Main Decisions of the EMBLAS Workshop
«Preparation of the Black Sea Regional Guidelines on Biological Monitoring»

(15-16 July 2014, Istanbul, Turkey)

Aim: elaboration of guidelines (manuals) for the Black Sea Zooplankton and Phytobenthos biological monitoring.

Participants:

In order to work efficiently the participants had been split into five topic groups before the workshop: Microzooplankton, Mesozooplankton, Macrozooplankton, Microphytobenthos and Macrophytobenthos.

Microzooplankton Group
1. Alexander Kurilov (OBIBSS) – Ukraine – Chairperson of the group
2. Nelli Gavrilova (IBSS) - Russia

Mesozooplankton Group
3. Borys Aleksandrov (OBIBSS) - Ukraine – Chairperson of the group
4. Alexandra Gubanova (IBSS) - Russia
5. Elena Arashkevich (IO) - Russia
6. Meri Khalvashi (MEFRI) - Georgia

Macrozooplankton Group
7. Tamara Shiganova (IO) - Russia – Chairperson of the group
8. Borys Anninskyy (IBSS) - Russia

Microphytobenthos Group
9. Elena Nevrova (IBSS) - Russia – Chairperson of the group
10. Anastasiia Snigirova (ONU) - Ukraine
11. Galina Kovalova (IAZ) - Russia
12. Tsiuri Gvarishvili (MEFRI) - Georgia

Macrophytobenthos Group
13. Galina Minicheva (OBIBSS) - Ukraine – Chairperson of the group
14. Nataliya Milchakova (IBSS) - Russia
15. Dmitry Afanasyev (ARIF, SFU) – Russia

Organizing Committee
16. Vasyl Kostiushyn (UNDP representative)
17. Liliya Spasova (UNDP representative)

Experts who participated in the workshop distantly (could not come to Istanbul):

- Philip Sapozhnikov (IO) - Methodology of sea bottom environmental conditions evaluation based on Microphytobenthos indicator groups.
- Ekaterina Kreneva (ARIF, SFU) - Checklist of Microzooplankton (Sea of Azov, Russian coastal waters of the Black Sea).

Organizations:

MEFRI - Georgian Marine Ecology and Fisheries Research Institute National Environmental Agency

IAZ -   Institute of Arid Zones, Southern Scientific Center, RAS
IO -   P.P. Shirshov Institute of Oceanology, RAS
ARIF, SFU -   Azov Research Institute for Fisheries, Southern Federal University, Russia
ONU -   Odessa National I.I. Mechnikov University
IBSS -   A.O. Kovalevskiy Institute of Biology of Southern Seas NAS, Sevastopol, Ukraine
OB IBSS - Odessa Branch, A.O. Kovalevskiy Institute of Biology of Southern Seas, Odessa, Ukraine

The workshop comprised both plenary sessions and working groups' sessions. The first plenary meeting started with three presentations:

1. Brief results of EMBLAS project, goals and main problems with finalizing of guidelines for biological monitoring (V. Kostiushin).

2. Brief history and some results of guidelines for the Black Sea biological monitoring (prepared by B. Aleksandrov).

3. Macrophytobenthos: approaches, methods and manuals for the Black Sea biological monitoring (G. Minicheva).

During general discussion some general points of the further work in groups were formulated:

• All guidelines (manuals) should be intended not for the specialists of highest qualification but for the people having basic knowledge on the matter and dealing with routine biological monitoring. In another words, the guidelines should be addressed not only to the highest level taxonomy specialists, but also to wide range of practical biologists working in state monitoring system, protected areas etc.;

• Beside the detailed methodology for relevant samples collecting and processing, all manuals should contain lists of species and their occurrence within the territorial waters of each of the Black Sea countries, as well as major determinants for species identification;

• An important part of guidelines should be list of qualitative and quantitative indicators of the Black Sea ecosystem ecological state, which could be recommended for improving of biological (ecological) monitoring and assessment methods.

It was also decided that from practical point of view it would be better to organize only three working groups instead of 5: Zooplankton, Microphytobenthos and Macrophytobenthos.

The main decisions made by all the working groups during the workshop were the following:

Microzooplankton Group

1. Microzooplankton guideline structure similar to Mesozooplankton manual structure was approved.

2. It was decided to expand Section 2 «Sampling and treatment» and to complement it with the Microzooplankton collecting method using nets with a mesh size of 50 microns (N. Gavrilova). This method was successfully applied in the study of metazoan microplankton and tintinnids; it requires more detailed description in addition to the procedures for collecting and processing of metazoan microplankton, already described in the «Manual ...».

3. The checklist of Microzooplankton (Annex 1) will be corrected by adding of: a) list of rotifers (should be transferred from Mesozooplankton manual); b) list of larval stages of planktonic and

benthic invertebrates as the temporal component of Microzooplankton; c) section 'protozoan microzooplankton' should be entered separately, where, in addition to ciliates, links to the relevant check-lists of dinoflagellates and other colorless flagellates, traditionally considered as phytoplankton, should be added.

4. One more Annex containing the basic guidebooks for identification of Microzooplankton species should be added; the guidebooks should be supplemented with references for identification of rotifers (to be transferred from Mesozooplankton manual).

5. An additional Annex containing indicators of environmental quality assessment using microzooplankton will be prepared. This annex will be useful during field work since indicators for microzooplankton are currently not developed completely because of complexity and poor knowledge of the subject (A. Kurilov).

Mesozooplankton Group

1. The final edition of the manual on Mesozooplankton should be prepared not later than by 15 August, 2014 (B. Aleksandrov, E. Arashkevich, A. Gubanova):
   - To complete the Mesozooplankton manual with neuston sampling methodology, as neuston is an important indicator of of marine pelagic and benthic communities state (B. Aleksandrov).
   - To make corrections in Table 1 "Wire length needed to reach standard horizons for net hauls on the base of angle of wire" (B. Aleksandrov).
   - To correct the limits of mesh size for Mesozooplankton net: 180 or 200 μm (E. Arashkevich).
   - To specify the type of Mesozooplankton net that is used in Romania and its characteristics (E. Arashkevich).

2. To amend Annexes to the guideline.

Annex 1. Mesozooplankton species composition and its distribution in the Black Sea countries' national waters.

To check and refine the list of the Black Sea Mesozooplankton species (checklist) and occurrence of taxa in the national waters of the Black Sea countries (all participants):
   - Mark with an asterisk all marine species;
   - Species composition of rotifers should be moved to Microzooplankton guideline.
   - Species composition of hydromedusae should be moved to Macrozooplankton guideline.
   - The bottom Sagitta and different species of bryozoans should be removed from Mesozooplankton checklist; all representatives of meroplankton are considered as larvae of larger taxonomic categories, such as polychaetes, bivalves, gastropods, decapods, bryozoans, phoronid.

Annex 2. Taxonomic references for identification of the Black Sea zooplankton species.
The list of recommended qualifiers should be revised, leaving only the most significant of them. The list of recent publications that facilitate the definition of Mesozooplankton species should be extended (B. Aleksandrov, E. Arashkevich, A. Gubanova).

Annex 3. Zooplankton indicators of environmental status.
To clarify the "qualitative" and "quantitative" indicators of the Black Sea ecosystem state by Mesozooplankton (all participants).

Annex 4. Exotic species in the Black Sea zooplankton community.

Macrozooplankton Group

Prepared by Tamara Shiganova, Boris Anninsky

After presentation by T.Shiganova on the draft manual on Macrozooplankton preparation and the joint discussion it was decided:

1. To prepare macroplankton manual in the same format as zooplankton one (T.Shiganova).

2. To include all 12 hydromedusae species in spite of their often small size at matured stage (T. Shiganova, B.Anninsky).

3. To develop several annexes with list of checked species, guide on invasive and dominant species with pictures of the most abundant species.

In addition, other annexes, the inclusion of which for mesozooplankton will be decided, should be prepared (T.Shiganova).

4. To check biomass estimation equations of ctenophores Mnemiopsis leidyi and Beroe ovata.

5. To add special equations for larvae of both species (B.Anninsky)

6. To check biomass estimation of Aurelia aurita (B.Anninsky).

Microphytobenthos Group

1. The team of authors of the guideline comprises: Elena Nevrova, Anastasiia Snigirova, Alexey Petrov, Galina Kovaleva. The authorship of Philip Sapozhnikov and use of his materials in the manual will be discussed.

2. 2. Structure of the guideline:
> 1. Introduction (1.1. Main tasks; 1.2. Terms definition; 1.3. Assemblages of the Black Sea microphytobenthos);
> 2. Sampling methods (2.1. Selection of research area and sampling season; 2.2. Planning of sampling; 2.3. Methods of qualitative sampling, 2.4. Methods of quantitative sampling; 2.5. Determination of environmental factors; 2.6. Methods of fixation and storage of samples);
> 3. Treatment of the material (3.1. Qualitative view of samples; 3.2. Method of unicellular microalgae preparation; 3.3. Quantitative calculation of microalgae; 3.4. Method of permanent slides preparation for diatoms; 3.5. Species identification; 3.6. Formation of species lists and database);
> 4. Data analysis (4.1. Assessment of species diversity of microphytobenthos; 4.2. Analysis of taxonomic structure of microphytobenthos community; 4.3. Analysis of species occurrence and selection of species with high and the highest rank of taxonomic exclusiveness; 4.4. Evaluation of reproducibility and reliability of species composition of diatoms; 4.5. Assignment of significant components of microphytobenthos communities; 4.6. Biomass determination using the true scope method; 4.7. Determination of volume and surface area of diatoms using method of 3-dimensional geometric models);
> 5. Environmental assessment using components of microphytobenthos communities;
> 6. Quality Assurance

> List of references;

Annexes:
- Annex 1. List of main publications recommended for species identification.
- Annex 2. Methods for calculating the cells` biovolumes on the basis of geometrical shapes and the correction coefficients for some species of diatoms.
- Annex 3. Standard formula for calculating the volume, full and side surface area of unicellular algae.
- Annex 4. Types of diatoms` frustules and their corresponding classes of 3-dimensional models.
- Annex 5. Morphological and functional indices of phytoplankton.
- Annex 6. Checklist of Microphytobenthic species of the Northern-Western Black Sea.

3. It was decided to expand the introduction, justifying the need for monitoring studies based on Microphytobenthic objects, which are the key element of the primary producers of the sea shelf and most sensitive to human impact. The working group also decided to include into the Microphytobenthos guideline both communities on fine and solid substrates (including the fouling of macrophytes). Separate recommendations would be specified for each type of substrate in the manual.

4. In the section on sampling methods the specific tools and equipment with appropriate links and images are proposed. It was agreed to use a) Peterson dredge (modified version with the special grids to avoid losing of silt or topsoil); b) microbenthometer (sediment core); c) variations of tubes, squirts. The following was discussed: a) ways of sampling by method of transects and method of stations` net; b) list of environmental factors that are recommended to consider during monitoring; c) planning of sampling at different scales in order to obtain maximum information with minimum efforts. The use of empirical relationship between minimum number of samples and maximum information on benthic diatom species richness may be recommended for studies of the new Black Sea habitats under similar environmental conditions (E. Nevrova, A. Petrov).

5. In the section of samples processing methods it was decided: a) to clarify the feasibility of using heavy liquids for better separation of microalgae cells from silt and clay (G. Kovaleva); b) to complement the procedure for preparation of slides and use of glutaraldehyde for armorless microalgae (A. Snigirova).

6. In the section on samples quantitative analysis using of two methods for biomass calculation was suggested: on the basis of "geometric shapes" and on the basis of 3D- models.
The problem of cells number recalculation is still not resolved: per cm2, m2 of bottom substrate or per cm3, m3 of sediment. The working group members are inclined to use the dimension cell/sm2 while studying microphytobenthos on solid substrates or at depth exceeding 5 m. In studies of microphytobenthos at smaller depths on fine soils distribution of algae in soil column (2 cm surface layer) should be taken into account.

7. It was decided to expand the section on mathematical analysis methods with description of statistical methods and algorithms and respective software packages (Alexey Petrov, IBSS).

8. Estimation of Microphytobenthos biodiversity using the Taxonomic Distinctness Indices (TaxDI) was proposed (E. Nevrova). Advantages of these Indices in comparison with traditional Indices i.e. Shannon, Pielou, Margalef etc. were discussed.

9. List of references includes 99 entries.

10. Details on preparation of annexes:

- Annex 1. List of main publications for species identification includes 60 entries.
- Annex 4. The group has decided to illustrate the types of diatoms frustules and their corresponding classes with 3D-models. To calculate more precise morphometric parameters of diatoms volume and surface areas using 3D-models it is recommended to approach directly the developer of the original program Anton Lyakh (IBSS) through the site: 3D-microalgae.org.
- Annex 5. It has been decided to use only quantitative indicators of the environment, namely: indicative and discriminative species, species belonging to monospecies branches of Bacillariophyta hierarchical tree, which are characterized by high and the highest ranks of taxonomic exclusiveness (E. Nevrova). The form of qualitative indicators of the state of environment is under discusion (A. Snigirova).
- Annex 6. Checklist of benthic microalgae will be based on published materials for the following parts of the Black Sea: the shelves of Bulgaria, Romania, Odessa coast, district of Zernov`s Phyllophora Field. Preparation of checklist for different groups of algae: responsible for diatoms – E. Nevrova; for other groups – A. Snigirova. It has been agreed to provide information about the author (link to reference), who found a species in the Black Sea for the first time, as well as its basionym and the most commonly used synonyms.

11.	The working group recommends to provide Trainings on the Microphytobenthos methods for specialists from the all the Black Sea countries as a suggestion for the future phases of EMBLAS Project.

The draft version of microphytobenthos guideline will be prepared not earlier than September 15, 2014.

Macrophytobenthos group

1. To supplement the manual prepared for the BSC (G.G. Minicheva Methods of sampling, treatment and estimation of Macrophytobenthos parameters.- 2008.- 15 pp.) with sections: "Underwater assessment methods" (Alexander Kurakin – OB IBSS) and "Herbarium preparation" (Natalia Milchakova – IBSS) before August 15, 2014.

2. To prepare the lists of taxonomic references for the Black Sea macrophytobentos species identification (before August 15, 2014).

3. To compile the list of qualitative and quantitative indicators (before August 15, 2014).

4. To revise and supplement the checklist of macrophytes species prepared by the MISIS project.

5. List of co-authors should contain the names of all the experts who participated in preparation of this document (October, 2014).

General decisions:

1. The final draft of Guidelines is to be sent to the Black Sea Commission Permanent Secretariat not later than on 15th of August, 2014 for further dissemination to experts in Bulgaria, Romania and Turkey with deadline for their feedback 15th of September, 2014. The finalized version of the Guidelines approved by experts is to be sent by the BSC PS to the

nominated Editor for revision and then the revised version of the Guidelines is to be published on the BSC web-site for open access of users.

2. Biological indicators of marine ecosystem state, which were included into the Guidelines are to be adopted in practice during the Black Sea Ecological Monitoring Programme development and EMBLAS II planning phase for research expeditions in 2015 year.