
 GO:0055114 oxidat...

Molecular Function: GO:0030060 L-mala...

Contributing signatures

Signatures from InterPro member databases are used to construct an entry.

HH: prediction
 HAMAP

Domain

Lactate/malate dehydrogenase, C-terminal (IPR022383)

Short name: *Lactate/malate_DH_C*

Domain relationships

Lactate dehydrogenase/glyoxyside hydrolase, family 4, C-terminal
 ↳ Lactate/malate dehydrogenase, C-terminal

Description

L-lactate dehydrogenases are metabolic enzymes which catalyse the conversion of L-lactate to pyruvate, the last step in anaerobic glycolysis [PubMed: 11111111].

Contributing signatures

Signatures from InterPro member databases are used to construct an entry.

HH: prediction
 Pfam
 PPF02866 (Ldh_1_C)

Active site

Malate dehydrogenase, active site (IPR001252)

Short name: *Malate_DH_AS*

Description

Malate dehydrogenase (EC:1.1.1.37) (MDH) [PubMed: 1610875] catalyzes the interconversion of malate to oxaloacetate utilizing the NAD/NADH cofactor system. The enzyme participates in the citric acid cycle and exists in all aerobic organisms.

While prokaryotic organisms contains a single form of MDH, in eukaryotic cells there are two isozyt mitochondrial matrix and the other in the cytoplasm. Fungi and plants also harbor a glyoxysomal fo glyoxylate pathway. In plants chloroplast there is an additional NADP-dependent form of MDH (EC:1.1.1.41) both the universal C3 photosynthesis (Calvin) cycle and the more specialised C4 cycle.

The pattern for this enzyme includes two residues involved in the catalytic mechanism [PubMed: 1610875]. It is involved in a proton relay mechanism, and an arginine which binds the substrate.

GO terms

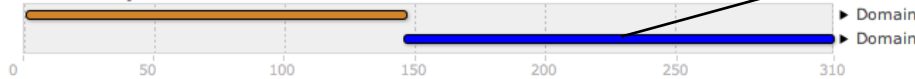
Biological Process: GO:0006108 malate metabolic process
 GO:0055114 oxidation-reduction process

Molecular Function: GO:0016615 malate dehydrogenase activity

Protein family membership:

- ↳ L-lactate/malate dehydrogenase (IPR001557)
- ↳ Malate dehydrogenase, type 1 (IPR010097)
- ↳ Malate dehydrogenase, type 1, bacterial (IPR023958)

Sequence features summary



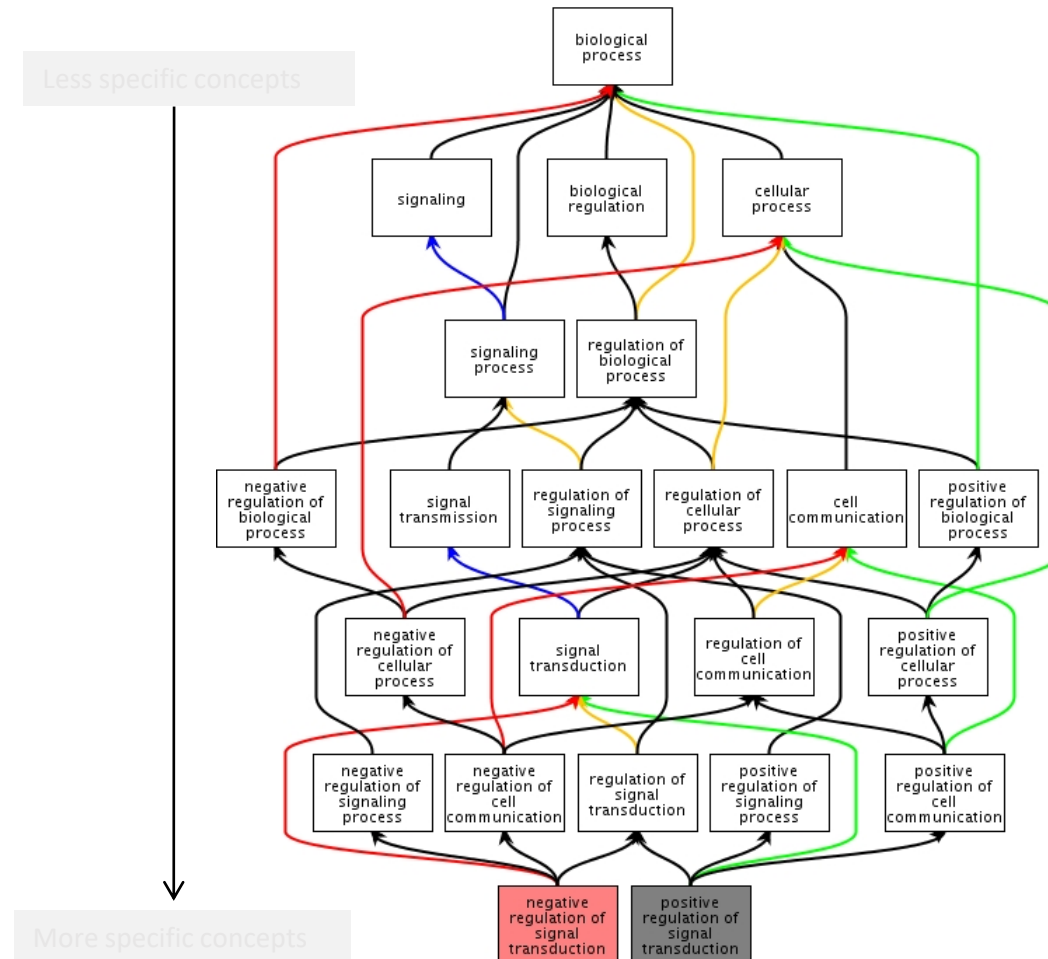
Sequence features

IPR001252 Malate dehydrogenase, active site

PS00068 (MDH)

The Gene Ontology

- A way to capture biological knowledge in a written and computable form
- A set of concepts and their relationships to each other arranged as a hierarchy

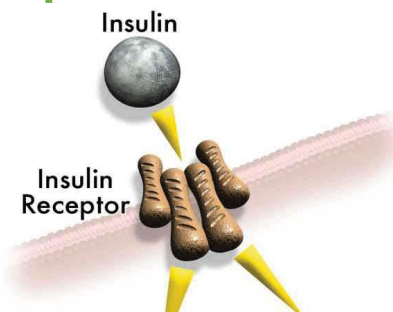


www.ebi.ac.uk/QuickGO

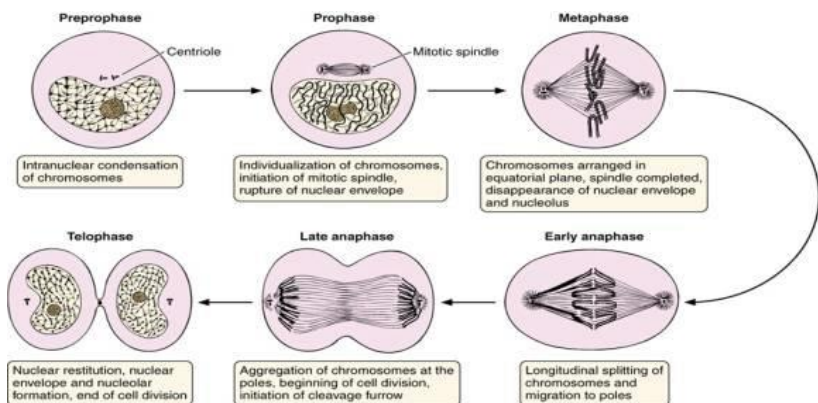
The Concepts in GO

1. Molecular Function

An elemental activity or task or job



- protein kinase activity
- insulin receptor activity



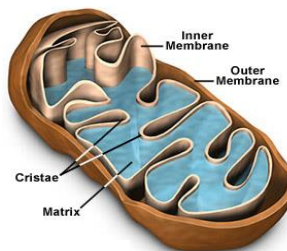
2. Biological Process

A commonly recognised series of events

- cell division

3. Cellular Component

Where a gene product is located



- mitochondrion
- mitochondrial matrix
- mitochondrial inner membrane

Anatomy of a GO term

The image shows a screenshot of a Gene Ontology (GO) term page for 'nucleus'. The page is annotated with callouts pointing to various parts of the interface:

- Unique identifier:** Points to the GO ID 'GO:0005634'.
- Term name:** Points to the term name 'nucleus'.
- Definition:** Points to the detailed text description of the nucleus.
- Synonyms:** Points to a table listing alternative terms for 'nucleus'.

The page structure includes a top navigation bar with tabs: Term Information, Ancestor Chart, Child Terms, Protein Annotation, Co-occurring Terms, and Change Log. The main content area is divided into sections: ID, Name, Ontology, Definition, Comment, and GONUTS. Below the main content, there are more tabs: Synonyms, Taxon Constraints, GO Slims, Cross-references, and Replaces. The Synonyms section contains a table with the following data:

Type	Synonym
exact	cell nucleus

InterPro2GO

```
>Seq1
MNGTEGPNFYVPFSNKTGVVRSPEAPQYYLAEP
WQFSMLAAYMFLILVIGFPINFLTLYVTVQHKKLR
TPLNYILLNLAVADLFMVFGGFTTLYTSLHG YFVF
GPTGCNLEGGFATLGGEIALWSLVVLAIER YVVVC
KPM SNFRFGENHAIMGV AFTW|
```



Family

Malate dehydrogenase, type 1, bacterial (IPR023958)

Short name: Malate_DH_Type1_bac

Family relationships

Malate dehydrogenase, type 1
→ Malate dehydrogenase, type 1, bacterial

Description

This enzyme catalyzes the reversible oxidation of malate to oxaloacetate. It exist as a homodimer and belongs to the LDH/MDH superfamily, specifically to the MDH type 1 family.

GO terms

Biological Process: [GO:0006099](#) tricarboxylic acid cycle
[GO:0053114](#) oxidation-reduction process

Molecular Function: [GO:0030060](#) L-malate dehydrogenase activity

Contributing signatures

Signatures from InterPro member databases are used to construct an entry.

HH: prediction
HAMAP
MF_01516 (Malate_dehydrog_1)

Domain

Lactate/malate dehydrogenase, C-terminal (IPR022383)

Short name: Lactate/malate_DH_C

Domain relationships

Lactate dehydrogenase/glycoside hydrolase, family 4, C-terminal
→ Lactate/malate dehydrogenase, C-terminal

Description

L-lactate dehydrogenases are metabolic enzymes which catalyse the conversion of L-lactate to pyruvate, the last step in anaerobic glycolysis [[PubMed: 11276087](#)]. L-lactate dehydrogenase is also found as a lens crystallin in bird and crocodile eyes. L-2-hydroxyisocaproate dehydrogenases are also members of the family. Malate dehydrogenases catalyse the interconversion of malate to oxaloacetate [[PubMed: 8117664](#)]. The enzyme participates in the citric acid cycle. This entry represents the C-terminal, and is thought to be an unusual alpha+beta fold.

GO terms

Biological Process: [GO:0053114](#) oxidation-reduction process

Molecular Function: [GO:0016616](#) oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor

Contributing signatures

Signatures from InterPro member databases are used to construct an entry.

HH: prediction
HAMAP
PF02866 (Ldh_L_C)

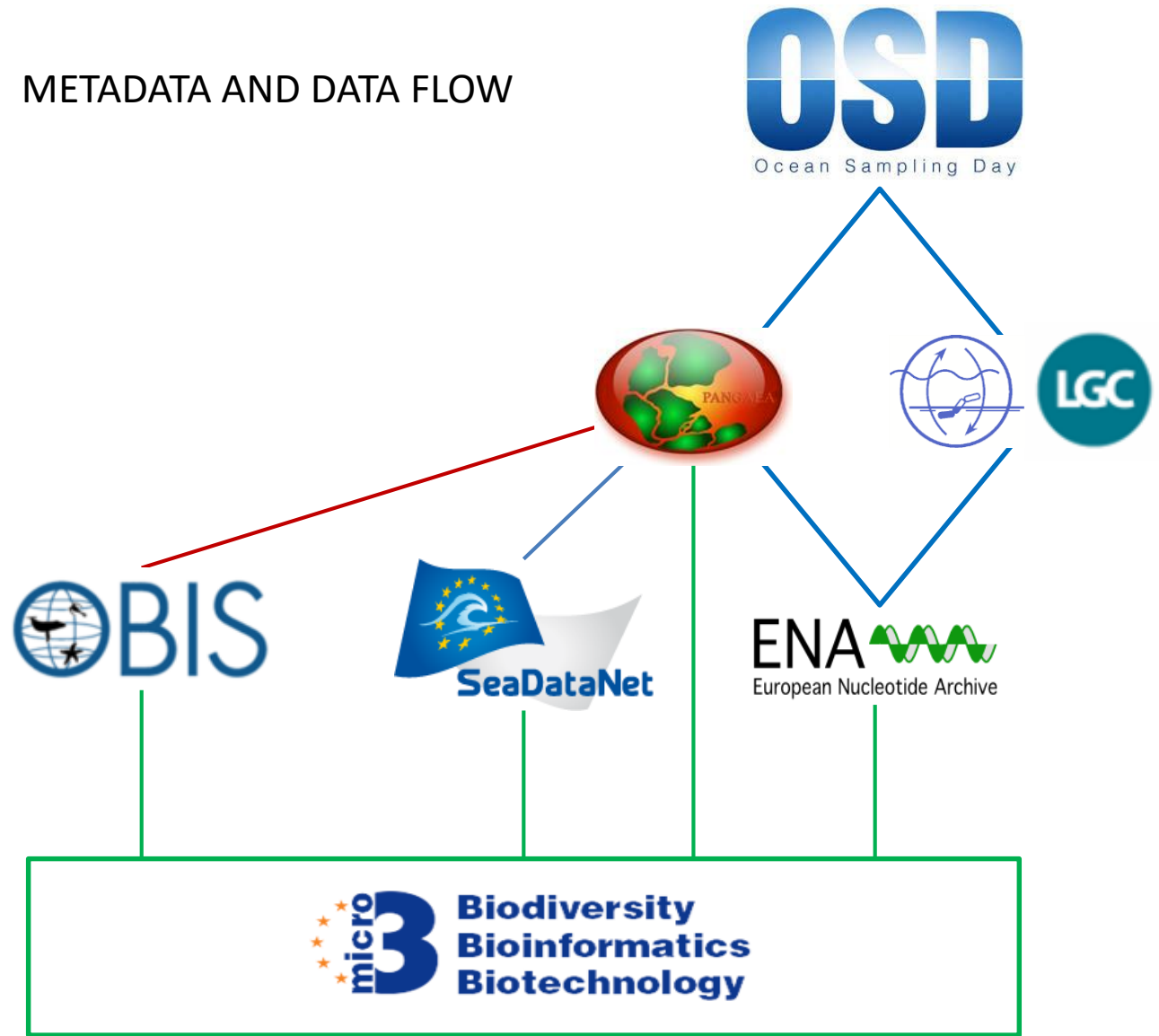
Biological Process

[GO:0053114](#) oxidation-reduction process
[GO:0006099](#) tricarboxylic acid cycle

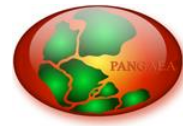
Molecular Function

[GO:0016616](#) oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor
[GO:0030060](#) L-malate dehydrogenase activity

METADATA AND DATA FLOW



Environmental data published at PANGAEA:

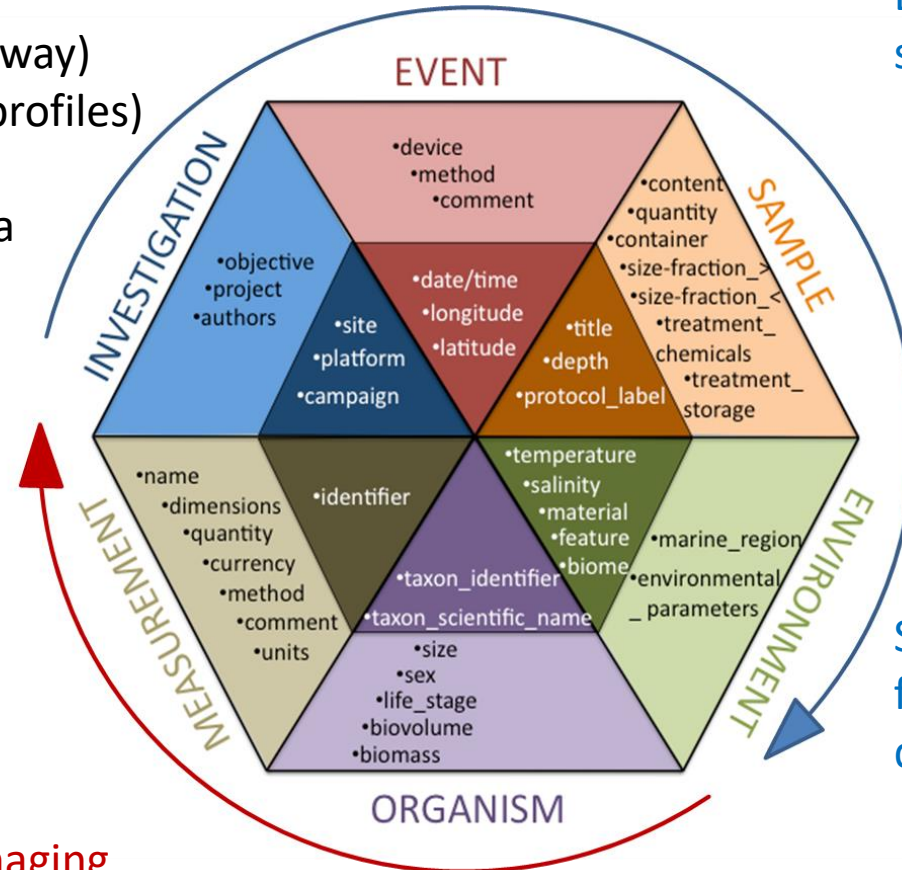


- Metadata
- Sensors (underway)
- Sensors (vert. profiles)
- Water samples
- Contextual data
- more to come



Contextual environmental data disseminated to EBI/ENA for each sequence submitted by Genoscope:

- Sample metadata
- Temperature
- Salinity
- Oxygen
- Nutrients
- Chlorophyll

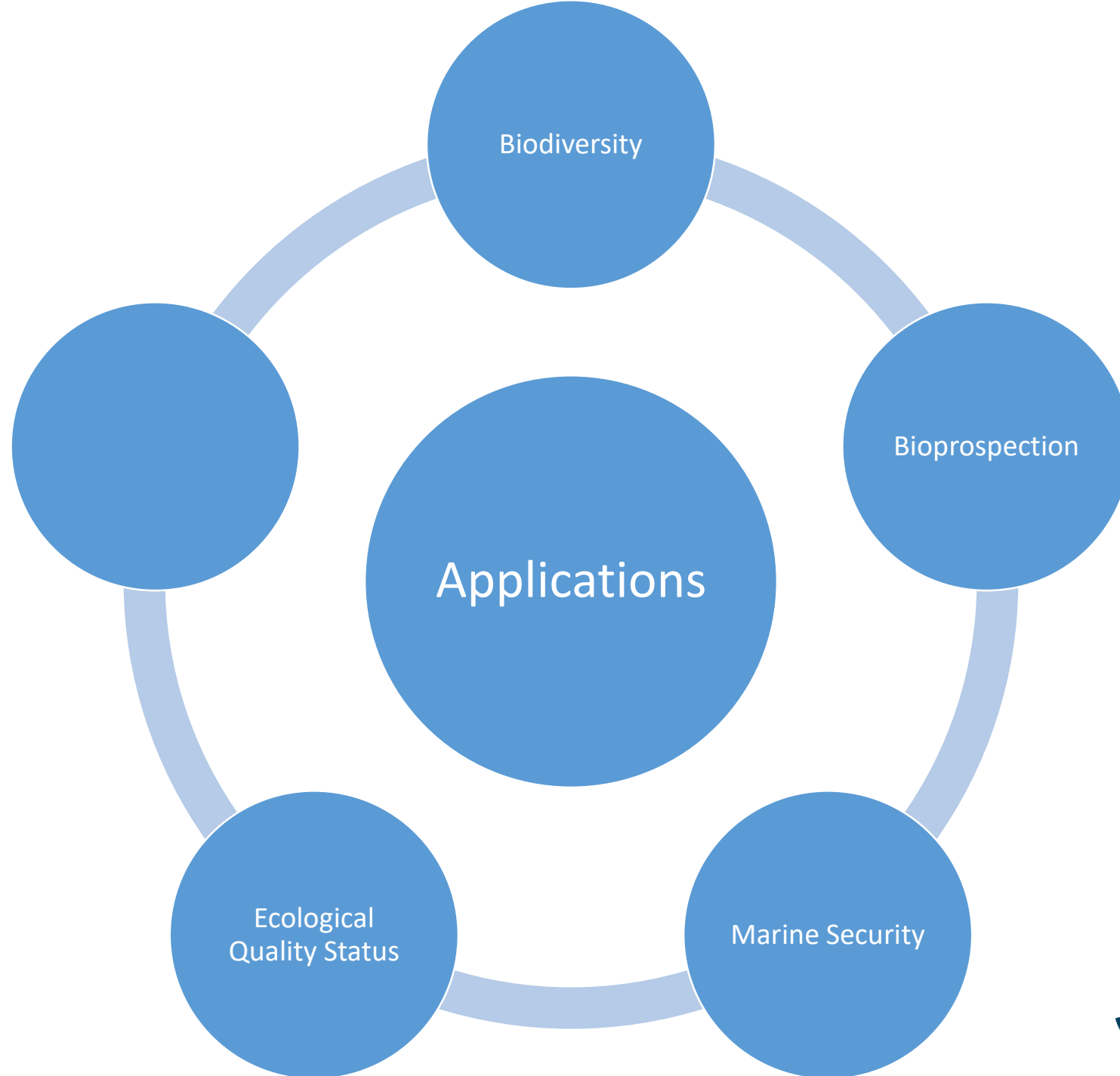


Selected environmental data from all sampling sites disseminated to SeaDataNet:

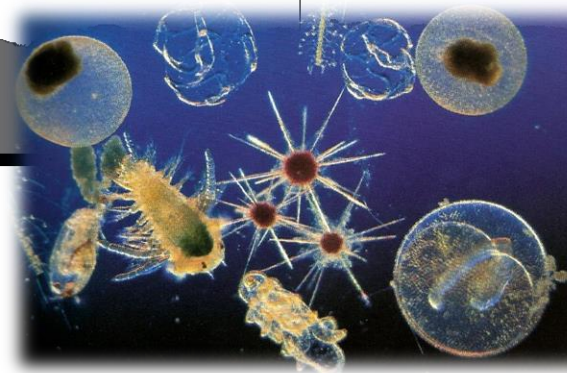
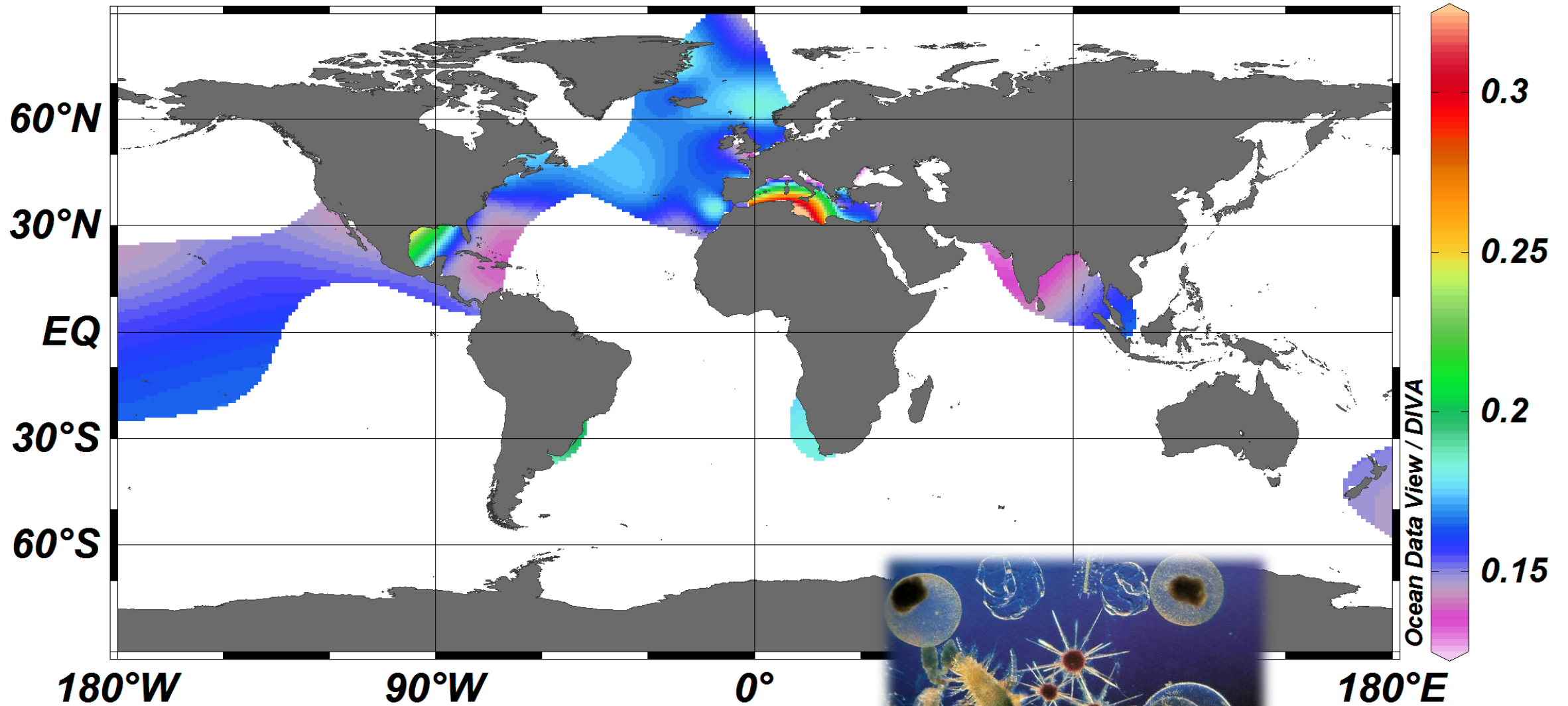
- Temperature
- Salinity
- Oxygen
- Nutrients
- Chlorophyll

Taxonomic and imaging data to be published at PANGAEA and disseminated to OBIS

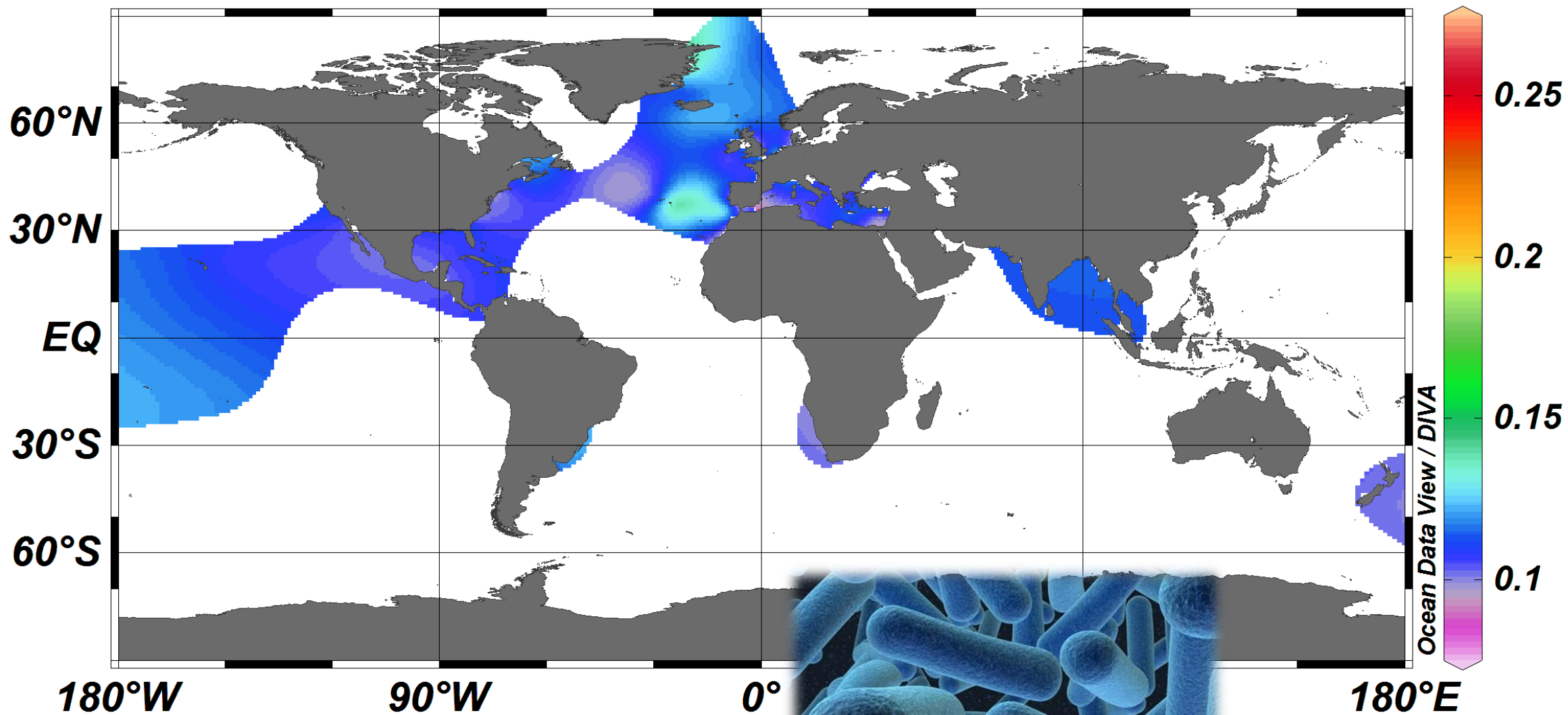




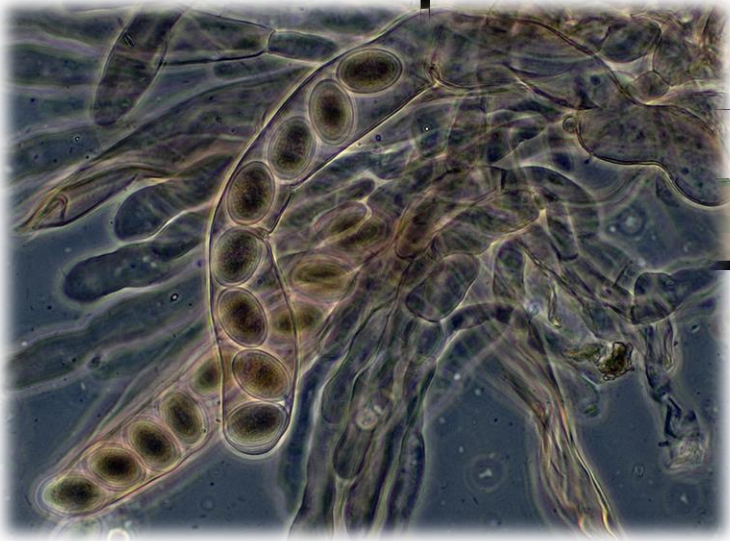
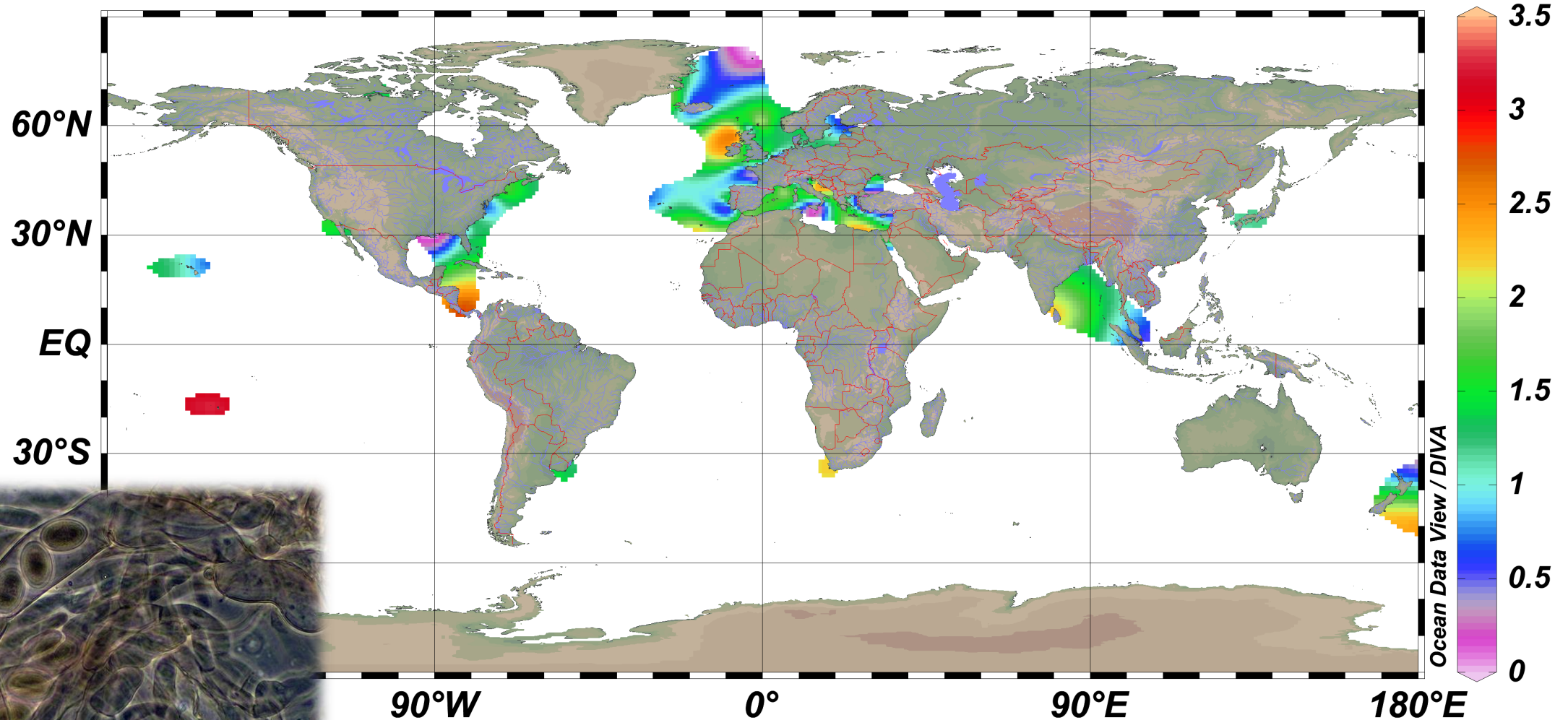
Diversity Index 18S



Diversity Index 16S



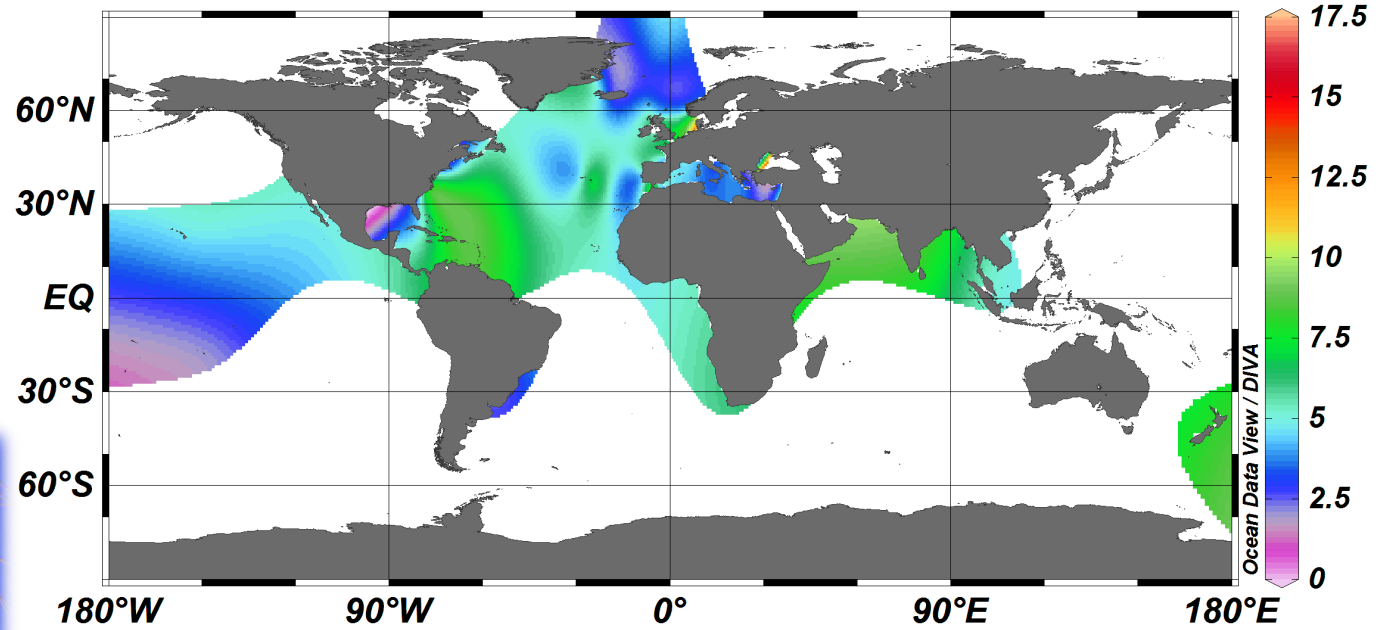
Fungi Shannon Diversity



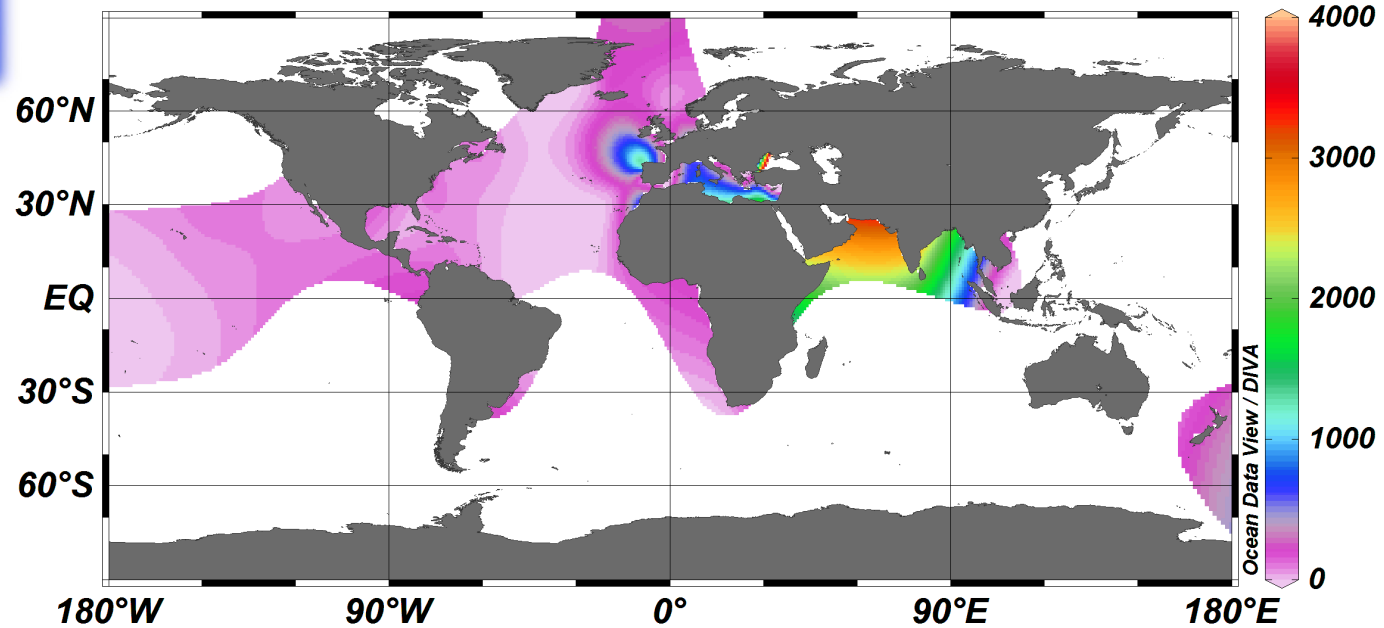
Cnidaria



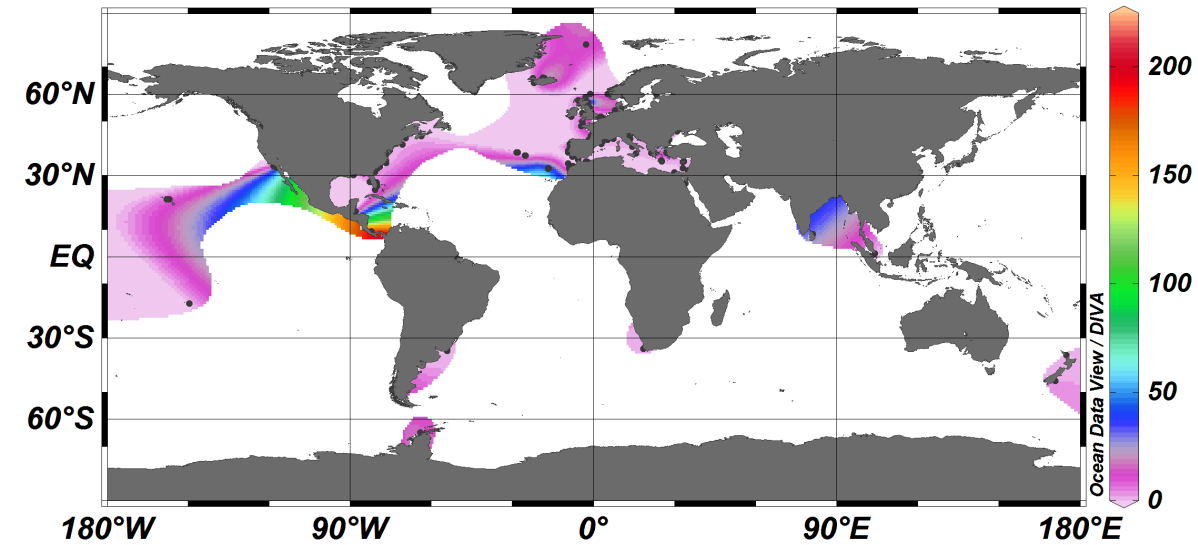
S @ Pressure [db]=first



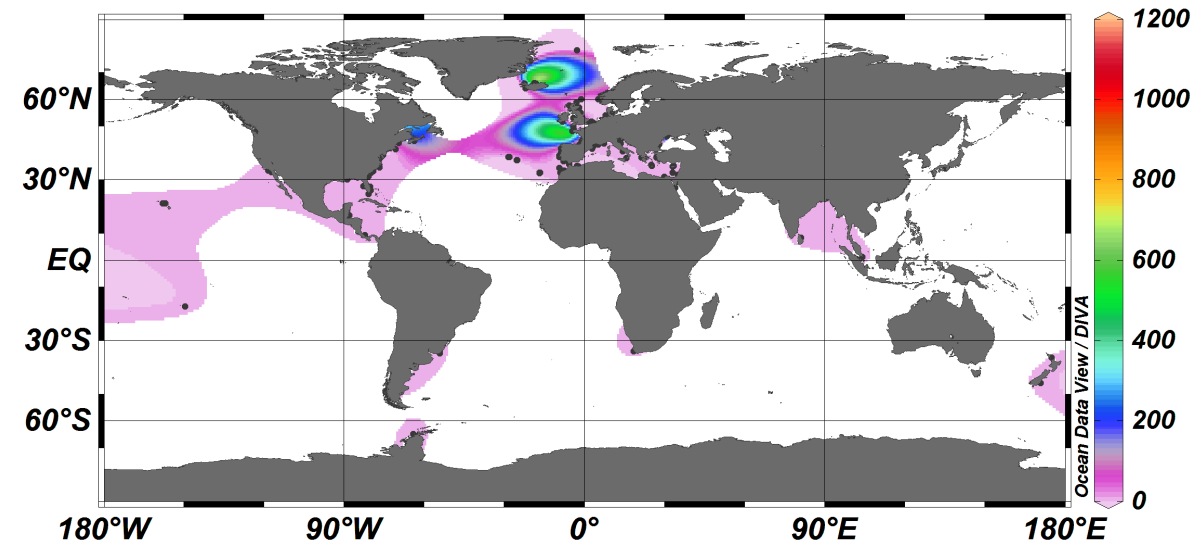
Total Hits @ Pressure [db]=first



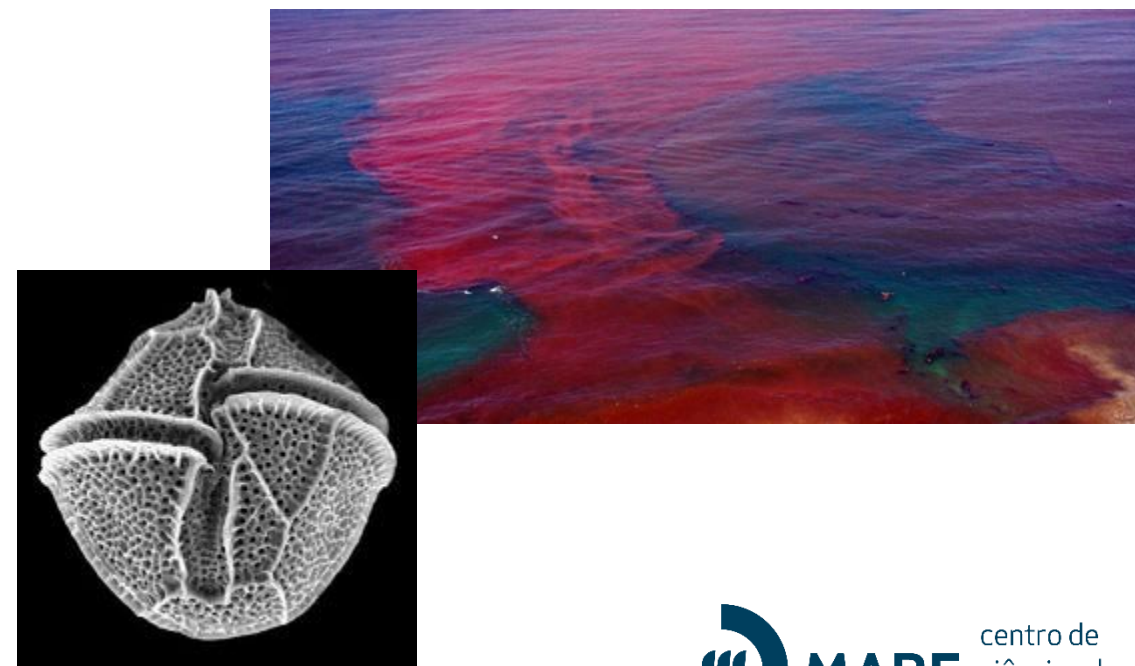
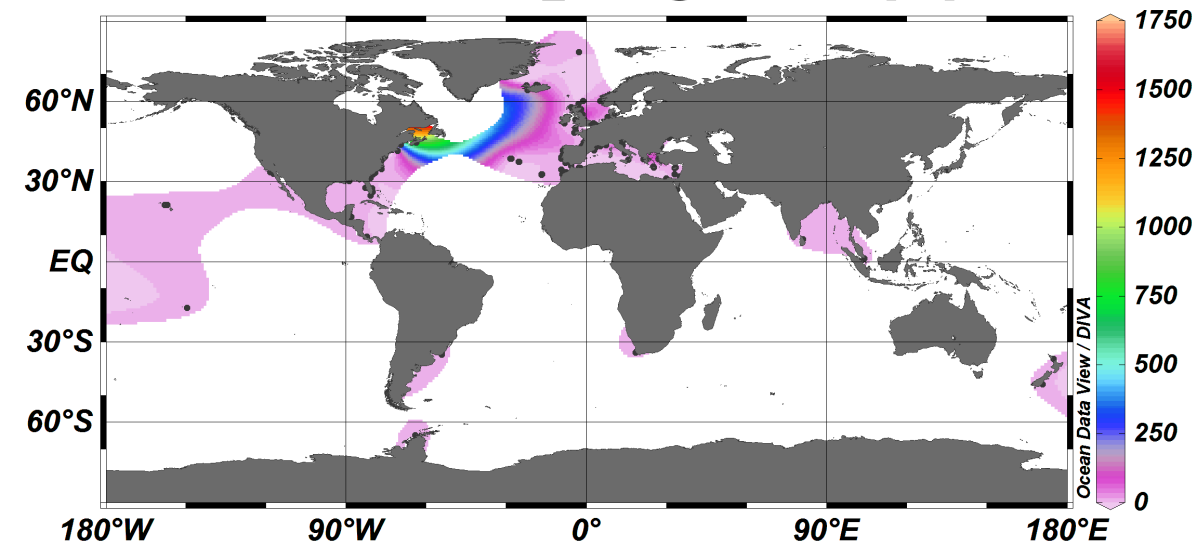
sum_ASP @ Pressure [db]=first



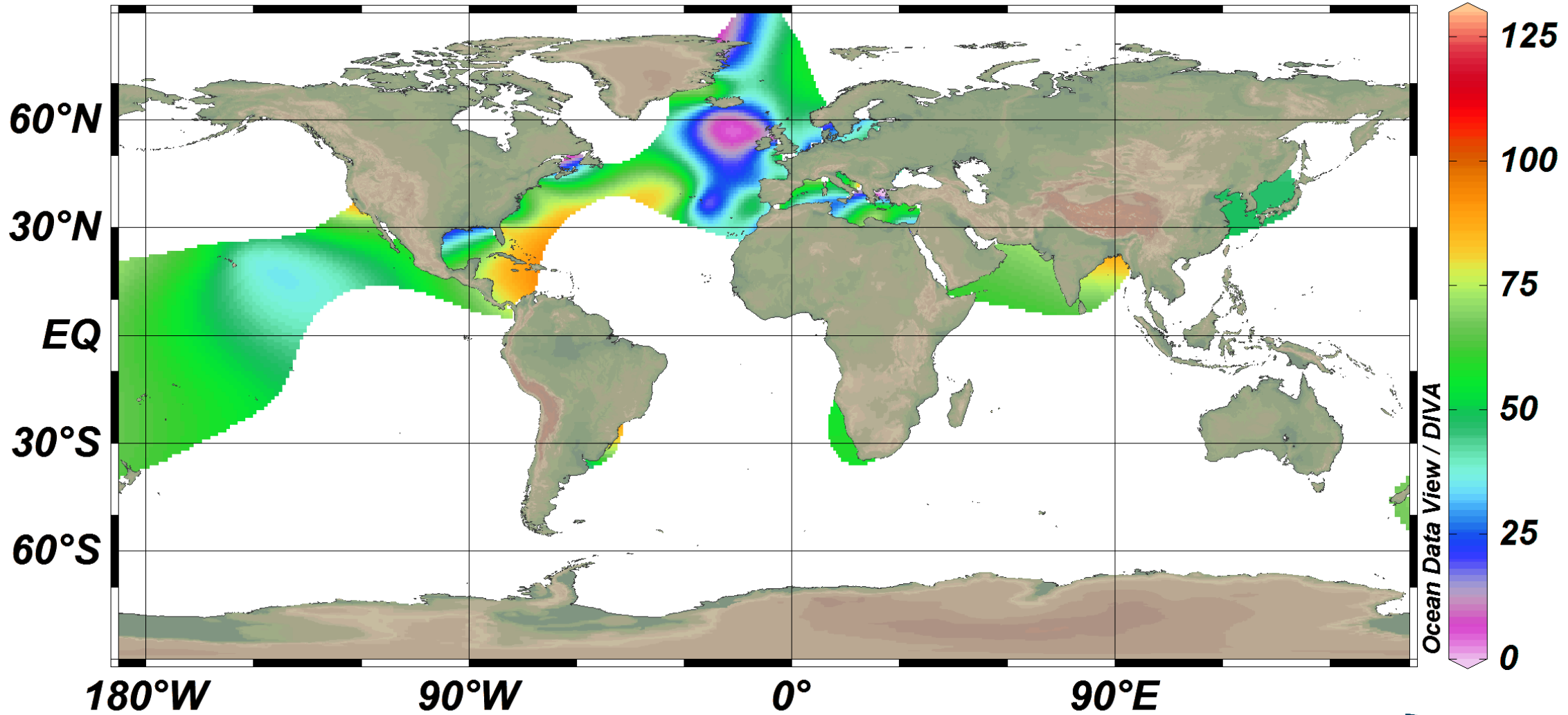
sum_psp @ Pressure [db]=first



sum_DSP @ Pressure [db]=first

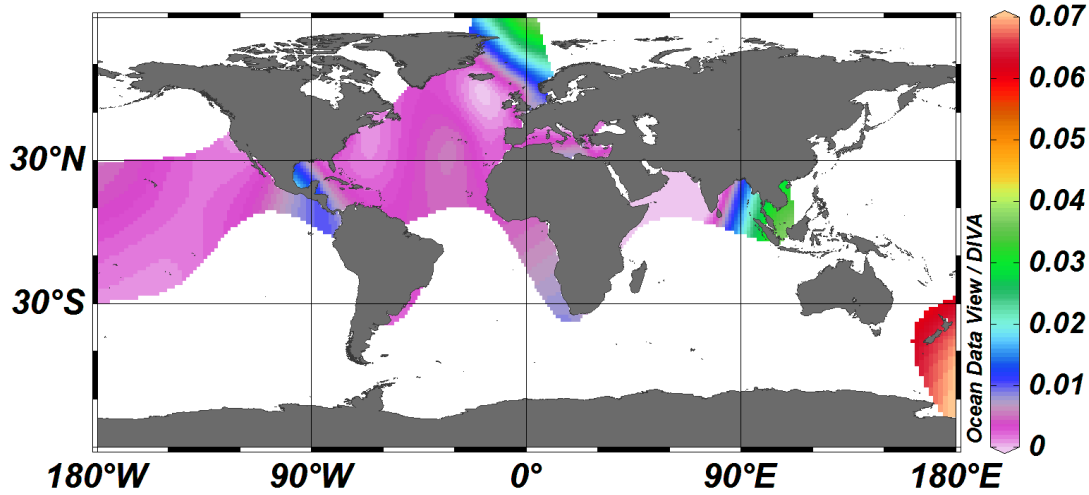


Small_multidrug_resistance @ Pressure [db]=first

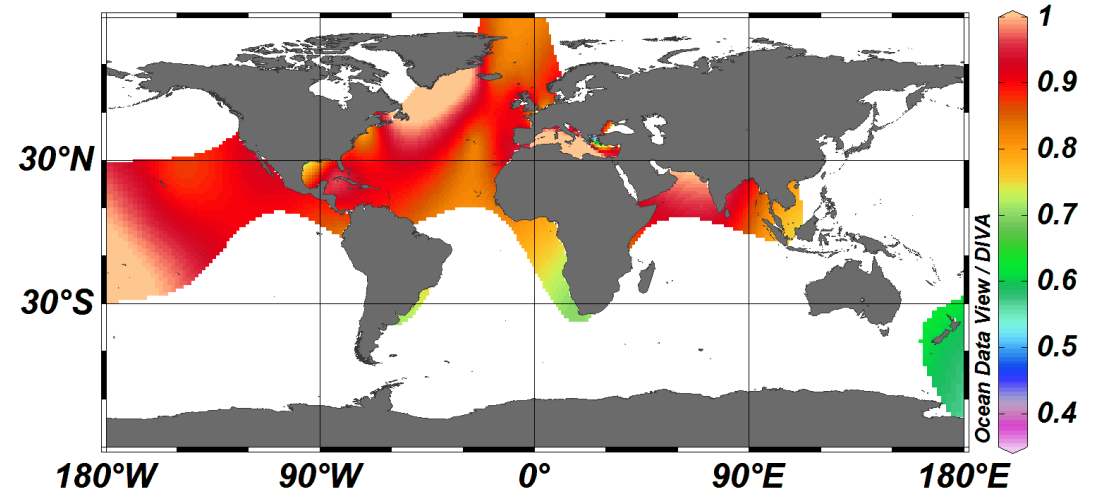


UniPept

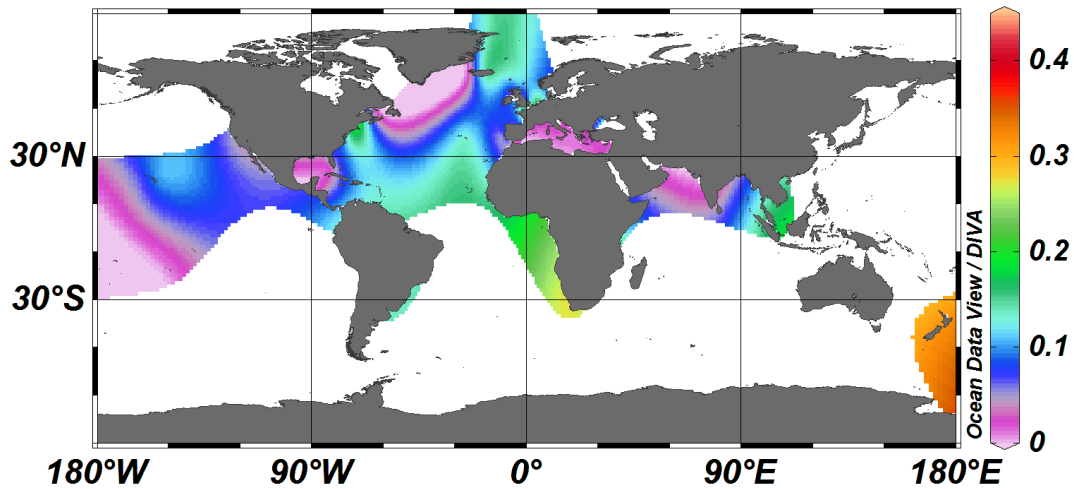
k__Archaea @ Pressure [db]=first



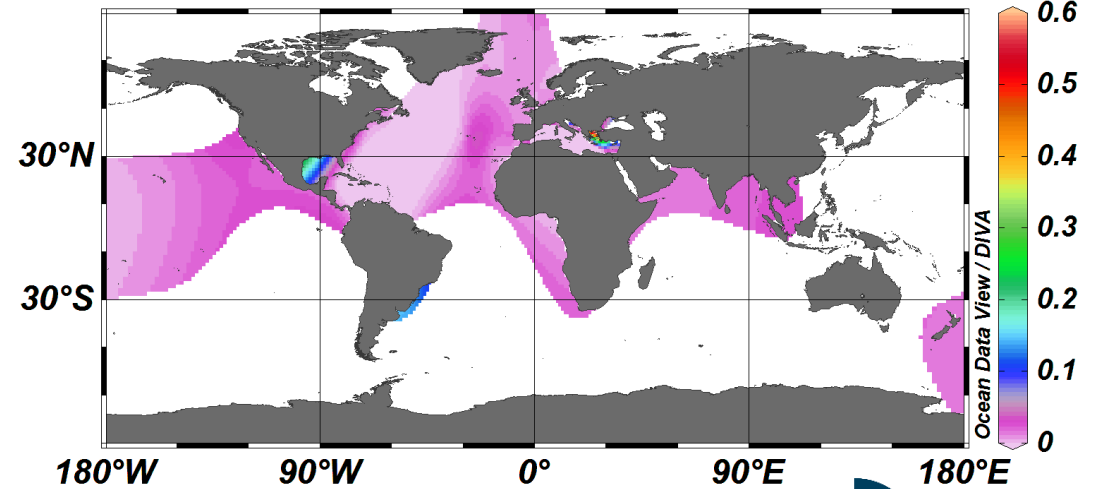
k__Bacteria @ Pressure [db]=first



k__Eukaryota @ Pressure [db]=first



k__Viruses @ Pressure [db]=first



A Global Map of Human Impact on Marine Ecosystems

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