European Commission
DG Environment

Cruise Report
MISIS JOINT CRUISE

Scientific Data Collected on board R/V AKADEMIK

22.07 – 31.07.2013

MSFD Guiding Improvements in the Black Sea
Integrated Monitoring System

Package activity number: 2

Initial Testing of Revised Monitoring Programmes, Joint Survey, Data Collection

Dissemination Level – Public

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Disclaimer

The Research Team

Research Vessel Akademik

Contents

I. Cruise summary

This cruise report outlines the scientific program conducted onboard *R/V AKADEMIK* during the MISIS Joint Cruise in the period 22.07 – 31.07.2013, in Romanian, Bulgarian and Turkish Black Sea waters. The report consists of summaries of scientific data collected during the oceanographic operations and shortly describes the inter-comparison exercises.

On July 22, 2013, the scientific teams from Romania (NIMRD and GEOECOMAR), Bulgaria (IO-BAS) and Turkey (SINOP University and TUBITAK) gathered in Varna, Bulgaria to begin a remarkable cruise onboard what was to become home for the next ten days, the *R/V AKADEMIK*. We sailed that afternoon from Varna to the first sampling station (M 01) located in the Romanian coastal waters (on transect Constanta – East). Before reaching the first sampling station, in the afternoon of July 22, the scientific equipment onboard was tested to ensure the proper implementation of the sampling program. Also, some organizational issues, including the appointment of the responsible scientists for physical-chemical and biological data, contaminants, marine litter, were discussed during a short meeting onboard.

The sampling program started on July 23, 2013, in the morning, and continued until July 25, 2013 in Romanian waters, where seven sampling stations (along the transect Constanta–East, bottom depths within 33 – 1000 m) were performed. CTD measurements were performed at each station; the sampling depths for nutrients, TOC, chlorophyll *a*, phytoplankton and zooplankton were selected based on temperature, density and fluorescence profiles. Waters samples for pollutants (heavy metals, pesticides, etc.) were taken only from the surface layer (actually from 1 m below the surface). For microzooplankton, the water samples were taken from surface, chlorophyll maximum (DCM), and cold intermediate (CIL) or bottom layers (for shallower stations). Zooplankton and ichthyoplankton samples were collected from all stations, while benthos samples were taken only from five stations (coastal and shelf stations). Also, sediment samples for TOC and pollutants were collected from the coastal and shelf stations (M 01 – M 05). The dredge was launched in order to collect biota samples for pollutants and to assess marine litter (two dredge launchings; the first one between stations M 01 and M 02, bottom depth of ~ 33 m, and the second one, which was repeated, between the stations M 04 and M 05, bottom depth of ~ 65 m). Also, for assessing marine litter, the ROV (Remote Operating Vehicle) was lowered in the Romanian coastal waters (station M01).

After a successful work done in the Romanian waters, we sailed to the shallowest sampling station from the Bulgarian waters (M 12 – 23 m, on transect Galata). On 26 July, 2013, in the morning, we started our scientific program in the Bulgarian waters, which lasted until 28

July, 2013. We performed five stations, along the transect Galata (bottom depths within 23 – 1169 m); the parameters analyzed were similar to those in the Romanian waters (sediment samples were collected only from four stations). Marine litter and pollutants in biota were studied by launching the dredge in the coastal and shelf waters (bottom depths within 23 – 69 m). In addition, in the coastal waters (bottom depth of ~ 40 m), the ROV was launched (on 26 July) in order to test the possibility to quantify marine litter at the sea bottom following the JRC draft Manual for Marine Litter assessment.

One of the shelf stations (M 10 – bottom depth of 75 m) was chosen for inter-comparison exercises for zoobenthos, contaminants and TOC in sediment. In the morning of 28 July, we reached the deepest station of the program, M 13 (bottom depth of 2018 m) for the inter-comparison exercise with regard the chemical (nutrients, TOC, contaminants) and some biological parameters (chlorophyll, phytoplankton, and zooplankton) from the water column.

After successful completion of the scientific program in the Bulgarian waters, in the evening of 28 July 2013, we sailed to the Turkish waters, for the last part of the cruise. On 29 July 2013, we started the sampling program on the transect Igneada; the first station performed being the deepest of that transect (M 14, bottom depth of 1124 m). Five stations (bottom depths within 27 and 1124 m) were done in two days (until 30 July); the interested parameters were similar to those analyzed previously (see the transects Constanta and Galata). Also, ROV was launched in the coastal Turkish waters (bottom depth of ~ 26 m) on 30 July.

An inter-comparison station (M18 – in the coastal waters, bottom depth of 27 m) for sediment (chemistry and biology) and phytoplankton was selected on that transect.

In the evening of 30 July the scientific program was finished, and we started to sail to Varna, the end point of our cruise. Although we planned to launch again the ROV and the dredge in the Bulgarian waters (on 31 July, in the Resovo area), the bad meteorological conditions did not allow us to follow the program. Consequently, during that day, we sailed to the Varna harbor, and we reached the end point next day, in the morning.

We wish to acknowledge the crew, technical and scientific staff for their amazing dedication and efforts in making the MISIS Joint Cruise such a successful scientific program and a wonderfully tight-knit community.

II. Cruise objectives

The MISIS Joint Cruise is the most important part of the 2nd activity of the MISIS project (PA2 - **Initial testing of the revised monitoring programs (field and laboratory work), management of data, assessments: Organization of Joint Black Sea Survey (cooperation with other projects – PERSEUS, Europe Aid, Coconet, for instance).**

The MISIS (**M**SFD GUIDING **I**MPROVEMENTS IN THE BLACK **S**EA **I**NTEGRATED MONITORING **S**YSTEM) Project aims to contribute to advances in the field of environmental protection by providing an improved monitoring program (parameters, data collection in terms of spatial and temporal dimension, reporting – in line with WFD and MSFD requirements), and facilitating the harmonization process in identification, designation and management of MPAs in the Black Sea region. In case that the beneficiary states agree to use the same methodologies and schemes of monitoring, the regional picture of pressures-impacts and associated Black Sea state will become much more adequate than at present. In other words, the improvement and harmonization of monitoring will provide for comparable data sets on the state of the Black Sea (eutrophication, pollution, biodiversity, habitats change, fishing resources, etc.), and also better knowledge on loads stemming from different sources.

Hence, in order to achieve the above mentioned objectives of the project, the Joint Cruise was crucial to achieve the Project objectives as outlined in the DoW:

1. Testing the revised monitoring programs;
2. Collecting additional data and producing homogenous data sets for the Black Sea coastal waters (where possible, open sea will be also included) based on a single sampling procedure and laboratory analysis of specified determinants and biological quality elements;
3. Organizing inter-comparison exercises to evaluate the performance of laboratories involved. Evaluations from previous inter-comparisons will be collected as a basis for comparison of performance (for instance, during the last years BSC and the project FP6 SESAME organized a number of such exercises).
4. Carrying out ecological assessment of the Black Sea, taking into consideration the requirements in the WFD and the descriptors of the MSFD;
5. Screening for new priority pollutants;
6. Providing general overview on the status of habitats.

III. Survey area

The study area was the Western Black Sea, including the Romanian, Bulgarian and Turkish waters. Three transects were chosen; they were considered representative for the purposes of the projects. Map of sampling stations and stations coordinates and depths are presented in Figure.1, Table 1.

The Romanian transect, Constanta-East, includes seven stations (as is listed in the table below); it starts with the station M 01 (in the neighbouring of the Constanta port, bottom depth of 33 m), and ends in the open sea waters (station M 07 – bottom depth of 1000 m). The sampling stations covered the coastal (M 01), shelf (M 02–M 05) and open waters (M 06–M 07).

The Bulgarian transect - Galata transect (travers Varna) - was selected as one of the regular monitoring transects of the IO-BAS monitoring network, and comprises five stations (M 12 to M 08, with bottom depths within 23–1169 m) covering the coastal (M 12), shelf (M 09–M 11) and open sea waters (M 08).

Both Constanta–East and Galata transect are part of the national monitoring networks, so the data obtained during this cruise could be compared with the previous data collected during national monitoring programs and other international projects.

In the Bulgarian waters, at depth of about 2018 m, an inter-comparison station was chosen (M 13) for chemical and some biological parameters in the water column.

The Turkish transect (Igneada) comprises five stations (M 14–M 18, with bottom depths within 27–1124 m), and similar to the Romanian and Bulgarian waters, they covered coastal (M 18), shelf (M 17–M 15) and open sea waters (M 14).

Both in the Bulgarian and Turkish waters, there were selected another two inter-comparison stations (one for each country; M 10, bottom depth of 76 m, and M 18, bottom depth of 2753 m, respectively) for sediment chemistry and benthos (both stations), and for phytoplankton (only M 18).

Station	Transect	Lat, °N	Long, °E	Bottom depth, m	Type	Date
M12 probe		43°08.592	028°03.828	20.1	coastal	22.07.13
M 01	Constanta	44°10.000	028°47.000	33.3	coastal	23.07.13
M 02	Constanta	44°10.000	029°.08.000	47.0	shelf	23.07.13
M 03	Constanta	44°10.000	029°.20.000	54.0	shelf	23.07.13
M 04	Constanta	44°10.000	029°.40.000	64.7	shelf	24.07.13
M 05	Constanta	44°04.800	030°.11.900	101.0	shelf	24.07.13
M 06	Constanta	43°55.000	030°.22.100	495.0	open waters	24.07.13
M 07	Constanta	43°47.800	030°.42.900	1000.0	open waters	25.07.13
M 12	Galata	43°10.000	028°.00.000	23.2	coastal	26.07.13
M 11	Galata	43°10.000	028°.20.000	39.9	shelf	26.07.13
M 10	Galata, inter-comparison	43°10.000	028°.30.000	76.1	shelf	26.07.13
M 09	Galata	43°10.000	028°.40.000	92.7	shelf	27.07.13
M 08	Galata	43°10.000	029°.00.000	1169	open waters	27.07.13
M 13	Inter-comparison	42°44.232	029°.20.599	2018	open waters	28.07.13
M 14	Igneada	42°00.252	028°.48.773	1124	open waters	29.07.13
M 15	Igneada	41°56.173	028°.34.350	101.0	shelf	29.07.13
M 16	Igneada	41°53.830	028°.22.760	75.6	shelf	29.07.13
M17	Igneada	41°51.235	028°.06.772	53.3	shelf	30.07.13
M 18	Igneada, inter-comparison	41°49.795	028°.00.275	27.2	coastal	30.07.13

Table 1: List of sampling stations, coordinates and depths

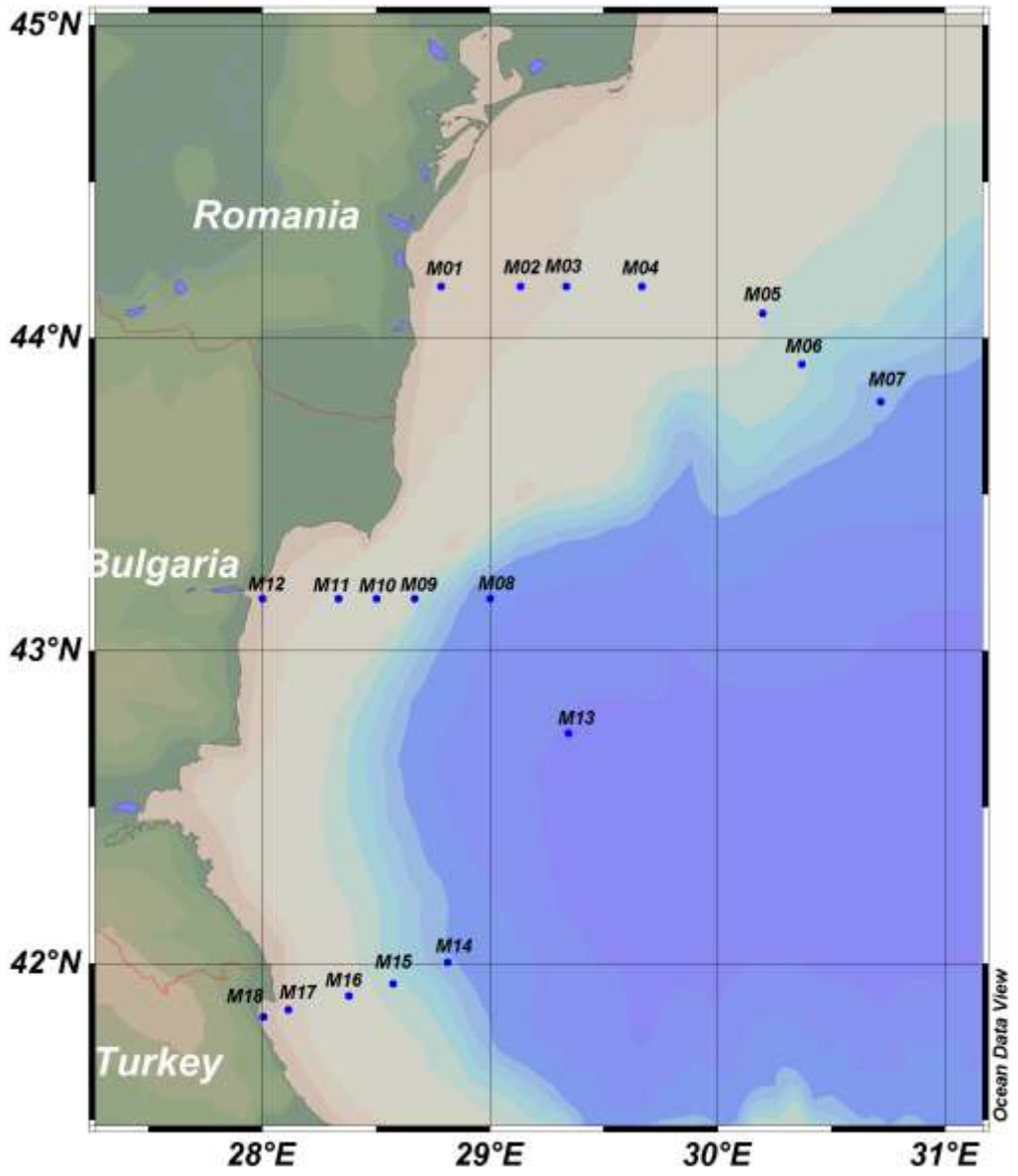

Figure 1: Map of study area

III. Survey area

3.1 Crew List

Table 2: R/V AKADEMIK Crew List

Name	Rank
Mihail Trifonov	Master
Penchi Karapchanski	Chief officer
Kolyu Kolev	Watch officer
Stoyan Stoyanov	Radio officer
Trifon Tonchev	Chief Engineer
Dimitar Dimitrov	Second Engineer
Gacho Gachev	Watch Engineer
Vasil Shovlekov	Electrician
Stanislav Grudev	Bosun
Vasil Chernev	Helmsman
Stefan Nanov	Helmsman
Filip Alexandrov	Motorman
Angel Ivanov	Motorman
Lyubcho Goranov	Motorman
Viktor Chakarov	Cook

3.2 Technical Staff

Table 3: R/V AKADEMIK Technical Staff

Name	Function	Organization
Anatoli Apostolov	CTD operator	IO-BAS, Bulgaria
Asen Krastev	Engineer	IO-BAS, Bulgaria

3.3 Scientific Staff

Table 4: Scientific Staff

Name	Function	Organization
Snejana Petrova Moncheva	Cruise Chief Scientist, Chief Scientist, Biology-phytoplankton	IO-BAS, Bulgaria
Kremena Blagovestova Stefanova	Scientist, Biology-zooplankton	IO-BAS, Bulgaria
Galina Petrova Shtereva	Scientist, Chemistry	IO-BAS, Bulgaria
Ognyana Hristova	Scientist, Chemistry	IO-BAS, Bulgaria
Boriana Djurova	Scientist, Chemistry	IO-BAS, Bulgaria
Anton Krastev	Scientist, Chemistry	IO-BAS, Bulgaria
Laura Boicenco	Project leader, Scientist, Biology-phytoplankton	NIMRD, Romania
Florin Timofte	Scientist, Biology-zooplankton	NIMRD, Romania
Adrian Filimon	Scientist, Biology-zoobenthos	NIMRD, Romania
Cristina Tabarcea	Scientist, Biology-zooplankton	NIMRD, Romania
Oana Vlas	Scientist, Biology-phytoplankton	NIMRD, Romania
Andra Oros	Scientist, Chemistry	NIMRD, Romania
Adrian Teaca	Scientist, Biology-zoobenthos	GEOECOMAR, Romania
Mihaela Muresan	Scientist, Biology-zoobenthos	GEOECOMAR, Romania
Dan Mihai Secrieru	Scientist, Geochemistry	GEOECOMAR, Romania
Dan Lucian Vasiliu	Scientist, Chemistry	GEOECOMAR, Romania
Levent Bat	Scientist, Biology-zoobenthos	Sinop University, Turkey
Murat Sezgin	Scientist, Biology-zoobenthos	Sinop University, Turkey
Fatih Sahin	Scientist, Biology-phytoplankton	Sinop University, Turkey
Hakan Atabay	Scientist, Chemistry	TUBITAK, Turkey

IV. Sampling program. Preserving and analytical procedures

CTD data – CTD measurements were done at all stations. The onboard equipment CTD - SBE 11 plus is equipped with SBE 32 Carousel Water Sampler (12 teflon batometers, 5 l each) (Figure. 2). In addition to standard sensors (pressure, temperature and conductivity), two auxiliary sensors measured dissolved oxygen and *in situ* chlorophyll *a*. The downcast data (pressure, temperature, conductivity, dissolved oxygen and chlorophyll – depth, salinity and density were derived) were binned to 1 m depth intervals using SBE Data Processing Software version 7.18 (Sea-Bird Electronics, Bellevue, Washington, USA).

Figure 2: CTD equipment

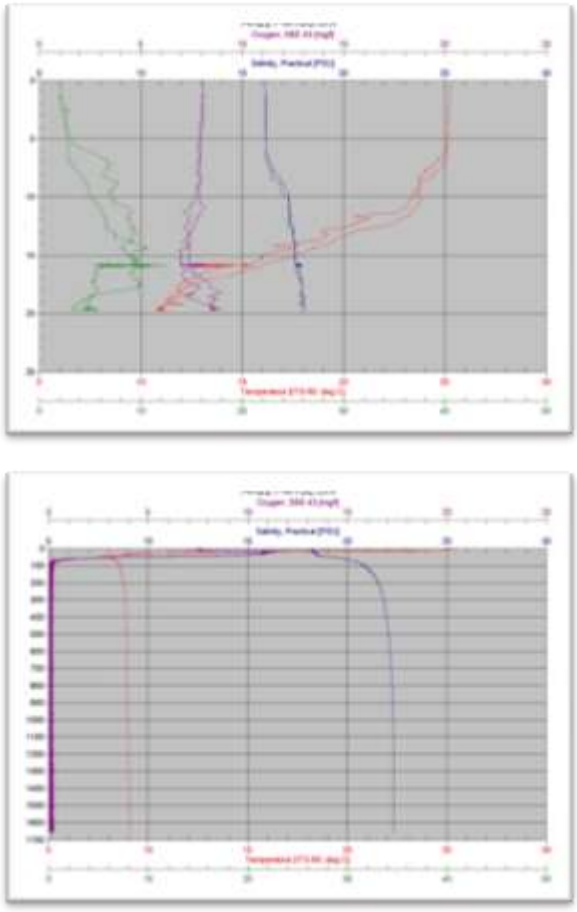

Figure 3: CTD profiles

4.1 Physical - Chemical

Dissolved Oxygen – DO measurements were done onboard by the GEOECOMAR and IO-BAS teams. Both teams analyzed DO according to Winkler method (Grasshoff *et al.,* 1999). GEOECOMAR measured DO (102 samples) at all stations, while IO-BAS at all stations on the Bulgarian transect, as well as at some selected stations as is shown in the Annex 1 (94 samples). The sampling depths were selected according to CTD profiles (Figure. 3), from the surface down to 16.0 Sig T layer (Annex 1).

H_2S – Sulphide measurements were done onboard by IO-BAS team according to methylene blue spectrophotometric method (with *p*-phenylenediamine). Water samples were collected

from the four open sea stations (M 07, M08, M 13, and M 14), from sampling depths below Sig T 16.0 layer (Annex 1).

Total Suspended Solids (TSS) – measurements were done only by TUBITAK from the Turkish transect and inter-comparison station M 13 (total of 22 samples). Variable volumes of seawater (within 1.2 – 3.0 L), collected by Seabird CTD Rosette system from different depths (surface, thermocline, Chl a maximum, CIL and bottom layers), were filtered onboard (filters pore size 0.7µm). The filters were kept frozen until subsequently analyses in laboratory (gravimetric method).

Nutrients (nitrates, nitrites, ammonia, phosphates, total phosphorus, silicates, and total nitrogen) – nutrients samples were collected by IO-BAS, GEOECOMAR, NIMRD and TUBITAK teams. The water samples (volumes within 0.25 – 0.5 l) were collected by Seabird CTD-Rosette system in 5 l plastic bottles during the up-cast at different depths according to CTD profiles. NIMRD, GEOECOMAR and TUBITAK have preserved the samples frozen (at – 20 ÷ -24 °C) until their subsequent analysis in laboratories, whilst IO-BAS team analyzed nutrients onboard (only for the inter-comparison exercise they preserved the samples frozen).

Only GEOECOMAR collected water samples from all transects, at all stations (162 samples). IO-BAS took samples at all stations from the Bulgarian transect, as well as at some selected stations from the other transects (102 samples), whilst NIMRD and TUBITAK sampled only from the Romanian and Turkish transect (49 and 39 samples, respectively) (Annex 1).

The analytical methods used by each team are shown in the chapter below (Table 5). It is noteworthy that besides the cruise partners, there were collected nutrients samples for another laboratory ("Romanian Waters" National Administration – ANAR, 6 samples, 3 replicates each) within the inter-comparison exercise (Table 5).

TOC (water column and sediment) – water samples for TOC were collected by NIMRD (at all stations – 104 samples) and TUBITAK (6 stations – the Turkish transect and the station M 13, 39 samples) from the same sampling depths as nutrients (Annex 1).

NIMRD sampled about 200 ml sea water in glass ampoules, while TUBITAK sampled 100 ml in glass bottles and added 200 µl 1:1 HCl. Both teams stored the samples at refrigerator temperature until their subsequent analysis in laboratory (analytical methods are shown in the chapter below – Table 5).

Sediment samples for TOC were collected using a Van Veen grab sampler by GEOECOMAR, NIMRD and TUBITAK teams. The undisturbed surface layer, 1.0–1.5 cm thick, was carefully

collected with a plastic tube and the samples were placed in polyethylene containers, sealed, labelled and transported to laboratories.

GEOCOMAR collected samples from all transects, at coastal and shelf stations (17 samples, including inter-comparison). NIMRD collected sediment samples only from the inter-comparison stations, while TUBITAK sampled from the Turkish transect (coastal and shelf) and inter-comparison station M10 (5 samples, including inter-comparison).

Figure 4: Seawater samples collection

4.2 Pollutants

Pollutants (heavy metals, PAH, OCPs, PCBs) in water – water samples for pollutants were collected from the surface layer (more precise, 1 m below the surface) from the 5 l Niskin bottles of the Rosette System. About 1 litre seawater was transferred into glass bottles, which were stored at refrigerator temperature until their subsequent analysis in laboratory.

Only NIMRD analyzed the pollutants in water at all stations (18 surface samples) (Annex 1). Total heavy metals (dissolved + suspended) will be analyzed by graphite furnace atomic absorption spectrometry (GF-AAS). GC-ECD method will be used for OCPs and PCBs, GC-MS method for PAHs, and the fluorescence method for TPHs.

Pollutants (heavy metals, OCP, PAH, PCBs) in sediment – sediment samples for pollutants were taken using a Van Veen sampler grab, from the surface undisturbed layer. GEOECOMAR collected sediment samples (17 samples) from all transects, at coastal and shelf stations for heavy metals, NIMRD collected 13 samples from all transects for POPs and 2 samples at inter-comparison stations (M10 and M 18) for heavy metals, and TUBITAK collected sediment samples from Turkish transect and inter-comparison station (M10), at coastal and shelf station (5 samples, including inter-comparison) for all pollutants (Annex 1). Also additional samples were collected for "Romanian Waters" National Administration (ANAR) for inter-comparison exercise (at stations M10 and M18) (Table 5).

The samples were stored frozen (at – 20 ÷ - 24 °C) and analyzed subsequently in laboratories (analytical methods are shown in the chapter below – Table 5, except the methods for OCPs and PCBs – GC-ECD, and TPH – fluorescence method).

Pollutants in biota – NIMRD collected 13 samples of molluscs - *Mytilus, Rapana and Scapharca* (4 from the Romanian transect, 6 from the Bulgarian transect, and 3 from the Turkish transect) (Annex 2). The dredge was launched in order to collect biota samples for pollutants (Figure. 5). One sample consisted of 10 – 15 individuals from the same species, shell length measured, whole soft tissue being separated onboard in clean conditions (Figure. 5), wrapped, frozen and subsequently analyzed in the NIMRD laboratory for heavy metals and POPs.

Figure 5: Biota samples collection for pollutants analyses

For heavy metals, the biota samples are freeze-dried, digested with nitric acid in Teflon vessels on hot plate, and metals are analyzed by GF-AAS. GC-ECD method will be used for OCPs and PCBs, and the GC-MS method for PAHs.

4.3 Biology

Chlorophyll a measurements were done by IO-BAS, GEOECOAMR, TUBITAK, and NIMRD teams (Annex 1). GEOECOMAR measured chlorophyll at all stations (70 samples), while IO-BAS and TUBITAK at stations from the transect Galata and Igneada, respectively (33 and 28 samples, respectively – including the inter-comparison stations). Solely, NIMRD collected samples only at inter-comparison stations (11 samples). The sampling depths were selected according to CTD fluorescence profiles, generally from the surface, thermocline, chl-a maximum layer and CIL.

The water samples (volumes within 1 – 5 l) were collected by Seabird CTD-Rosette system in 5 l plastic bottles during the up-cast. Immediately after collection, the samples were filtered onboard using two types of filters: Whatman GF/F, 0.7 µm pore size (IO-BAS and TUBITAK), and nitrocellulose membrane Millipore, 0.8 µm pore size (GEOECOMAR and NIMRD). Then, the filters were frozen at -22 ÷ -24 °C until their subsequent analysis (analytical methods are given in the Table 5, chapter below).

Phytoplankton determinations were done by IO-BAS, NIMRD and SINOP teams (Annex 1). Seawater samples (volume of 1 l) were collected by Seabird CTD-Rosette system in 5 l Niskin bottles from different depths according to CTD fluorescence profiles (similar to Chl *a* samples) and transferred in 1 l plastic bottles. The samples were preserved onboard with 20 ml formaldehyde - borax solution/bottle and Lugol solution for additional replicates (3 samples for each team).

In addition to inter-comparison stations, each team involved collected samples from their national waters as follows: NIMRD - transect Constanta-East (41 samples, including inter-comparison stations), IO-BAS from the transect Galata (39 samples, including inter-comparison stations), and SINOP from the Igneada transect (30 samples, inter-comparison stations included) (Annex 1).

Pigments (HPLC) **-** Seawater samples for pigment analyses were collected by TUBITAK (at all stations - 72 samples), from different depths, according to CTD fluorescence profiles.

Seawater samples (volumes within 0.5 - 5 L, collected by Seabird CTD-Rosette system) were filtered onboard (filter type: Whatman GF/F, pore size of 0.7 µm, and 25 mm diameter). The filters were stored in liquid nitrogen tank onboard, while in the laboratory, they were frozen at -80 ^{0}C until their subsequent analysis.

The method chosen in this study (Barlow et at., 1993) is a modification of the reverse-phase method described in Mantoura and Llewelyn (1983).

Zooplankton

Microzooplankton samples were collected only by NIMRD at all stations (54 samples) from different depths (surface, Chl a maximum, and CIL or bottom layers). The samples (collected by Seabird CTD-Rosette system) were filtered onboard (1 L) through 33 µm mesh, and then preserved in 98 % ethylic alcohol. In the laboratory, all tintinnids will be identified and counted under an inverted microscope (Olympus XI 51) at different magnifications, using the identification keys and information given by different authors. Abundance will be

expressed as number of cells per litre for each species and for total tintinnids. The length and width will be measured using Imaging Software Cell*.

Mesozooplankton samples were collected by vertical Juday closing net (diameter of 36 cm - 0.1 m^2 mouth opening area, and mesh aperture of 150 µm) from 2 meters above the bottom of oxygen minimum zone (Sig T 16.2) to the surface, at discrete layers depending of water stratification and thermocline depth. The samples were preserved in buffered with disodiumtetraborate (borax) ($Na_2B_4O_3.10H_2O$) 4% formalin solution.

Gelatinous zooplankton sampling was performed with a Hansen egg net (70 cm opening and 300 µm mesh). One vertical tow was performed at each station from the bottom to the surface (shelf waters) or from Sig T 16.2 to the surface (open water stations). All the organisms from the sample were counted and measured onboard for the determination of density and biomass.

Figure 6: Zooplankton collection

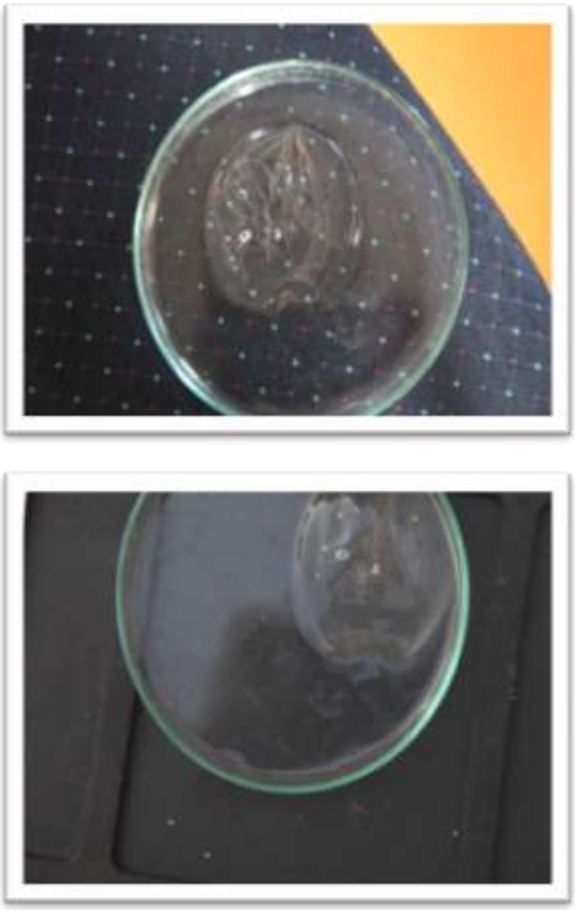

Figure 7: Gelatinous measurements

Mesozooplankton samples were taken by NIMRD (26 samples), IO-BAS (24 samples), and SINOP (20 samples) teams from the corresponded transects (NIMRD – Constanta-East, IO-BAS – Galata, and SINOP – Igneada) and inter-comparison station M 13 (Annex 3).

Gelatinous zooplankton samples were collected only by NIMRD and IO-BAS teams. NIMRD collected 12 samples from the Romanian and Turkish transects, while IO-BAS collected 6 samples from the Bulgarian transect (Annex 3).

Ichthyoplankton – the sampling was done using a Hansen vertical net (diameter of 70 cm and mesh size of 300 µm). The samples were preserved in buffered with disodiumtetraborate (borax) ($Na_2B_4O_3.10H_2O$) 4% formalin solution after the gelatinous species processing (the remaining part of the samples).

Ichthyoplankton samples were collected by NIMRD and IO-BAS teams. NIMRD collected 12 samples from the Romanian and Turkish transects, while IO-BAS collected 6 samples from the Bulgarian transect.

Zoobenthos (macrozoobenthos and meiozoobenthos)

Macrozoobenthos

The common protocol of sampling has foreseen that all the team shall use the same instrument of collecting. Thus, all macrozoobenthos samples were collected by a Van Veen grab with surface of 0.135m^2. At each station, the teams collected 3 replicates.

Total 30 macrozoobenthos samples were collected by GEOECOMAR from the Romanian and Bulgarian transects (at coastal and shelf stations), and also from inter- comparison stations. NIMRD team collected 6 samples (3 samples from each inter-comparison station), while the SINOP team collected 9 samples from the Turkish transect (coastal and shelf stations), and the inter-comparison station M 10 .

The benthos samples collected by GEOECOMAR were tagged and photos were taken when the sample was onboard. There were taken only the first top 15 – 20 cm of the sample (depends of the substrate type; at some stations the sample was collected integrally). Only the inter-comparison samples were integrally collected by all teams, while the SINOP team collected integral samples every time.

GEOECOMAR and NIMRD performed a pre-washing of the samples through 0.5 mm mesh size sieves for sediments excess removal. The preserving was done with formaldehyde 4% buffered with seawater and the samples were stored in labelled plastic bags and containers until their subsequent examination in laboratory. A macro-visual description of each sample was done before preserving and the main communities and species were identified on spot.

The samples collected by the SINOP team were pre-sieved with a 0.5 mm mesh and the retained fauna were put in jars containing 10% seawater–formalin solution. The samples brought to the laboratory were washed through 1 mm and 0.5 mm sieve mesh sizes. The material obtained was examined under stereo binocular dissecting microscope and zoobenthic organisms were sorted into higher systematic groups. These samples were delivered to specialists for taxonomic identifications.

Figure 8: Benthos samples collection and pre-treatment

Meiobenthos

The meiobenthos samples were also collected using a Van Veen grab (surface of 0.135 m^2). The subsampling was done by using a corer of 12.52 cm^2. Two replicates were taken from each station.

The samples were collected by GEOECOMAR and SINOP teams. GEOECOMAR sampled from the Romanian transect (coastal and shelf stations), and also from inter-comparison stations (total of 14 samples), while SINOP team sampled from the Bulgarian and Turkish transects, including inter-comparison stations (10 samples).

GEOECOMAR preserved the samples integrally, as soon as they were collected, by using 4 % formaldehyde buffered with seawater, except the inter-comparison stations, where the samples were preserved in 95 % ethylic alcohol. The SINOP team preserved all samples in 95% ethylic alcohol. A detailed analysis of samples will be performed subsequently in laboratories.

Sediment description – was done by GEOECOMAR at all stations.
After reception of the grab (surface of 0.135 m^2) on the deck, the following procedures were done:

- inspection of the content through the open windows. The smell**,** colour, visual sediment description (type, surface disturbed/undisturbed, presence of organic/inorganic debris, etc.) were recorded. Also, photos were taken for each sample (Fig.9);
- measuring of temperature (T);
- collection of samples through the open windows (in order to preserve the deposit as undisturbed as possible) for further chemical and biological analyses.

Figure 9: Sediment photos

Marine litter

Marine litter surveys were performed by two different methods: a beam trawl (Figure. 10) and Remotely Operated Vehicle (ROV) Mariscope (Figure. 11) following the methodology of the Draft MSFD Guidance of the ML TG, 2013.

The IO-BAS beam trawl parameters are: 2.5 m opening width and 50 mm mesh. The trawling was performed at sites between depth 20 and 70 m at speed 3 knots. The area covered was computed from the geographic coordinates of the starting and ending points of the hauls. The marine litter was sorted and measured onboard according to the items categories listed for Sea-floor (Annex 5.1) of the Guidance.

Figure 10: Beam trawl for sampling marine litter

The Remotely Operated Vehicle (ROV) Mariscope was deployed at 2 stations for a pilot survey and data were recorded for further analysis at IO-BAS laboratory.

Figure 11: Remotely Operated Vehicles (ROV) Mariscope

Figure 12: Marine litter items collected during the surveys

V. Inter-comparison exercises

The main objective of these exercises was to collect samples for chemical and biological parameters by the MISIS partners, following their routine methodology of sampling and analysis for assessment of the comparability of data collected during the MISIS Joint Cruises under PA2. These exercises are expected to produce valuable results for making recommendations for further improvement and harmonization of research (monitoring) methodology in the Black Sea.

5.1 Chemistry

For inter-comparison exercise in terms of water column chemistry, we chose an open sea station (M 13) in order to evaluate our laboratories' performances for low nutrient waters. For sediment chemistry, two stations (M18 - coastal waters, and M 10 - shelf waters) were chosen. Regarding the contaminants in biota, only NIMRD collected samples, so no inter-comparison was done.

Two groups were organized, as follows: physical-chemical parameters in water column and sediment, and contaminants in sediment.

Seawater samples were collected by Seabird CTD-Rosette system in 5l plastic bottles during the up-cast. Each team used a separate bottle to collect three water replicates for each of the parameters in following order: dissolved oxygen (DO), nutrients, and pollutants. There were chosen two sampling depths for this exercise as follows: 1 m below surface and 46 m (coinciding with CIL).

Sediment samples were collected using a Van Veen grab sampler. The undisturbed surface layer, 1.0–1.5 mm thick, was carefully collected with a spatula, very well homogenized, and the samples were placed in polyethylene containers for heavy metals, respectively aluminum foils for POPs, sealed, labelled and transported into the laboratory. Each sample will be split into three replicates and analysed accordingly by each laboratory. Samples were preserved frozen (at – 20 ÷ - 24 °C).

The participants, responsible persons, parameters and analytical procedures are shown in Table 5. Sampling procedures are presented in the chapter above.

Table 5: Sampling procedure - Chemistry

Group	Responsible person	Station/sampling depth	Parameters	Participants	Analytical procedure
Physical-chemical parameters	Luminita Lazar (NIMRD)	M 13 (1m below surface layer and Cold Intermediate Layer (CIL) - 46 m	DO	GEOECOMAR	Winkler (Grasshoff et al, 1998) – onboard
				IO-BAS	
			Nutrients (PO4, TP, NO2, NO3, NH4, SiO4, TN)	ANAR	Spectrophotometric (SR EN 26777:2002/C91:2006 (NO2); SR EN ISO 6878:2005 (PO4, TP); STAS 9375-73 (SiO4) TN – not analyzed
				GEOECOMAR	Spectrophotometric Grasshoff et al, 1998)* TP and TN – not analyzed
				IO-BAS	Spectrophotometric Grasshoff et all, 1998)* and **
				NIMRD	Spectrophotometric Grasshoff et all, 1998)* and **
				TUBITAK	Autoanalyzer,NO_2+NO_3 analysis Cd reduction flow injection method (S.M. 4500-NO_3-I:2005); NH_4 analysis flow injection method (S.M. 4500-NH3 H:2005); PO4 analysis flow

					injection method; silicate analysis colorimetric method (S.M. 4500-SiO_2:2005 F), TN and TP analysis Persulphate Method for Simultaneous Determination of Total Nitrogen and Total Phosphorus (S.M. 4500- P A, 4500- P J.)
			TOC (water)	NIMRD	High-Temperature Combustion Method
				TUBITAK	
		M 10 and M18	TOC (sediment)	GEOECOMAR	Walkey-Black method (1947), adopted and modified from Jackson (1958)
				NIMRD	Automate method with Solid Sample Combustion Unit SSM, using TOC analyzer Shimadzu
				TUBITAK	Loring and Rantala, 1992; Verardo et al.,1990)
Pollutants sediments	Dan Secrieru (GEOECOMAR)	M 10 and M 18	Heavy metals	ANAR	SR EN ISO 15586/2004 and 17852/2008 (Hg)
				GEOECOMAR	Sediments are freeze-dried, digested with nitric acid in microwave digestion system (partial digestion method), and metals are analyzed by GF-AAS
				NIMRD	GF-AAS

				TUBITAK	Hg: EPA 3051A: Microwave pre-treatment with acid decomposition - ISO 20280, Other heavy metals, EPA 6020 A 2007 – 02
	Andra Oros (NIMRD)		PAHs	ANAR	GC-MS SR EN ISO 17993-2004
				NIMRD	GC-MS
				TUBITAK	TUBITAK analyzed PAHs using adopted and modified methods determination of petroleum hydrocarbons in sediments, reference methods for marine pollution studies No. 20, UNEP 1992 and Standard Operation Procedures, 2000. National Research Center for Environment and Health, Institute for Ecological Chemistry–Neuherberg/Almanya

* two different types of nitrates reduction; homogeneous reduction by hydrazine in the presence of copper ions as catalyst (Mullin and Riley, 1955) – NIMRD and GEOECOMAR, and heterogeneous through Cd column –ANAR, IO-BAS, and TUBITAK.

**NIMRD analyzes TN using oxidative combustion-chemiluminiscence method, while IO-BAS and TUBITAK by spectrophotometric method after alkaline oxidation (Grasshoff et al., 1999)

5.2 Biology

Chlorophyll a and Phytoplankton

For phytoplankton inter-comparison exercise, two stations were chosen; one of them, the open sea station M 13 was selected for low Chl *a* concentration and algal biomasses, while the coastal station, M 18, was selected for Chl *a* high concentrations and phytoplankton biomasses.

Samples were collected by Seabird CTD-Rosette system in 5l plastic bottles during the up-cast. Each team used a separate bottle to collect three water replicates from Chl *a* Maximum Layer at both inter-comparison stations (43 m and 16 m, respectively). At station M 13, each team collected one replicate from a separate bottle from other three different depths as follows: 1 m below surface, thermocline - 13 m, and CIL - 50 m).

The participants, responsible persons, and analytical procedures are shown in Table 3. Sampling procedures are presented in the chapter above.

Table 6: Sampling procedure - Chlorophyll a and Phytoplankton

Group	Responsible person	Station/sampling depth	Parameters	Participants	Analytical procedure
Phytoplankton	Snejana Moncheva (IO-BAS)	M 13 (CHL a Maximum layer – 43 m) and M 18 (CHL a Maximum layer – 16 m)	Chlorophyll *a*	GEOECOMAR	0.8 µm pore size membranes; solvent – acetone; extraction - homogenization and sonication; spectrophotometry, Jeffrey-Humphrey (1975) equations
				IO-BAS	0.7 µm pore size GF/F filter; solvent-acetone;

					extraction-homogenization; spectrophotometry, Jeffrey-Humphrey (1975) equations
				NIMRD	0.8 µm pore size membranes; solvent – acetone; extraction - homogenization; spectrophotometry, SCOR-UNESCO (1966) equations
				TUBITAK	0.7 pore size GF/F filter; solvent-acetone; extraction- homogenization; spectrophotometry, Jeffrey-Humphrey (1975) equations
			Phytoplankton	IO-BAS	Sample concentration – Decantation Uthermol; Counting Chamber - Sedgwick Rafter, Uthermol; Type of microscope - Nikon inverted+image analysis; Volume of subsample – 1 ml; Magnification – 20X, 40X No. cells counted per sample – 400.
				NIMRD	Sample concentration – Decantation Uthermol; Counting Chamber - Uthermol; Type of microscope – Olympus Inverted; Volume of subsample – 0.1 ml

				SINOP	Sample concentration – Decantation Uthermol; Counting Chamber - Segwick Rafter, Uthermol; Type of microscope - Nikon inverted+epiflourescense attachment Volume of subsample – 0.1 ml; Magnification – 20X, 40X.

Zooplankton– Samples (three replicates per each partner) were collected by Juday closing net in integrated layer 120-0 (at station M 13) and preserved in 4% formaldehyde solution.

The participants, responsible persons, and analytical procedures are shown in Table 7. Sampling procedures are presented in chapter above

Table 7: Sampling procedure - Zooplankton

Group	Responsible person	Station/sampling depth	Parameters	Participants	Analytical procedure
Zooplankton	Florin Timofte (NIMRD)	M 13 (tow 120 – 0m)	Mesozooplankon	IO-BAS	according to "Black Sea zooplankton manual".
				NIMRD	according to "Black Sea zooplankton manual".
				SINOP	according to "Black Sea zooplankton manual".

Zoobenthos

During inter-comparison exercise, performed on the Bulgarian and Turkish transects, the partners collected 3 replicates each for macrozoobenthos, and 2 replicates each for meiobenthos.

The participants, responsible persons, and analytical procedures are shown in Table 8. Sampling procedures are presented in chapter above.

Table 8: Sampling procedure – Zoobenthos

Group	Responsible person	Station/sampling depth	Parameters	Participants	Analytical procedure
Zoobenthos	Murat Sezgin (SINOP)	M 10 and M 18	Macrozoobenthos	GEOECOMAR	ISO 5667-19:2004 Water quality - Sampling -- Part 19: Guidance on sampling in marine sediments Manuals on sampling and analysis, including guidelines on equipment, site selection, abundance, biomass, blooms and taxonomic identification, developed and used for soft-bottom macrozoobenthos (Todorova and Konsulova, 2005)
				NIMRD	
				SINOP	
	Derya	M 10 and M 18	Meiobenthos	GEOECOMAR	Elmgren R, Radziejewska T (1989),

	Urkmez (SINOP)			SINOP	Recommendations for quantitative benthic meiofauna studies in the Baltic, Balt Mar Biol Publ 12: 1–20; Somerfield PJ, Warwick RM, Moens T (2005) Meiofauna techniques. In: Eleftheriou A, McIntyre A (eds) Methods for the study of marine benthos. Blackwell, Oxford, pp. 229–272

VI. Annexes

Annex no. 1 - CTD casts, nutrients, pollutants, phytoplankton sampling from water column. Pollutants and zoobenthos sampling from sediment

Station	Start CTD		End CTD	CTD sampling	Hydro-chemistry **NIMRD, GEECOMAR, IO-BAS, TUBITAK**	Pollutants water **NIMRD**	Pollutants sediment **NIMRD, GEOECOMAR, TUBITAK**	Phyto-plankton **NIMRD, IO-BAS, SINOP**	Chl a **GEOECOMAR, IO-BAS, TUBITAK**	HPLC **TUBITAK**	Zoo-benthos **GEOECOMAR, SINOP, NIMRD**
	Day	Hour	Hour	depths, m							
~301	22.07.13	15:40	15:45	18,10,0	-	-	-	-	-	-	-
M01	23.07.13	05:26	05:42	32,14,0	X	X	X	x	X	x	X
M02	23.07.13	10:24	10:31	46,16,5,0	X	X	X	x	X	x	X
M03	23.07.13	15:55	16:06	52,25,9,0	X	X	X	x	X	x	X
M04	24.07.13	04:00	04:15	63,40,15,0	X	X	X	x	X	x	X
M05	24.07.13	12:11	12:25	98,76,41,16,0	X	X	X	x	X	x	X
M06	24.07.13	16:35	16:57	200,190,174,166, 114,87,42,15,0	X	X		x	X	x	
M07	25.07.13	05:02	05:24	300,250,200,165, 154,147,139,131, 112,0	X	-	-	-	-	-	-

M07B	25.07.13	06:08	06:23	87,75,46,16,0	X	X		X	X	X	
M12	26.07.13	05:02	05:07	22,12,0	X	X	X	x	X	x	X
M11	26.07.13	10:03	10:11	38,25,15	X	-	X	x	X	x	X
M11B	26.07.13	10:33	10:34	0	X	X	-	X	X	X	-
M10	26.07.13	16:47	17:00	74,50,25	X	-	X	x	X	x	X
M10B	26.07.13	17:26	17:31	11,0	X	X	-	X	X	X	-
M09	27.07.13	09:57	10:07	90,75,65,43,0	X	-	X	x	X	X	X
M09B	27.07.13	10:39	10:45	21,17,10,0	X	X	-	X	X	X	-
M08	27.07.13	14:42	15:11	500,250,200,173, 157,145,142,122, 106,84,75	X	-		-	-	-	
M08B	27.07.13	15:43	15:56	156,75,65,39,10	X	-	-	X	X	X	-
M08C	27.07.13	16:23	16:31	12,0	X	X	-	X	X	X	-
M13	28.07.13	05:06	05:22	51,13,0	X	X		x	X	x	
M13B	28.07.13	06:45	06:57	49,43,14,0	X	-	-	-	-	-	-
M13C	28.07.13	07:52	08:00	42	-	-	-	X	X	X	-
M13D	28.07.13	08:56	09:06	47,42,0	X	-	-	-	-	-	-
M13E	28.07.13	10:07	11:32	1650,1500,1250, 1000,750,500, 250,200,155, 109,101,95	X	-	-	-	-	-	-

M13F	28.07.13	12:24	12:38	95,90,89,83,77, 63,50,16,0	X	-	-	-	-	-	-
M14	29.07.13	05:06	05:57	1100,750,500, 250,200,135, 129,127,122, 118,111,107	X	-		-	-	-	
M14B	29.07.13	06:28	06:45	99,83,65,19,0	X	X	-	X	X	X	-
M14C	29.07.13	07:15	07:27	100,66,18,0	X	-	-	-	-	-	-
M15	29.07.13	10:26	10:37	96,74,34	X	-	X	x	X	x	X
M15B	29.07.13	10:58	11:05	35,20,0	X	X	-	X	X	X	-
M16	29.07.13	16:08	16:21	74,65,24	X	-	X	x	X	x	X
M16B	29.07.13	16:40	16:44	24,0	X	X	-	X	X	X	-
M18	30.07.13	05:08	05:20	25,15,0	X	x	X	x	X	x	X
M18B	30.07.13	05:50	05:58	16,0	-	-	-	x	x	x	-
M18C	30.07.13	06:24	06:30	16	-	-	-	x	x	-	-
M18D	30.07.13	07:25					-	-	-	-	-
M18E	30.07.13	08:19					-	-	-	-	-
M17	30.07.13	14:58	15:05	51,21	X	-	X	x	X	x	X
M17 B	30.07.13	15:39	15:43	0	X	X	-	X	X	X	-

Annex no. 2 - Biota sampling for pollutants

Station	Cast	Day	Start			End			Dredge length (M)
			Latitude (° ')	Longitude (° ')	Time (hh:mm)	Latitude (° ')	Longitude (° ')	Time (hh:mm)	
M01-M02		23/07/13	44°10.125'N	028°48.973'E	09:16	44°10.121'N	028°49.349'E	09:21	500
M04-M05	A	24/07/13	44°10.103'N	029°40.399'E	07:22	44°10.099'N	029°40.756'E	07:27	490
M04-M05	B	24/07/13	44°10.096'N	029°41.903'E	07:51	44°10.101'N	029°42.918'E	08:06	1350
M12-M11	A	26/07/13	43°09.999'N	028°10.026'E	07:24	43°09.999'N	028°10.026'E	07:29	440
M12-M11	B	26/07/13	43°10.005'N	028°10.922'E	07:43	43°10.013'N	028°11.892'E	07:58	1300
M12-M11	C	26/07/13	43°10.058'N	028°16.684'E	08:46	43°10.074'N	028°17.349'E	08:56	895
M11-M10		26/07/13	43°15.329'N	028°18.425'E	15:09	43°15.470'N	028°18.771'E	15:14	535
M10-M09		27/07/13	43°08.790'N	028°27.147'E	08:00	43°09.066'N	028°27.110'E	08:05	512
M18	A	30/07/13	41°50.763'N	028°01.808'E	12:35	41°50.558'N	028°02.046'E	12:40	500
M18	B	30/07/13	41°49.810'N	028°02.540'E	13:27	41°50.234'N	028°02.191'E	13:37	920
M17		30/07/13	41°52.495'N	028°07.455'E	17:46	41°52.999'N	028°07.689'E	17:56	980

Annex no. 3 - Zooplankton sampling

Station	Date	Time (Ichthyo and Jelly)		Mesozooplankton tows (down to upper layer in meters)						Macro ad Ichthyoplankton (down to upper layer in meters)	ZPK	ICH
		Start (Local time)	End (Local time)	1	2	3	4	5	6			
M01	23/07/13	09:20	09:45	10-0 m	31-15 m					28-0 m	2	1
M02	23/07/13	13:50	14:00	15-0 m	45-15 m					40-0 m	2	1
M03	23/07/13	19:20	19:40	10-0 m	25-10 m	52-25 m				50-0 m	3	1
M04	24/07/13	no	No	15-0 m	30-15 m	62-30 m				60-0 m	3	1
M05	24/07/13	16:00	16:15	15-0 m	30-15 m	85-30 m				95-0 m	3	1
M06	24/07/13	20:50	21:10	15-0 m	57-15 m	102-57 m	167-102 m	185-167 m		180-0 m	5	1
M07	25/07/13	10:30	10:45	15-0 m	65-15 m	105-65 m	130-105 m	165-130 m		165-0 m	5	1
M08	27/07/13	18:15	20:00	10-0 m	30-10 m	50-30 m	74-50 m	107 -74 m	174-107 m	174-0 m	6	1
M09	27/07/13	13:00	14:20	20-0 m	55-20 m	80-55 m	90-80 m			85-0 m	4	1
M10	26/07/13	20:00	20:30	15-0 m	35-15 m	74-35 m				70-0 m	3	1
M11	26/07/13	no	No	20-0 m	37-20 m					35 -0 m	2	1
M12	26/07/13	08:00	08:30	20-0 m						18-0 m	1	1
M13-Inter	28/07/13	08:30	10:40	120-0 m	three replicates for all the partners						9	0
M13	28/07/13	15:00	16:10	15-0 m	45-15 m		55-45	74-55	110-74	120-0 m	5	1

						m	m	m				
M14	29/07/13	08:30	11:30	20-0 m	60-20 m	75-60 m	105-75 m	135-105 m		140-0 m	5	1
M15	29/07/13	14:00	no	20-0 m	40-20 m	58-40 m	98-58 m			95-0 m	4	1
M16	29/07/13	no	20:10	10-0 m	30-10 m	73-30 m				70-0 m	3	1
M17	30/07/13	18:00	no	15-0 m	30-15 m	50-30 m				50-0 m	3	1
M18	30/07/13	08:20	08:45	10-0 m	25-10 m					20-0 m	2	1
Total samples											70	18

Annex no. 4 - Trawl net operations (marine litter)

Station	Cast	Day	Depth station	Speed (knots)	Depth trawl	Start			End			Dredge length (M)
						Latitude (° ')	Longitude (° ')	Time (hh:mm)	Latitude (° ')	Longitude (° ')	Time (hh:mm)	
M07-M08		26/07/13	1000.0	3.0	25.0	43°47.180'N	030°41.720'E	08:16	43°46.966'N	030°40.706'E	08:31	1350
M11		25/07/13	25.9	1.8	10.0	43°14.989'N	028°19.112'E	14:30	43°14.890'N	028°18.751'E	14:40	520
M15-M16		29/07/13	96.2	2.0	20.0	41°55.791'N	028°33.418'E	13:10	41°55.741'N	028°33.201'E	13:15	315

Annex no. 5 - ROV operations (marine litter)

Station	Cast	Day	Depth	Start			End		
				Latitude (° ')	**Longitude** (° ')	**Time** (hh:mm)	**Latitude (° ')**	**Longitude (° ')**	**Time (hh:mm)**
M01	A	23/07/13	33.3	44°10.024'N	028°46.987'E	07:35	44°10.024'N	028°46.987'E	08:55
M11	A	26/07/13	26.5	43°15.252'N	028°19.783'E	12:40	43°15.252'N	028°19.783'E	13:57
M18	A	30/07/13	25.9	41°51.777'N	028°01.781'E	10:30	41°51.777'N	028°01.781'E	11:45