Innovative monitoring tools for the integrated assessment of the environmental status and multi-layer biodiversity

Roberto Danovaro
Main objectives: development (objective 5.1) and validation (objective 5.2) of innovative monitoring systems to refine and deepen our ability to detect environmental/biotic variables utilized in marine monitoring.

- Application of the latest developments in molecular biology (second generation sequencing, high-throughput approaches) to:
  - identify species (from microorganisms to large invertebrates)
  - detect functional genes indicating potential danger for humans.
- Earth observation and ecosystem model output, automated ship-board instruments and underwater image/sound analysis devices.
- Identification of key variables and indicators at the three main levels of biodiversity investigated: a) species and functional groups, b) habitats and c) ecosystems.
- Evaluation of the effectiveness of the newly developed instruments and methods involved.
- Benchmark the innovative techniques against current ones (WP1), analysis for their cost-effectiveness (WP2) and performance (WP3) for environmental monitoring.
Specific Objective 5.1: Development of innovative monitoring systems and tools able to provide real-time information on biodiversity needed for the assessment of good environmental status.
- Task 5.1.1: Applying remote sensing to assess marine biodiversity (from species distribution to habitat types and their heterogeneity).
- Task 5.1.2: Assessing the value of utility of molecular approaches for biodiversity quantification in monitoring systems.
- Task 5.1.3: Applying innovative tools to gather information on the diversity of potentially-dangerous algae for human health.

Specific Objective 5.2: Validation of new and innovative monitoring systems and tools developed.
- Task 5.2.1 Validation of the metagenetic and metagenomic approaches to assess structural and functional biodiversity (from phytoplankton to macro-invertebrates)
- Task 5.2.2 Validation of the identification of toxic genes of harmful algae.
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WP5 Overall structure

REMOTE SENSING
Phytoplankton blooms
Habitat complexity

MULTIBEAM
Habitat heterogeneity

IMAGING
Habitat complexity and species distribution

ACOUSTIC
Plankton – Benthos assemblages

BIOSENSORS
Organisms movement and growth

WP5 Innovative monitoring techniques

METAGENETIC
Structural multilevel biodiversity

METAGENOMIC
Functional multilevel biodiversity

MICROARRAY
Potential harmful algae & risk for Human health

ARTIFICIAL SUBSTRATES
Mol vs Classical analyses
Specific Objective 5.1: Development of innovative monitoring systems and tools able to provide real-time information on biodiversity needed for the assessment of good environmental status.

Task 5.1.1: Applying remote sensing to assess marine biodiversity (from species distribution to habitat types and their heterogeneity).

- Existing and innovative tools will be combined to have an integrated assessment of the environmental status (ferrybox devices for plankton monitoring, ocean colour algorithms, remote logging equipment, particle imaging for identifying phytoplankton, marine ecosystem models, freely-available operational models from the GMES marine service, models developed in WP4, ecosystem model ERSEM, optical tools, phytoplankton pigment chemotaxonomic analysis, zooscan).

These innovative approaches will be tested on fisheries surveys for 1-2 years (in monitoring networks from the partners).

Leader: CEFAS, Partners: AZTI, NIOZ, SYKE, NILU, MHI-NASU, JRC, PML

- Novel tools for the remote measurement of benthic biodiversity (and further assessment) at different spatial scales will be developed by using high definition multibeam.

Leader: OceanDTM, Partners: HCMR, AZTI, PML

- Habitat complexity and the species inhabiting different habitat types, with particular focus on benthic assemblages, will be assessed by means of visual imaging systems (i.e., ROV equipped with HD and 3D cameras) and image analysis. Existing techniques already used in other field (such as acoustic devices used by industries for petroleum investigations) will be applied with a synergistic approach (ROVs/AUVs) to assess GES. Leader: APN, Partners: HCMR, KUCORPI, PML, CEFAS, IO-BAS, AU, UHULL

- The application of innovative acoustic monitoring will allow in situ detection and assessment of plankton communities and marine mammals. Leader: IO-BAS, Partners: AZTI, PML

- High frequency, non-invasive biosensors to measure movement and shell growth in bivalves with powerful mathematical analysis and recent technological advances in real time data transfer and analysis. Leader: APN. Participants: CNRS, CONISMA, PML

Outputs: Deliverable D 5.1 (Month 12), D 5.2 (Month 36), D 5.3 (Month 24)
Milestone: MS 20 (Month 12)
Task 5.1.2: Assessing the value of utility of molecular approaches for biodiversity quantification in monitoring systems.

... possibility to obtain information in real time. For this task we will take advantage of the recently developed high-throughput sequencing technologies (e.g. 454 and Illumina) and genomic analysis software to perform metagenetic (and in selected samples of metagenomic analyses); ...

The metagenetic approach will be used to assess the biodiversity of both planktonic and benthic components (i.e., phytoplankton, prokaryotes, meiofauna and macrofauna) by taxonomic assignment of the obtained sequences, allowing us to evaluate biodiversity and to identify species indicators of presence of toxins.

This task will include: i) the development of standardized and comparable protocols to set up metagenetic analyses on prokaryotes and eukaryotes, ii) the development of a pipeline set up to identify the prokaryotes playing a key role in the functioning of marine ecosystems; iii) bioinformatics analyses of data gained through metagenetic analyses on prokaryotes and eukaryotes for the analysis of multi-level diversity of pelagic and benthic components; iv) the development of new diversity indices based on metagenetic diversity (using approach similar to AMBI, link to WP3); v) development of the first data base of metagenetic diversity on whole sample (including prokaryotes and all metazoa, i.e. protists + meiofauna + macrofauna).

Leader: CSIC, Partners: APN, AZTI, CNRS, CONISMA, IO-BAS, Cefas, KAUST

Outputs: Deliverable D 5.1 (Month 12), D 5.4 (Month 36)
Milestone: MS 20 (Month 12)

PLANKTON (CSIC): Phyto (Protist) + Prok + small euk: Catalan (CSIC) + Red Sea KAUST + Atlantic ATZI (?tbc)
BENTHOS (Conisma) Meio-macro: Adriatic Conisma W Med CNRS + Atl Bay of Biscay ATZI+ CNRS + Norwegian (APN) + Red Sea KAUST
Task 5.1.3: Applying innovative tools to gather information on the diversity of potentially dangerous algae for human health.

A specific effort will be paid to introduce recently-developed and innovative tools for the “real-time” detection of potentially dangerous species for human health. Among these, our interest will be focused on toxic species of dinoflagellates and other key taxa determining Harmful Algal Blooms (HABs), which are potentially dangerous for human health of for the higher trophic levels (including fish, mammals) in EU coastal waters. The innovative tools developed in existing EU project (i.e., MIDTAL) will be applied in key areas by using a high throughput, automation system of microarray-based detection assay, which consists of a series of genetic probes targeting genes that are specific to all harmful algae of interest. A collection of these genes will be gathered from data in public datasets and completed with the information obtained in objective 5.1. Using this newly developed custom microarray, the presence and expression level of genes that are indicators of the toxic effect of the species determining the algal bloom can be measured. The main advantage of this microarray based method is that it does not need any cultivation, providing an immediate response in situ, thus enabling the start of a real time decision making on the strategies to be adopted to preserve human health.

Participants: Leader: CONISMA, Partners: CSIC, APN, Cefas

Outputs: Deliverable D 5.1 (Month 12)  
Milestone: MS 20 (Month 12)

HAB: Adriatic Conisma + BS IO-BAS + Baltic KUCORPI (lagoon?) + Catalan (CSIC) + Norwegian Sea APN + S North Sea PML
Specific Objective 5.2: Validation of new and innovative monitoring systems and tools developed.

Task 5.2.1 Validation of the metagenetic and metagenomic approaches to assess structural and functional biodiversity (from phytoplankton to macro-invertebrates)

This task will be dedicated to compare traditional approaches (i.e., taxonomic analysis, size and biomass-spectra and functional traits approaches) with innovative metagenetic and metagenomic approaches. The consistency of diversity patterns and the sensitivity to changes toward environmental gradients will be verified. For macroinvertebrates, soft-bottom (using van Veen grabs) and hard-bottom (using ARMS and ASU devices) substrata will be considered. In particular, in the framework of existing monitoring networks: i) key-samples will be collected (AZTI) and devices such as ARMS and ASU will be deployed in the four regional seas; ii) multi-level biodiversity will be assessed with traditional approaches (AZTI, CONISMA, CSIC, KAUST, with NOAA as observer); iii) multi-level biodiversity will be analyzed with innovative tools (i.e., metagenetic or metagenomic approach) for all possible biodiversity components (from prokaryotes to phytoplankton and metazoans; AZTI, CONISMA, KAUST, CSIC, with NOAA as observer); iv) AMBI and M-AMBI indices, used in assessing benthic health, will be assess for soft-bottom benthic communities (AZTI); iv) new indices will be developed on the basis of data gathered with innovative approaches and will be compared with AMBI and M-AMBI (AZTI), in collaboration with WP3; v) indices based on data gathered by traditional and innovative approaches will be compared, in collaboration with WP6. The information generated here will be used in the cost-benefit analysis to be done in WP2. The result from this task will be published in several manuscripts. Participants: Leader: CONISMA, Partners: CSIC, AZTI, KAUST, PML, CNRS

Outputs: Deliverable D 5.5 (Month 46) - Milestone: MS22 (Month 36)

**ARMS vs ASU:** Med Conisma; Atl ATZI; BS IO-BAS, Baltic KUCORPI, Eng Channel PML (?tbc), Red Sea KAUST

**Metagenetic vs classical benthic invertebrates:** same as above

**Multilevel benthic biodiv:** Med Conisma(CSIC picoeuk) + BS IO-BAS + Engl Chan (S North Sea) PML (+ APN naturally stressed area?)
Task 5.2.2 Validation of the identification of toxic genes of harmful algae.
This task will be dedicated to compare data obtained with traditional approaches (i.e., taxonomic analyses, etc.) with those obtained by innovative high throughput assays (i.e., microarray assays) and to test which approach provides the most adequate information to the risk of exposure. In particular, in this task: i) water samples will be collected during summer periods, ii) replicates of key-samples will be examined by light, epifluorescence or electron microscopy (traditional approach) and by microarray assays (innovative approach); iii) the sensitivity of both approaches to changes toward environmental gradients will be tested; iv) the information gathered by the two approaches will be compared in terms of sensitivity of the methods, amount and type of information gathered, rapidity of obtaining information and monetary cost-benefit analysis.

Leader: CONISMA, Partners: APN, IO-BAS.

Outputs: Deliverable D 5.5 (Month 46)
Milestone: MS22 (Month 36)

Mol tool for toxic genes: Adriatic Conisma + Black Sea IO-BAS + Baltic KUCORPI (lagoon?) + Catalan (CSIC)
First step: DEVELOPMENT

i) the development of standardized and comparable protocols to set up metagenetic analyses on prokaryotes and eukaryotes,

ii) the development of a pipeline set up to identify the prokaryotes playing a key role in the functioning of marine ecosystems;

iii) bioinformatics analyses of data gained through metagenetic analyses on prokaryotes and eukaryotes for the analysis of multi-level diversity of pelagic and benthic components;

iv) the development of new diversity indices based on metagenetic diversity (using approach similar to AMBI, link to WP3);

v) development of the first data base of metagenetic diversity on whole sample (including prokaryotes and all metazoa, i.e. protists + meiofauna + macrofauna).
Second step: VALIDATION

i) Comparison of traditional approaches (i.e., taxonomic analysis, size and biomass-spectra and functional traits approaches) with innovative metagenetic and metagenomic approaches;

ii) Check for consistency of diversity patterns and the sensitivity to changes toward environmental gradients will be verified (both on soft- and hard-bottom substrata).
WP5 - Innovative monitoring techniques

• Molecular tools and methodologies for the detection of specie from microorganisms to large invertebrates or toxin genes
  – NGS (Next Generation Sequencing)
    • Metagenetic
    • Metagenomic
  – Microarrays
Microarrays

• POA (Phylogenetic arrays)
  – Targeting DNA/RNA with hierarchical probes
  – Output of MIDTAL (completed EU project) and μAqua

FGA (Functional gene arrays)
GeoChip 3.0 can be used to analyze the structure, functional activity and dynamics of microbial communities, but also to link microbial communities with ecosystem processes and functions.
Deliverables

D 5.1 - Responsible CONISMA (Month 12)
Report on the set up of the field and experimental activities.

D 5.2 - Responsible IO-BAS (Month 36)
Report on the application of acoustic devices and visual imaging to assess abundance and diversity.

D 5.3 - Responsible APN (Month 24)
Report on the application of non-invasive biosensors.

D 5.4 - Responsible CSIC (Month 36)
Report on the optimization of protocols and results of the molecular analyses of biodiversity.

D 5.5 - Responsible CONISMA (Month 46)
Report on the validation of the application of remote sensing devices and metagenomic approaches
MS 20 – Responsible CONISMA (Month 12)
Workshop of WP5, defined all experimental protocols.

MS 21 – Responsible CSIC (Month 24)
Workshop of WP5 to present the results of the application of innovative monitoring techniques.

MS 22 – Responsible APN (Month 36)
Optimized protocols for molecular and microarray analyses available.

MS 23 – Responsible CONISMA (Month 46)
Workshop of WP5 participants, dedicated to presentation of results of the validation.
## Timetable

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<td>D 5.5, MS23 (CONISMA)</td>
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Second step: VALIDATION

In particular, in the framework of existing monitoring networks:

i) key-samples will be collected (AZTI) and devices such as ARMS and ASU will be deployed in the four regional seas;

ii) multi-level biodiversity will be assessed with traditional approaches with innovative tools (i.e., metagenetic or metagenomic approach) for all possible biodiversity components (from prokaryotes to phytoplankton and metazoans (AZTI, CONISMA, CSIC, KAUST, with NOAA as observer)

iii) AMBI and M-AMBI indices, used in assessing benthic health, will be assess for soft-bottom benthic communities (AZTI);

iv) new indices will be developed on the basis of data gathered with innovative approaches and will be compared with AMBI and M-AMBI (AZTI), in collaboration with WP3;

v) indices based on data gathered by traditional and innovative approaches will be compared, in collaboration with WP6.
Kick-off meeting, 6th-9th November 2012, Mallorca