EMBLAS-Plus
Environmental Monitoring in the Black Sea

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Summary of EMBLAS Project findings, gaps and recommendations

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CONTENT

1. SUMMARY OF FINDINGS

EU/UNDP projects EMBLAS-II and EMBLAS-Plus were carried out in the period of 2014-2020 with the main goal to improve environmental monitoring and support implementation of the EU marine and water policy in the Black Sea region. The projects involved key governmental and scientific organisations responsible for monitoring of sea environment in Georgia, Russian Federation, Ukraine and numerous scientific organisations from the other Black Sea riparian countries, European Union and China. The investigations covered coastal, territorial and open sea water areas and the findings were used to address 8 out of 11 descriptors as defined in the EU MSFD. The Joint Black Sea Surveys (JBSS) in 2016, 2017 and 2019 included unique monitoring of transects from Constanta (Romania) via Odesa (Ukraine) to Batumi (Georgia) and in the central sea part of the Russian Exclusive Economic Zone. A longer-term (12 months) monitoring studies of selected parameters were carried out at island Zmeiniy, Odesa (Ukraine), Kerch Strait, Gelendzhik Bay (Russian Federation), Batumi and Cape Verde (Georgia). For an overview of sampling stations, see Figure 1.

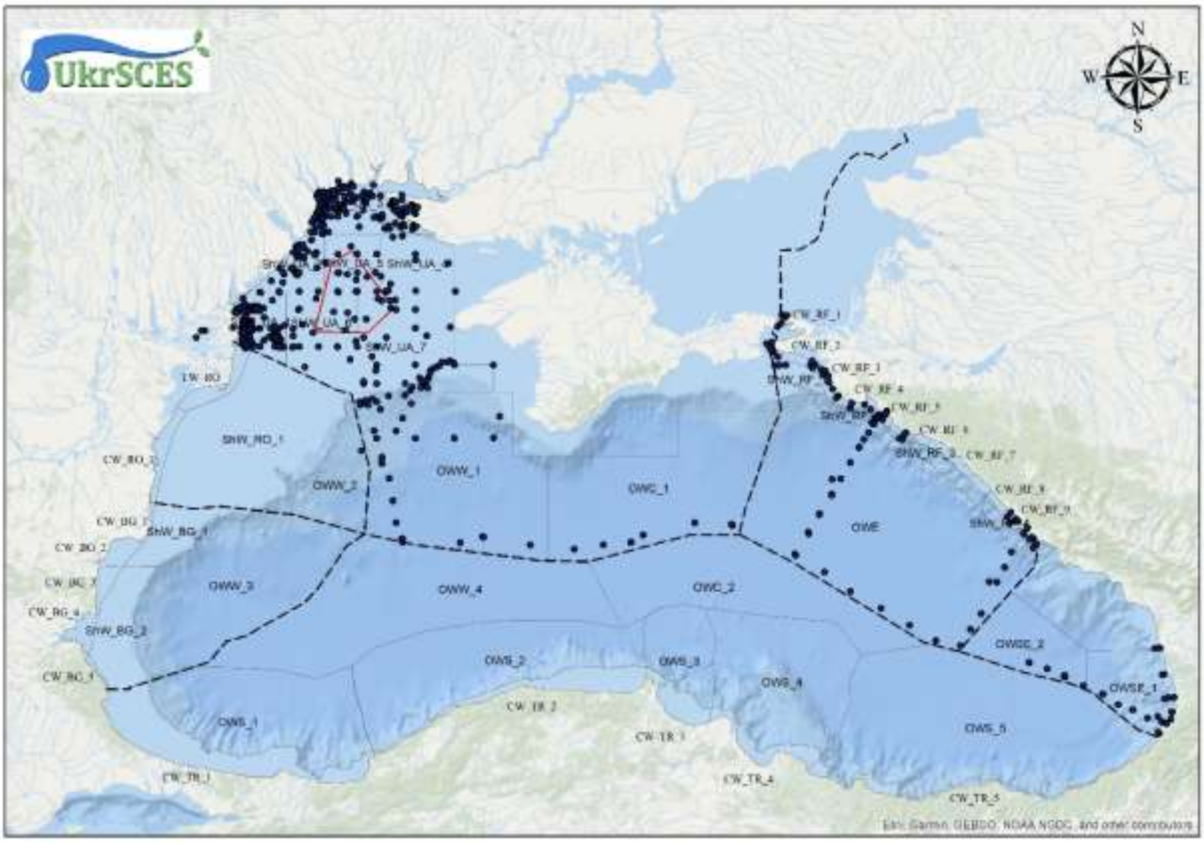

Figure 1: Positions of sampling stations monitored during the JBSS 2016, 2017 and 2019. Grey lines represent borders of Coastal Water Bodies (CW) and Marine Assessment Units (ShW, OW). The red polygon shows borders of the Zernov's Phyllophora Field.

The newly obtained and historical data on biological, chemical and physico-chemical parameters were collected using harmonised Data Collection Templates and stored in the open access Black Sea Water Quality Database (http://blackseadb.org/; 438,101 data entries as of January 2021). A novel wide-scope chemical target screening allowed for monitoring of more than 2,400 substances in each sample. Chemical non-target screening data collected within the JBSS were stored in the NORMAN Database System – Digital Sample Freezing Platform (https://norman-data.net/Verification/) and were used for retrospective screening of 65,000+ substances in each sample. A baseline of pollution of sediments and sea water by microplastics was established for the first time.

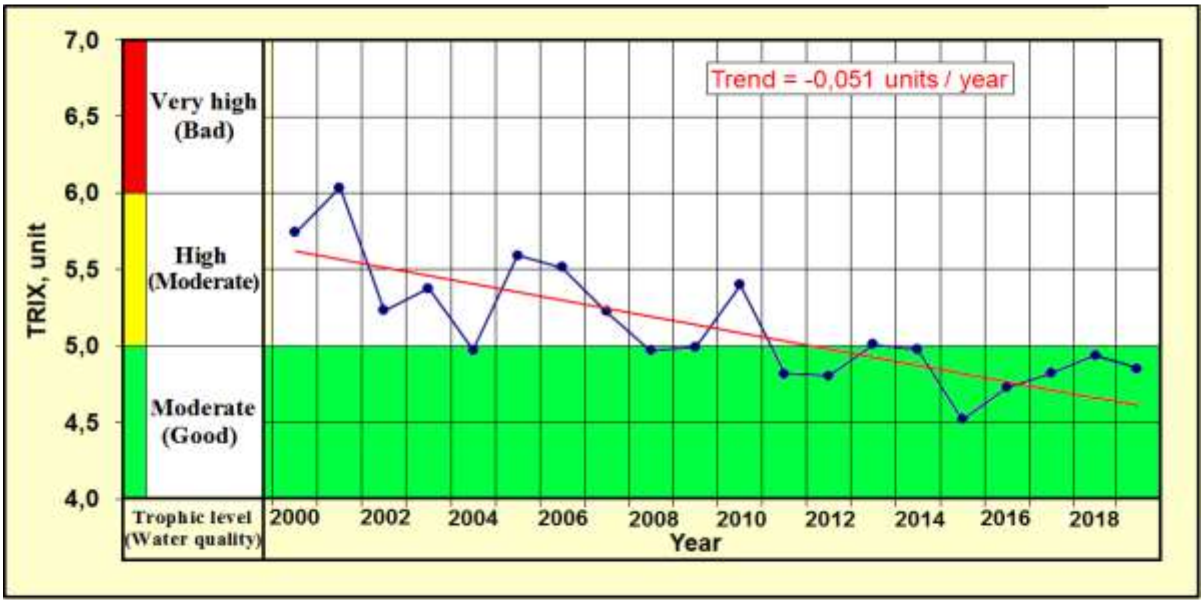

Figure 2: Decreasing trend of pollution by nutrients in the North-Western shelf of the Black Sea from 2000 to 2019 expressed in units of TRIX indicator.

A correlation between microbial activity in the deep-sea anoxic zone and degradation of persistent chemicals and plastics has been revealed. Environmental DNA of all living organisms, from microbes to marine mammals, was systematically screened at the sea region scale for the first time. The surveys brought a new insight on the dynamics of the border between hydrogen sulphate (oxygen-free anoxic) and oxygenated zone, which at certain areas was as close as 52 m to the surface of the sea.

The results showed that eutrophication status of the North-Western shelf, most significantly impacted by the inflowing large European rivers Danube, Dniester and Dnieper, is gradually improving in the last two decades (Fig. 2). This was supported by status assessment of macrophyte indicator in the marine protected area Zernov's Phyllophora Field from 1964 till 2019 (Fig. 3). A general trend towards improved environmental status of the Black Sea based on biological indicators has been observed.

The occurrence of two most prominent non-indigenous species M. leidyi and B. ovata has been shown to decrease steadily in the period from 1999 till 2019 (Fig.4).

The populations of marine mammals were for the first time systematically monitored by aerial surveys in cooperation with CeNoBS (ACCOBAMS) project.

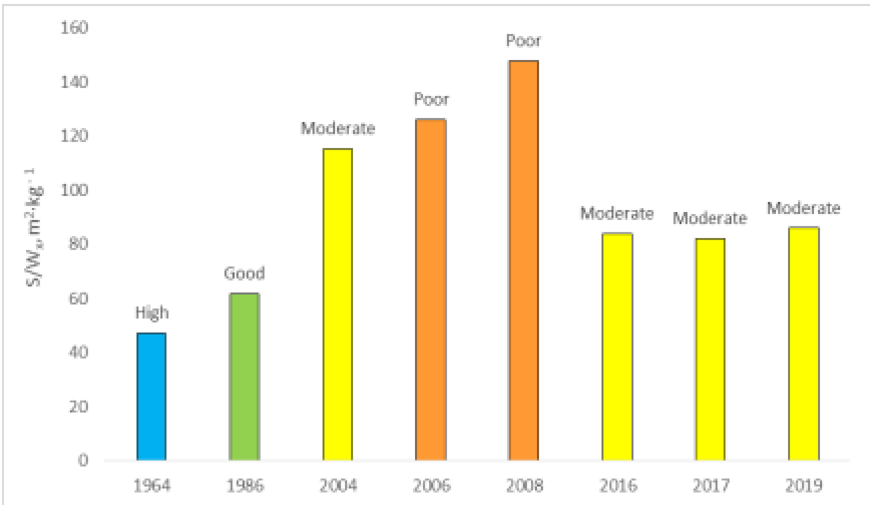

Figure 3: Ecological **status of the Zernov's Phyllophora Field from 1964** till 2019 expressed in units of macrophyte indicator (S/W)x.

Uniquely in Europe, candidate Black Sea Specific Contaminants were prioritised in sea water (51 substances), biota (75 substances) and sediments (22 substances) using NORMAN Prioritisation Framework.

As an outcome, chemicals were found to represent one of the major threats for the Black Sea ecosystem and Programme of Measures for reduction of the most dangerous prioritised chemicals should be set up.

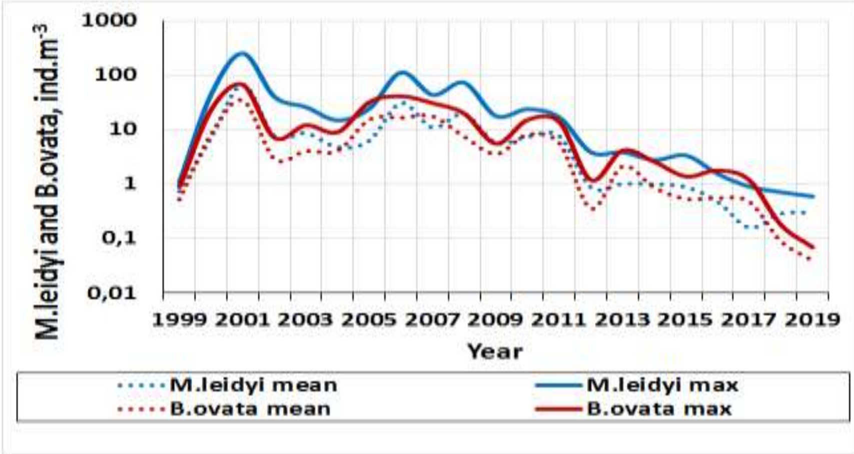

Figure 4: Decreasing trend in occurrence of non-indigenous species M. leidyi and B. ovata in the Black Sea in the period from 1999 to 2019.

A specific study on the presence of antibiotic resistance bacteria and genes indicated that the Black Sea region is under threat of a serious outbreak unless the levels of antibiotics inflowing into the Black Sea are reduced. Monitoring of Floating Marine Macro Litter (FMML), carried out in close cooperation with the EC JRC, has shown that the Black Sea is more than twice as polluted by marine litter as Mediterranean Sea (Fig. 5).

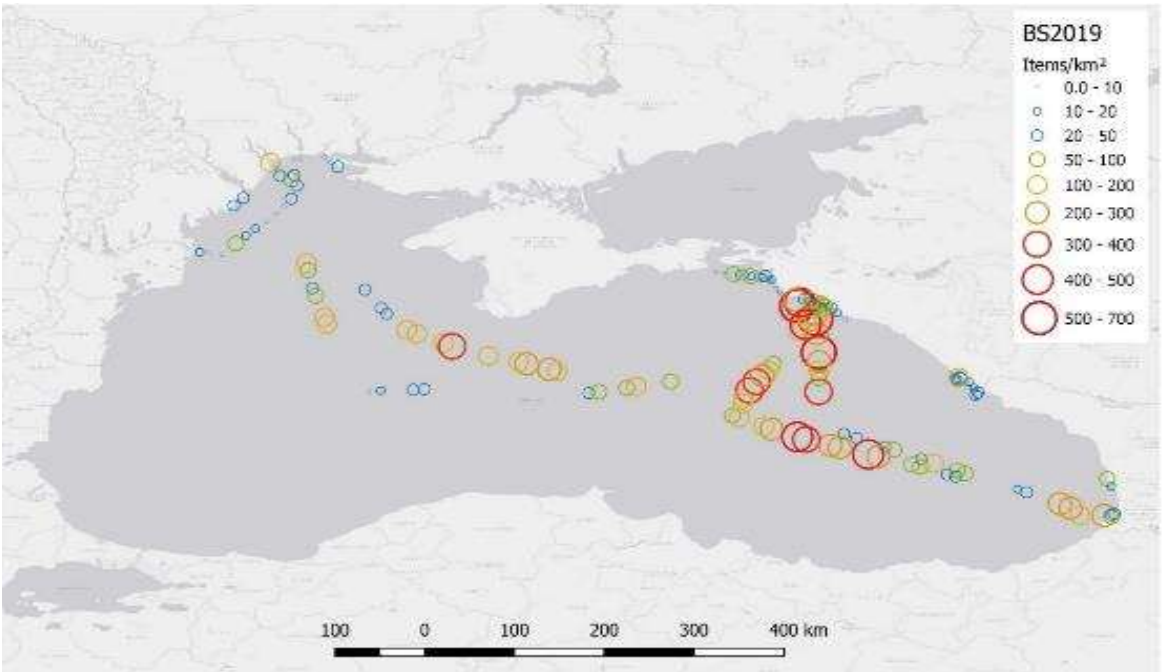

Figure 5: Pollution by Floating Marine Macro Litter monitored during the JBSS 2019. The coloured circles indicate No. of detected items/km^2.

A set of indicators harmonised among the EMBLAS-participating countries has been established for assessment of status of biodiversity parameters phytoplankton, zooplankton, macrophytes, benthic invertebrates and eutrophication; and supported with the Threshold Values setting up the boundaries between assessment classes (Fig. 6). Coastal water bodies (areas up to 1 nautical mile from the shore) have been delineated using common methodology according to requirements of the WFD. In cooperation with the EU-funded ANEMONE project, Marine Assessment Units (MAUs) have been established in territorial and EEZ waters of all Black Sea countries. An indicative assessment of the status of the Black Sea coastal water bodies and MAUs based on the results obtained within the EMBLAS project has been performed. A typical representation of the assessment is shown for phytoplankton in Figure 7.

WFD - ecological status (coastal waters)	High	Good	Moderate	Poor	Bad
MSFD - environmental status (territorial and open sea waters)	GES		Not GES		

Figure 6: Colour coding of environmental and ecological status of coastal water bodies (WFD) and open sea waters (MSFD).

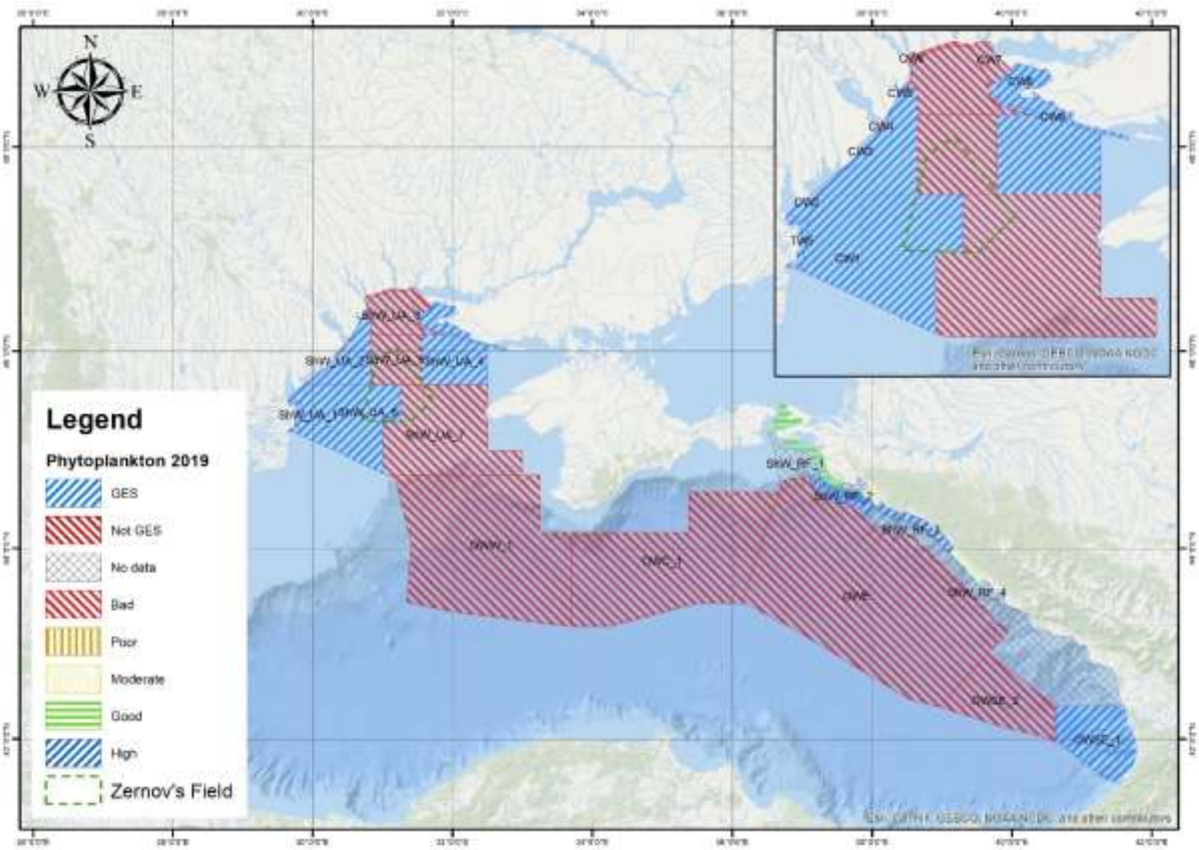

Figure 7: Indicative assessment of environmental and ecological status of the Black Sea areas investigated during the JBSS 2019 based on phytoplankton indicator – Shannon index. For status colour coding, see legend.

As a final outcome of the project, MSFD- and WFD-compliant monitoring networks have been proposed for Ukraine, Georgia and Russian Federation, with lists of monitoring stations, parameters and monitoring frequencies. A template for economic evaluation of costs of national monitoring has been developed and first estimates have been calculated in 2017. A need for regular (annual or bi-annual) multi-country Joint Black Sea Surveys monitoring open sea region has been stressed as a priority.

The EMBLAS projects achieved very good visibility by regulators at the EU and national levels, scientists, civil society organisations and general public via numerous public presentations, press conferences, videos, documentaries and books. Thousands of school children participated in the citizens' monitoring of selected biological species (sentinels) via on-line mobile application Black Sea SaveBook and a well attended Facebook group "Fans of the Black Sea" has been established. More than 5,000 people got actively involved in the public campaign "Sort Plastic – Save the Sea". Local NGOs were implementing small pilot projects addressing the marine litter pollution and environmental protection. A tradition of Clean Beach campaigns has been established and ca. 13,000 people took part in the clean-ups in the three countries during the projects. An overview of major environmental problems of the Black Sea has been summarised in a series of infographics developed in cooperation with Cousteau Society (Fig.8).

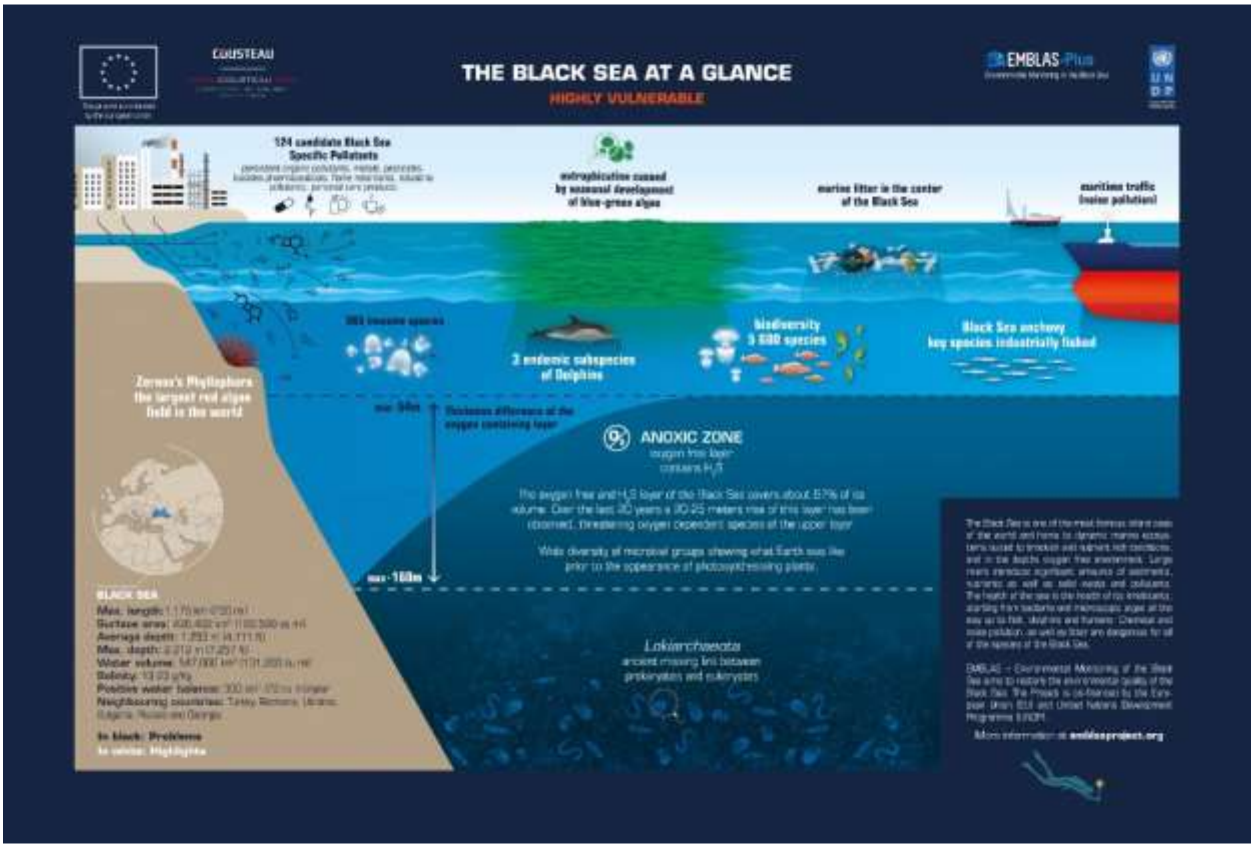

Figure 8: Example of an inforgraphics providing overview of major environmental issues of the Black Sea identified within the EMBLAS projects.

In a brief wrap-up, EMBLAS projects filled up the void of data and information needed for fact-based decision making to improve the environmental status of the Black Sea. The findings identified major problems related either to the anthropogenic activities or climate change. The Black Sea Commission, European Commission and its services, UNDP and its regional network, national governments, international organisations active in the Black Sea region, civil society organisations – were all involved in the journey. It is to be hoped that this spirit of cooperation will last and the main remaining challenge – establishment and implementation of regional Programme of Measures, will be pursued in the years to come. Here, the detailed list of gaps and recommendations addressed below might be a guide.

2. GAPS AND RECOMMENDATIONS

2.1 HYDROGRAPHY

Observations during the JBSS 2016, 2017 and 2019 and assessment of historical data indicated an impact of the climate change in the Black Sea region. Temperatures in the so-called 'Cold Intermediate Layer' (CIL) have overwhelmingly exceeded 8 °C isotherm in Western and Central regions since 2009. The temperatures of CIL waters were down to 7.2 °C only during cold winters, as e.g., in 2017. In contrast to 2017, both in 2016 and 2019 there was no CIL in "classical" definition as a layer bound by the 8 °C isotherm. The minimum temperature in the CIL in 2016, 2017 and 2019 was 8.28 ± 0.026, 7.62 ± 0.028 and 8.4 ± 0.1 °C, respectively.

The spatial and temporal variability of the position of upper border of the hydrogen sulphide zone in the deep part of the Black Sea is primarily determined by synoptic and seasonal variations of the hydrological structure of the sea waters. At the end of summer 2019 the upper border of the hydrogen sulphide zone in the Western and Central regions of the Black Sea fluctuated near the depth of 70 m. It went down to 135 m in Eastern region and to 150 m in South-Eastern part of the Black Sea. The upper boundary of hydrogen sulphide layer varied from σ_t =15,4 to σt =16,0 in the central part of the sea in 2017. This is significantly less than the value σ_t =16.18, which was obtained by Bezborodov at al. (1993) via processing the array of observations over the period from 1924 to 1990. Maximum lifting boundary of the H_2S-zone in units of σ_t (σ_t =15.4; 52 m) was recorded at the sampling station No. 7, located in the central part of the Eastern cyclonic gyre. A synchronized decrease of the depths of CIL, isoline 16,2 as well as disappearance of O_2 and onset of H_2S were also observed at the sampling station No. 7. It clearly shows significant influence of hydrological structure to processes on-going in the Black Sea.

The JBSS has demonstrated that there is a need for setting up a regional monitoring programme with higher density of sampling stations in order to identify the influence of hydrological processes on the hydrochemical and hydrobiological parameters.

The monitoring should cover deep part of the sea with an appropriate grid of stations and take into account the spatial and temporal variability of the processes in the sea. It should be supported with the suitable mathematical models, responsive to appropriate physical, chemical and biogeochemical production–consumption processes in the ocean.

2.2 PHYTOPLANKTON

Analysis of the biomass and taxonomic structure of the phytoplankton of the coastal waters of the North-Western region in 2016, 2107, and 2019 did not reveal significant changes compared to the period 2000-2012 that occurred after the eutrophication of the 70-80s. This indicates the stable state of the North-Western region ecosystem within the last two decades.

In the Eastern Central basin, the 3-year average total phytoplankton biomass was of 9.2 g/m^2 which is comparable to the values observed in the Black Sea after the mid-90s (10.6 g/m^2). It is 3-times higher than during the period before mid-80s when the eutrophication of the deep basin was minimal (reference period). EMBLAS studies confirmed the previously observed long-term trend towards an increase in the contribution of diatoms and coccolithophores and a decrease in the contribution of dinoflagellates. Thus, the taxonomic structure of phytoplankton biomass clearly differs from that during the reference period.

Assessment of the Black Sea status using biomass threshold values showed that most of the samples collected in 2019 in coastal and shelf waters indicate good environmental status of the adjacent waters. Open waters have been mainly assigned as having NotGES.

Since the end of the last century, there has been a longer period of mass development of E. huxleyi in the Black Sea (Sukhanova, 1995). Lately, the number of E. huxleyi in offshore areas has reached bloom concentrations every year, and a tendency to increase its abundance has been maintained, which is confirmed by satellite observations (Cokacar et al., 2001; Burenkov et al., 2006). The most intense and prolonged E. huxleyi bloom in the Black Sea was recorded in 2012 (Yasakova & Stanichny, 2012). This phenomenon is thought to be associated with a decrease in eutrophication, with climate changes during recent years (abnormally hot summer – early autumn), and with an increase in the phosphate concentration at depth (Mikaelyan et al., 2011, 2013).

Gaps

- Different periods of observations and differences in monitoring methodology negatively affect the comparability of data collected in different years and in different regions of the Black Sea.
- Monitoring in a limited period, for example, only in the warm season does not allow evaluation and comparing annual cycles of phytoplankton. Variations in seasonal dynamics in different regions of the Black Sea exacerbate this problem.
- Different histories and level of knowledge of phytoplankton studies in various Black Sea regions, limited availability of data for the reference period (historical data), as well as changes in sampling and processing methods make it difficult to assess long-term trends in phytoplankton.
- The lack of harmonised interoperable databases containing data on primary processing of samples limits the use of various structural and integral phytoplankton indicators for assessing the ecological and environmental status of various regions of the Black Sea.
- Assessment of phytoplankton biodiversity in different regions is associated with both the objective and subjective difficulties. One of them is the complexity of taxonomic identification of phytoplankton species. The varying amounts of sample material collected during monitoring also can affect the accuracy of estimates of biodiversity in the region.
- Based on the importance of regular studies of phytoplankton in the Black Sea, it can be assumed that at present the main gap in our knowledge is insufficient understanding of the physical processes that affect phytoplankton and biological productivity. Without fundamental understanding of the natural mechanisms governing the growth of phytoplankton in the deep-water basin and shelf waters, it is impossible to separate the effects of climate change, eutrophication and pollution from natural changes in the ecosystem.

Recommendations

- High spatial and temporal variability of phytoplankton requires the development of principles and criteria aimed at improving ecological monitoring in different regions of the Black Sea.
- Limited monitoring capabilities (for example, in the open waters of the Black Sea) require the use of additional research methods that allow for the most complete assessment of the spatial and temporal variability of phytoplankton. First of all, these are satellite scanner data that can be refined using field studies.
- Efforts should continue to maximize the amount of historical data in on-line databases, as well as to analyse the comparability of this information with current, newly obtained data. A joint database on the phytoplankton of the Black Sea can be created as a part of the Black Sea Water Quality Database. Its maintenance and development should be included in upcoming international projects in the Black Sea region.
- Continue the search for indicators and integral indices of phytoplankton allowing the most efficient assessment of the ecological and environmental status of various regions of the Black Sea.
- Further adjustment of the ecological status rating scales for various phytoplankton indicators is necessary, taking into account changes in the Black Sea ecosystem and accumulation of new and historical data.
- It is recommended to carry out joint fundamental research of key ecosystem processes in the framework of international projects. It is recommended to conduct regular (at least once in 3 years) joint survey with the participation of all countries in all regions of the Black Sea, as this is the best opportunity to identify long-term trends in phytoplankton.

2.3 ZOOPLANKTON

According to the historical data and results collected during the EMBLAS project, the main tendencies in changes of the mesozooplankton community in the Black Sea are: (i) decreasing of percentage of Noctiluca scintillans in total zooplankton biomass and (ii) increasing of percentage of Copepoda. These tendencies indicate decreasing of the pressure of negative eutrophication factor and show positive changes in the forage base of commercial planktophagous fishes and ecological status of the Black Sea waters. These tendencies are in good agreement with the conclusion of Polishchuk (2006) that the Black Sea ecosystem is in a state of de-**eutrophication and the establishment of a new "ecological norm".**

Abundance of species, which are indicators of an improvement in the status of the marine environment, have increased in coastal waters of Georgia over the past years.

At the assessment of status and trends it is advisable to use the following zooplankton metrics: total biomass, percentage of Copepoda in total biomass, percentage of Noctiluca scintillans in total biomass and the Shannon index.

Gaps

1. Spot sampling does not provide information about seasonal changes in the zooplankton community structure. Thus, to achieve a complete picture of the ecological status of the study area seasonally repeated sampling cruises are needed.
2. The problem of assessing the threshold values for zooplankton indicators of North-Western part of the Black Sea is in some cases associated with insufficient availability of the results of systematic monitoring of zooplankton for a number of water areas in the Deepwater Shelf, Shallow Shelf, as well as the lack of a data from Zmiinyi Island coast. The spatial heterogeneity of the distribution of zooplankton may determine the need for threshold values for more local water areas.
3. The problem in assessing seasonal threshold values may be impacted by annual succession that occur in the current decade under the influence of climatic factors.
4. Due to the lack of historical data for South-Eastern region, it was not possible to prepare scales/threshold values for different seasons. The threshold values should be reviewed and updated.

Recommendations

1. In order to summarize all primary zooplankton data, it is recommended to create a common database with harmonised and interoperable results of historical and new studies. This would allow to calculate metrics used in zooplankton integral indices (diversity indices, biomass of zooplankton, biomass of Copepoda, biomass of Noctiluca scintillans etc.).
2. Exclude the metric ratio between invasive and native Copepoda biomass from the system of hydrobiological monitoring in the Black Sea.
3. Perform at least four sampling cruises (in phenological winter, spring, summer and autumn) annually to achieve more complete information. These cruises should be performed at the same stations and regions to provide comparable data.

2.4 BENTHIC HABITATS

The status of benthic habitats has been assessed by averaging the M-AMBI*(n) values of the entire monitoring period. If at least 75% of the samples in each habitat were in good status then the overall habitat was in good status. It was found that all investigated habitats were in good ecological condition (GES), even if it was observed that in some stations M-AMBI*(n) had values below the set threshold. Regarding the temporal variation over the analyzed period (2016-2019), the trend was stable.

The multivariate indices AMBI and M-AMBI*(n) proved to be reliable tools suitable for assessment of environmental status of the investigated habitats. It can be used to assess GES at various spatial scales (from station to habitat or ecosystem).

Gaps

- The assessment of ecological status using multimetric indices such as AMBI and especially M-AMBI*(n) needs increasing sampling effort and sampling strategy for each habitat, especially when it has a widespread. A small number of samples within such habitat with various substrates or intensity of different ecological pressures is unlikely to produce a correct assessment. A better sampling strategy (spatial coverage) must be conceived for the future.

- It is necessary to pay more attention to species classification in ecological groups. For the Black Sea, habitats' peculiarities should be taken into account. Therefore, inclusion of the dominant species in one or the other ecological category should be reconsidered using statistical tools or to be based on experts**' judgement**.
- Lack of reference/baseline conditions.
- Need for further common thresholds for uni- or multivariate indices used for ecological quality assessment of unassigned habitats.

Recommendations

- Need for combining *in situ* sampling, acoustic backscatter and habitats modelling. This would help to better assess the habitats size distribution (one of the MSFD requirements).
- Better coordination of monitoring efforts at national and sub-regional level in order to take advantage of shared infrastructure and expertise.
- Inter-calibration/comparison of the taxonomic expertise and the methodological standards in order to achieve comparable results between the countries in the Black Sea region under MSFD reporting in the future.
- Common methodology for the Black Sea region for identification and classification of benthic habitats for elaboration of habitats distribution maps and planning of monitoring activity within each type of habitats.
- A necessity of increasing the competence in samples collection and analysis (taxonomic expertise), which weighed much in the quality of the results obtained.
- Set the reference values for good environmental status based on *true* historical data (statistical analysis of time series data and selection of best threshold values) or on expert judgement.
- All current threshold values should be *adapted/improved* once that new and better data will be obtained.

2.5 MACROPHYTOBENTHOS

Assessment of ecological status of the coastal waters of Georgia, Russian Federation and Ukraine within the framework of the EMBLAS project showed that the inter-annual differences in the period 2016-2019 were insignificant and were largely determined by annual differences in climatic conditions, place and time of sampling. In this regard, it can be concluded that the status based on macrophytobenthos indicators in the studied period was **"Moderate" at Zernov's Phyllophora Field (ZPF), "Moderate"- "Good" at** the **Caucasian coast, and "High"- "Good" at the Georgian coast.**

Evaluation of historical data and values of macrophytobenthos indicators obtained in the period 2016-2019 allowed for determining long-term trends in the ecological status at the national monitoring sites. Improvement of the ecological status of the studied marine ecosystems in Ukraine and Georgia from NotGES to GES was observed in the periods 2004 – 2008 versus 2016 – 2019, respectively. Similar trend was observed in the Caucasian coastal waters.

Being at a considerable distance from the shore and depth, the structure-functional organization of phytocenoses of ZPF are formed under the influence of the quality of the marine strata and bottom sediments, which depend on the quality of the runoff of three major European rivers (Danube, Dniester, Dnieper) that enter the Black Sea in the North-Western shelf. Therefore, the basis for ecological management of the major marine protected area of Ukraine – ZPF, is the quality of water from the inflowing rivers.

In the 60s and 80s of the last century, the GES was characteristic for ZPF. At the beginning of the new millenium, the status decreased to NotGES. An improvement in the environmental status has been observed during the project, and the status of ZPF in 2016-2019 corresponded to GES.

At the North-Eastern Caucasian coast, the ecological status of the coastal waters at the Abrau Peninsula was Good, whereas the status of coastal waters in the Novorossiysk Bay was Moderate and Poor. The ecological status of Caucasian coastal waters has not changed significantly over the period from 2002 to 2019.

At the South-Eastern (Georgian) coast long-term comparative analysis showed that the status at the monitoring site Green Cape was “Moderate“ in 2004, “High” in 2016-2017 and “Good” in 2018-2019. Using environmental assessment criteria, the status changed from NotGES in 2004 to GES in 2016-2019.

Gaps

Zernov’s Phyllophora Field

– It was recommended to conduct national monitoring/sampling at ZPF twice a year - in the first decade of April and then in the first decade of September. Unfortunately, this recommendation was not implemented within the NPMS UA 2019. For this reason, data on macrophytes floristic diversity of ZPF were obviously reduced.

Caucasian coast

– In 2016 and 2019, the studies were carried out at different sites. This makes it difficult to compare changes that occurred during this period.

Georgian coast

– Most of Georgia’s national monitoring sites were located in areas of low anthropogenic loading, and only the “Batumi port” monitoring site reflects the higher degree of anthropogenic load. In this regard, there is a risk of overestimating the status assessment for the Georgian coastal waters as a whole.
– The absence of historical data for the floristic composition and structural indicators of macrophytes communities is a relevant problem for determining long-term changes in indicators of macrophytobenthos and corresponding status assessment of the Georgian coastal waters.

Overall assessment

– Since different morpho-functional indicators result in different ecological and environmental status for one monitoring station, it is necessary to develop recommendations on the use of specific indicators for specific tasks, such as (i) assessment for various national subdivisions; (ii) seasonal variability; (iii) long-term changes, etc.

Recommendations

Zernov’s Phyllophora Field

– In relation to the natural feature of the spatial distribution of phytocoenoses, the monitoring of ZPF should be carried out at minimum two stations in each of the ZPF Ukrainian national subdivisions: Shallow Shelf (Sh4) Off-shore shelf (depth 15-24 m) and Deepwater Shelf (Sh6) ZPF (depth 25-55 m).
– The monitoring stations that were completed in 2016, 2017 and 2019 should be kept; especially Historical Monitoring St. 10 (20-25 m recommended depth) and Historical Monitoring St. 9 (35-40 m recommended depth) in order to obtain comparable sample material for future assessments.
– With regard to the known patterns and results of the seasonal dynamics of phytocenoses in 2017, the national monitoring in ZPF should be carried out twice per year: in the first decade of April and in the first decade of September.
– Sampling of macrophytes in ZPF should be carried out by the transect method using periphyton frames and diving operations.
– If, according to technical conditions, the collection of macrophytes in the ZPF is carried out from the vessel using ‘**Grab’**, it is recommended to recalculate the macrophytes biomass to the collection method using ‘**Frame’** by applying 25-fold increase coefficient.

Caucasian coast

– Further monitoring of three regions on the Caucasian coast, for which morphological and functional criteria for determining the ecological and environmental status were established: 1. Kerch Strait region; 2. Abrau Peninsula; 3. Novorossiysk Bay and the Sochi region.
– Since the intensity of the functioning of macrophytes communities significantly differs depending on the depth, the studies carried out on the Caucasian coast should use the depth of 1.0 m as a standard at the assessing of the ecological status of the coastal shelf.

Georgian coast

- Macrophytobenthos monitoring stations located in the Coastal Water Body which is under anthropogenic influence, as well as in the transitional zone, should be included in the national monitoring programme of Georgia.

Overall assessment

- To obtain inter-comparable data on the state of macrophytobenthos and the corresponding environmental conditions of the Black Sea coastal waters, it is necessary to fully harmonise national monitoring schemes of all Black Sea countries. A particular attention should be paid to the use of general principles: (i) selection of monitoring sites with high and low anthropogenic load; (ii) sampling period; (iii) sampling methods; (iv) indicators; (v) methods of analysis and interpretation of the data.

2.6 MARINE MAMMALS

There are three cetacean species inhabiting the Black Sea, each of them represented by a locally distributed subspecies. In 2016-2019, their status and distribution in the selected areas designated by the CeNoBS project, in cooperation with EMBLAS, were assessed by aerial and ship-based surveys and observations, added by photo identification (photo-ID) research. Based on comparison of newly obtained results to those from studies conducted 15 years ago, the status and distribution patterns of all populations seem stable. Also, present-day cetacean populations are roughly similar in distributions to the years 1976-1987, the period preceding the collapse of the overall cetacean population.

Decline of cetacean populations was believed to contribute to the trophic cascade of the Black Sea ecosystem (Daskalov, 2003; Llope et al., 2011). On the opposite, their recovery may be indicative of stabilization of the marine ecosystem. Thus, cetacean species may be regarded as sentinel species for the Black Sea, and the status of their populations can be used as an indicator for basin-wide environmental state.

Gaps

Habitat use and spatio-temporal structure, as well as sex and age segregation of cetacean populations are the least studied aspects of cetacean distribution in the Black Sea. Passive acoustic monitoring, as well as individual tracking by satellite telemetry or other tagging means, are necessary for advances in this sphere. Another important aspect, which should be comprehensively covered, is quantitative assessment of threats: first of all - incidental bycatch, and also chemical pollution, oil spills, habitat loss and underwater noise.

Recommendations

Implementing basin-wide multi-sectoral monitoring programme including regular basin-wide aerial surveys as a key element, supported by regional and national ship-based surveys, photo identification of local stocks (mainly coastal small groupings), passive acoustic monitoring and records of strandings and bycatch are necessary for obtaining timely updated reliable information on status of cetacean populations. Such monitoring is crucial for assessment of D1C1, D1C2, D1C4 and D1C5 criteria under the MSFD provisions. Also, demographic studies based on photo identification / mark-recapture approach and analysis of age structure of stranded and bycaught individuals are necessary for assessment of the D1C1 and D1C3 criteria. Region-wide cooperation, concerted effort, sharing knowledge, standardized research methods and creating data resources of common use as elements of holistic approach are crucial for monitoring highly mobile basin-wide populations of marine mammals.

2.7 MICROBIAL COMMUNITIES OF THE BLACK SEA

The first large-scale metagenomic study of Black Sea microbial communities was conducted in the course of EMBLAS-II and EMBLAS-Plus projects. The research contributed to understanding of the overall structure and function of Black Sea microbial communities, their vertical distribution in water column and in sediments and their role in ecosystem response towards environmental pollution.

Taxonomic diversity of microbes inhabiting both seawater and sediments was examined, the potential of microbial communities to degrade persistent organic pollutants and microplastics was investigated **and antibiotic resistance genes' (ARGs) dissemination survey was performed for the first time.**

According to the World Health Organisation, antibiotic resistance is one of the biggest threats to global health. The main danger in the context of antibiotic resistance is antibiotic resistance genes (ARG), because they can be distributed by horizontal gene transfer and switch the phenotype of pathogenic bacteria from non-resistant to the resistant one and can be acquired even from distantly related phyla (Pruden et al., 2006). Hence, ARGs are assumed as emerging contaminants that can be found in various environments from hospitals/clinics to the pristine natural environments.

ARGs targeting a wide spectrum of antibiotics, both broad spectrum (e.g., beta-lactams, macrolides) and last resort (vancomycin and colistin) antibiotics were found in the Black Sea surface water. Significantly higher ARG abundance was detected in the water of the coastal zone, especially in Ukraine, compared to the open water, indicating the problem of a very high concern in the region.

The obtained sequences were uploaded in the open access NCBI database.

Metagenomic study of the water column and sediments during the JBSS 2016 has shown that deep-sea sediments contain bacteria able to degrade organic pollutants, which was confirmed by relatively low levels of pollution by organic chemical pollutants in those samples. Also, it has been found that Black Sea sediment microbial communities possess ability for plastic degradation, which can be comparable to the other biodegradation functions in its magnitude.

A significant abundance of bacterioplankton and several features of its metabolism: high reproduction rate, the ability to decompose organic matter, and eventually completely utilize not only almost all known natural organic compounds, but also many xenobiotics, determine the extremely important function of bacterioplankton in the biogeochemical cycles of elements and self-cleaning processes occurring in marine ecosystems. The results showed no increase in number and biomass of bacterioplankton over the past 50 years, except for the maximum values in the North-Eastern part of the open Black Sea.

Gaps

- Microbial communities are known to play a crucial role in biogeochemical cycles, food webs and in functioning of marine ecosystems in general. Microbes are numerically dominant in marine environments and are characterized by pronounced diversity of metabolic potential. Nevertheless, this ecosystem component has been ignored by the MSFD so far. To date prokaryotes are understood only as "microbial pathogens" (MSFD Annex III, Table 2 Pressures and impacts: Biological perturbations – Introduction of microbial pathogens). Such understanding represents a small tip of the large iceberg of microbial communities' interaction with the other marine ecosystem components and thus prokaryotes' significant role in GES indicators is not taken into account.
- Taking into account microbial taxonomic and functional diversity and rapid response to environmental perturbations, the data on prokaryotic communities can potentially be incorporated into MFSD Descriptors D1. biodiversity, D4. food webs, D5. eutrophication, D8. contaminants. Such an approach would result in more comprehensive GES analysis.

Recommendations

- Being involved in key biochemical processes running in marine ecosystems as a sensitive and fast responding to environmental perturbations, microbial communities can be considered as potential sentinels of environmental changes.
- Microbial communities are the essential component of MFSD Descriptor 1. Biodiversity. As the majority of marine prokaryotes are challenging or even impossible to grow in culture, 16S rRNA metagenomic assessment is the preferential method to uncover microbial communities' taxonomic diversity. Yet, in order to make the results of such analyses more interpretable in terms of monitoring, indices relevant for the status of the Black Sea environment should be developed. Thus, it was proposes to test the ratio of specific functional prokaryotic groups relative abundance in future monitoring of the Black Sea. The suggested ratios were: (1) oxygenic photosynthesizers/oxygenic photosynthesizers and (2) sulphur reducing/sulphur oxidizing bacteria. The proposed indicators can be used for specific monitoring of Black Sea euxinic zone position, as a part of multimetric assessment.
- Taking into account microbial biodegradation potential, the data on prokaryotic communities can be used in monitoring of MFSD Descriptor 4. Food webs and 8. Contaminants. It has been shown that

both microbial taxonomic and metabolic patterns become reorganized when exposed to pollution with hydrocarbon-containing contaminants. Therefore, the ecosystem response towards environmental pollution can potentially be monitored with integrative indicator incorporating (1) the data on microbial community taxonomic shift towards the dominance of taxa previously shown to increase in the case of pollution, and (2) the data on the expression of genes coding specific enzymes (alkane monooxygenases, polyaromatic hydrocarbon-ring-hydro- xylating dioxygenases and dehydrochlorinase). **Contrary to direct chemical analysis, the study of microbial communities' response allows for monitoring of pollutants' fate in the environment,** including its removal from the food webs. The proposed indicator might not be applicable yet for routine monitoring in the Black Sea region, as it is still time consuming, expensive and requires more research on the timing and scale of microbial community shift in response to pollution. Yet, it could be considered as an option.

– As the Black Sea can be considered a hot-spot for plastic pollution it is highly important to conduct follow-up studies for microbial plastic degradation both in its seawater and sediments. Such studies can be based on both culture-dependent and molecular techniques. It is important to contribute to our understanding of various plastic degradation steps starting from bio-degradation and ending in mineralization in order to have a clear picture of the fate of plastics in the marine environment. However, it is widely known that a small fraction of the microbial community can be grown in vitro, which makes it difficult to extrapolate the results from lab experiments. One possible way to overcome these limitations is to set up substrate recruitment experiments directly in the Black Sea environment using floating PET objects. This can potentially allow to follow up all processes of microbial plastic degradation. Another way is to utilize the power of metagenomics (metatranscriptomics and metabolomics studies) of Black Sea microbial communities with the purpose to discover novel pathways for plastic degradation. Based on such data, more detailed surveys can be developed to target a range of enzymes active in microbial plastic degradation.
– To date, the distribution of ARGs can be considered the easiest to interpret and most straightforward indicator of the state of Black Sea environment, based on the data on microbial communities. The protocol used for detecting ARGs in the Black Sea was specific enough to reveal the current state of ARGs abundance and distribution. The results indicate that ARGs can reveal an alarming ecological problem (e.g., in Ukrainian coastal area), therefore the used protocol could be included in the national Black Sea monitoring programme. The Data Collection Templates developed in the course of the EU ANSWER project can be used as a template for harmonised ARG data collection and storage in the follow-up monitoring in the Black Sea region. This would also ensure inter-comparability of data obtained in different European sea regions. Taking into account current low culture of prescription (empiric treatment, ungrounded treatment) and consumption (self-prescription, over-usage in human care and veterinary) of antibiotics in Ukraine, it is important to collect data on antibiotic resistance on a regular basis. As the Black Sea is influenced by Dnieper and Danube river run-off to a large extent, it could also be recommended to monitor ARGs abundance in these water bodies.

2.8 ENVIRONMENTAL DNA

DNA metabarcoding is a novel technique, which allows taxonomic identification of an organism based on the traces of its DNA released into the environment. It helps to overcome the drawbacks of traditional morpho-taxonomic analysis, which heavily depends on the experts' experience. The first large-scale metabarcoding study of the Black Sea biodiversity has been performed in the course of EMBLAS projects.

Metabarcoding proved to be a promising tool for Black Sea biodiversity assessment, even though there is a need for further validation of the methodology. High diversity of phytoplankton, zooplankton, fish and zoobenthos has been detected. Importantly, the genera, which include species involved in harmful algal blooms (Gymnodium, Scrippsiella, Alexandrium) were found with 18S sequencing in considerably high abundance in water samples. The members of these genera have been shown to cause Paralytic shellfish poisoning (PSP) and Diarrhetic shellfish poisoning (DSP) (Hallegraeff et al., 2003). Both invasive and rare fish species have been detected with COI and 12S analysis. For example, Trypauchen vagina occurrence was detected for the first time in the Black Sea waters. Metabarcoding approach appeared to be useful for the detection of high conservation concern species, such as the members of *Acipenser* genera (sturgeons).

Gaps and recommendations

Limitations of metabarcoding technique were also identified. The major bottlenecks so far are the scarcity of Black Sea biodiversity sequencing data in the databases and the lack of studies testing specific molecular markers for detection of particular groups of organisms. Therefore, one of the priorities should be barcoding of individual Black Sea species with the purpose to fill this gap. This will make DNA metabarcoding a useful tool for biodiversity monitoring and invasive species detection.

Taking into account the need to develop a common biodiversity monitoring platform for the entire Black Sea region, it is highly desirable to start incorporating eDNA approach into monitoring practices. This will set the starting point for automation of biodiversity monitoring in the Black Sea region, which in turn will potentially increase the quality and comparability of the data needed for the Initial Assessment and monitoring of MFSD indicators.

So far, only metabarcoding species presence-absence data is recommended to be used in data analysis, whereas the estimation of relative abundance is considered unreliable.

Metabarcoding protocols should be developed and validated for Black Sea region.

The data on taxonomic distribution of fish communities should be tested with more specific 12S gene.

COI and 18S should also be tested for the bulk specimen sample (homogenized individuals) of zoobenthos to get the full picture of their utility.

Development of specific assays for early detection of invasive species is highly promising approach, as metabarcoding is more sensitive in terms of its capability to detect various life-stages, which are harder to identify with morpho-taxonomic approach.

The data collected with metabarcoding approach should be integrated in indices for biodiversity monitoring after the development and validation of the Black Sea metabarcoding protocols.

2.9 BIOASSAYS AND BIOINDICATION METHODS

Biomonitoring of waters in the Ukrainian part of the North-Western Black Sea (NWBS) shelf was carried out in the summer-autumn season of 2019, using bioassay and bioindication methods. The bioassay was based on responses of larvae of Black Sea mussels at early stages of development. The bioindication method used laboratory culture of microalgae and so-called 'organism-monitor' (algae of microphytobenthos).

In the period of 2016-2019, a deterioration of the ecological status of surface waters and near-bottom waters was observed in the area of the marine protected area Zernov's Phyllophora Field. The area adjacent to Cape Malyi Fountain in the coastal zone of the NWBS was found to provide favourable conditions for the life of hydrobionts. The worst ecological conditions for the life of aquatic organisms were determined in the aquatic and bottom environment of the sea near Zatoka, Chornomorsk, Dacha Kovalevsky's, and Kobleve.

The study has demonstrated that the coastal and open sea areas of the Black Sea require regular ecological monitoring.

2.10 NON-INDIGENOUS SPECIES

Assessment of the share of non-native species in taxonomical groups and their role in the investigated regions were based on the principles and methodologies of the MSFD. The introduction of non-native and invasive species into open and especially coastal waters is among the highest risks for the marine environment, which may be even further increased in relation to the global climate change and cause serious adverse environmental, economic and public health impacts. The non-native invaders induced considerable changes in the structure and dynamics of marine ecosystems and the food web by outcompeting original inhabitants.

The most pronounced event was the invasion by *M. leidyi*, which caused cascading effects at higher trophic levels, from a decrease in zooplankton to collapsing planktivorous fish and drastic declines among large pelagic fish and dolphins.

After arrival of B. ovata, the Black Sea ecosystem began to recover progressively (Shiganova et al., 2014; 2018). An additional factor that favoured the recovery of the ecosystem was a decrease in level of eutrophication, which resulted from reduced anthropogenic nutrient inputs (Cociasu et al., 2008). That was accompanied by decreasing in total phytoplankton biomass and harmful algae blooms. A combination of these factors in the late 1990s led to a general improvement of the Black Sea ecosystem (Shiganova et al., 2014).

The observations during 2016-2019 showed that the abundance of the most harmful invader M. leidyi significantly dropped after arrival of B.ovata. However, in 2019 with very low presence of B.ovata and higher temperatures M. leidyi could reach higher abundance and biomass during its peak, even in December. In this case biomass of edible zooplankton was lower and both of these parameters resulted in 'Bad' ecological status of the studied areas. The introduction of the predator *Beroe ovata* into the Black Sea demonstrates a meaningful example of internal biological control.

The number of species in the Black Sea is presently relatively small (3,786 as of December 2020). The contribution of non-native species in total abundance amounted for 3.8% (UA region), 9.9% (RF) and 2.6% (GE), while their participation in total number of species was 4.3% (UA), 5.1% (RF) and 6% (GE). The Black Sea natural system is still resilient for the new incoming species and permit naturalisation of most of them without currently noted significant impact. Here, the exception might be geographical expanding of D. quadrilobata and S. gynobranchiata across the basin. Some of the zoobenthic non-native species have become valuable food items (Rhithropanopeus harrisii, Anadara inaequivalvis, Mya arenaria, etc.), despite the displacement of native species.

Summarizing the new finding, it can be concluded that at least five new non-native species were introduced in the Black Sea with the ballast waters. This did not consider new Mediterranean species, spreading not only around the Bosphorus area, but also in the other areas of the Black Sea due to increase of surface water temperature.

All non-native species in the Black Sea have been established in the coastal, shelf and continental slope waters, pelagic species are capable to spread in the open sea as a temporal expansion.

2.11 COMMERCIAL FISH AND SHELLFISH STOCKS

A methodology tackling MSFD Descriptor 3 - Commercial Fish and shellfish (*"Populations* of all commercially exploited fish and shellfish are within safe biological limits, exhibiting a population age and size distribution that is indicative of a healthy *stock")* has been introduced and suggested to be followed up in the continuation of the EMBLAS projects. Fisheries have a wide impact on the marine ecosystem, including on its biological diversity (Descriptor 1), food webs (Descriptor 4) and sea-floor integrity (Descriptor 6).

It has been stated that the fishing mortality in the Mediterranean and Black Sea has remained virtually unchanged since 2003. It remains extremely high, indicating that most selected stocks are severely overfished.

2.12 EUTROPHICATION

Assessment of the anthropogenic nutrient loading causing eutrophication of sea waters, and its direct and indirect effects reflected by indicators of Descriptor V of the MSFD (2008/56/EU) was carried out. According to the MSFD, the human-caused eutrophication must be minimized, especially its harmful consequences, which may include loss of biodiversity, damage of ecosystems, harmful algae blooms and lack of oxygen in bottom layers. Set of indicators has been developed for the assessment of the status of eutrophication (European Commission, 2010). They are following main phenomena related to eutrophication: algal blooms, decreasing of water transparency, development of hypoxic or anoxic conditions under the pycnocline in shelf zone in the warm time of the year, oxygen concentration reduction in bottom waters caused by its usage for biochemical combustion of organic matter stored there, hypoxia and anoxia as a result of eutrophication and lack of oxygen and weak up-and-down water exchange caused by vertical stratification.

The EMBLAS studies indicated that the observed levels of nutrients in the upper sea layer showed no evident changes compared to the 2000s. However, in relation to historical data, the average DIP (mineral form of phosphorus) values within the EMBLAS observation period were significantly lower than the average value of 0.51 µmol/L for the period 1990-2005 (Orlova, 2008) and corresponded to a GES value of 0.37 µmol/L. There is a tendency of decrease in the annual average concentration of both total phosphorus (TP) and its mineral form (DIP). The average TP values for the identified shelf areas were less than the average Target value (1.06 µmol/L) and corresponded to 'Good' ecological status.

Relative to the 1992 historical data, during the EMBLAS observation period a 1.2-fold decrease in the amount of mineral nitrogen and an increase in TN concentration due to its organic form were noted. In the variability of nitrogen concentration, and in contrast to the concentration of phosphorus, these are general trends in its long-term changes on the North-Western and North-Eastern shelf of the Black Sea.

Regarding Chl-a, there is no reason to conclude that its level in the North-Eastern part of the open waters of the Black Sea differs significantly from previous decades. Here, it should be noted that the Target and **Reference Chl**-a values for coastal waters by the Black Sea countries are not consistent enough, so the level of the adopted Reference Chl-a values varies from 0.25 µg/L (Turkey) to 4.0 µg/L (Georgia).

According to the observational data from the EMBLAS period, an improvement in water transparency was noted in relation to its average value of 7.8 m and extreme values (2 and 13 m) obtained from historical data in 1992. An increase in water transparency was clearly noted also in the South-Eastern sea region. In relation to the average value of water transparency of 4.6 m, obtained from the historical data of 1992-2000, the current value of the average transparency of the shelf waters according to the EMBLAS 2016 and 2017 observations is higher, which indicates some improvement in the regime of the photosynthesis process in the bottom layers of the shelf.

According to the EMBLAS observations, an improvement in the oxygen regime was noted in the area of the Zernov's Phyllophora Field, relative to 1992-2000. On the other hand, the decreased values of the average oxygen content of the recent period in the upper sea layer in relation to the period of the 90s can be explained by climatic changes, an increase in air temperature in the winter period, a decrease in the frequency of cold winters and a weakening of winter vertical convection of waters.

According to regular weekly observations in 2000-2019 in the coastal waters of the Odessa region on the North-Western shelf of the Black Sea, there is a general trend towards a decrease in the annual average concentration of mineral DIP and TP, as well as DIN, and increase in the concentration of TN, due to its organic form.

According to the regular observations on the North-Eastern shelf of the Black Sea in the coastal waters of the Anapa-Sochi region in 1996-2019, and in contrast to the North-Western shelf, there is a tendency towards increase in the annual average and maximum concentration of DIP.

According to observations in 2016 and 2019 in the South-Eastern shelf of the Black Sea in the coastal region Sarpi - Green Cape, surface waters correspond to GES.

In the Central Eastern deep sea water in 2019, the waters were predominantly in NotGES; the main reason being the bloom of phytoplankton and the high concentration of phytoplankton biomass.

In the deep sea water of the Central Eastern part of the Black Sea, the nutrient level in the upper layer did not change significantly compared to the 2000s, and small differences for phosphates and silicates can be associated with hydrological and biological characteristics of specific years.

2.13 CONTAMINANTS

Laboratory of EC JRC Ispra, Italy, provided support in analysing 109 contaminants in seawater samples collected within JBSSs in 2016, 2017 and 2019. The obtained results revealed the presence of several WFD priority substances as well as Watch list compounds and other emerging pollutants (e.g. triazine pesticides, pharmaceuticals, PCBs, flame retardants) at environmentally relevant concentrations in the Black Sea water samples. An indicative risk assessment using environmental quality standard (EQS) values for coastal waters from the EQS Directive has shown that all of the investigated water bodies and marine assessment units were at risk.

Similar to observations in the JBSS 2017, phosphorus flame retardants (PFRs) were detected at each site also in the JBSS 2019. The sum of the concentrations of all PFRs was the highest at the area close to the Danube estuary and in Georgian coastal waters. PAHs were present at all sampling sites, and their elevated concentrations were registered at the sites impacted by the Danube, and at several Ukrainian and Georgian sampling sites.

Based on the data from JBSSs 2016, 2017 and 2019, benzo(a)pyrene, fluoranthene, PFOS, heptachlor and heptachlor epoxide were WFD priority substances causing that investigated WBs/MAUs were 'at risk'. Their regular monitoring would be needed to gather sufficient amount of data to assess their risk to environment.

Based on the data from the JBSS 2019, there is a need to add the Watch list compounds estrone, 17-beta-estradiol, imidacloprid and EHMC to investigative monitoring programmes of the Black Sea countries and assess their risk.

Additionally, a wide-scope target screening of more than 2,400 substances in each sample has been carried out. Based on the results for sea water and sediments, several priority pollutants and emerging contaminants could be classified as "Black Sea Specific Contaminants" candidates and should be monitored in the future on a regular basis. In particular, Cd, Hg, DEHP, di-butyl-phthalate, para-para-DDT, 1-H-benzotriazole, 2-hydroxy-atrazine, 2-hydroxy-propazine, allethrin I, apophedrin, desethyl-atrazine, carbamazepine, carbendazim, carboxin, chloridazone, diphenamide, endothal, imidacloprid, picolinafen, propazine, pyrazophos, pyrethrin I, telmisartan and terbuthylazine could be candidates for future monitoring studies based on their high frequency of detection and exceedance of toxicity threshold values. Overall, more contaminants and at higher concentration levels were detected in coastal and shelf sediment samples compared to the open sea ones.

In parallel, a suspect screening (presence/absence/semi-quantification) of 65,691 substances was carried out. According to the risk assessment, nine substances were proposed as BSSC candidates in seawater: hexadecanamide, N,N-dimethyl-, laurocapram, N,N-diethylhexadecan-1-amide, diisobutyl phthalate, diisononyl adipate, octanoic anhydride, 1-(2-cyclohexylethyl)cyclohexyl acetate, ethanol, 2-[2-(4-methylphenoxy)ethoxy]- and octadecyl hydrogen phthalate. Moreover, eight substances were proposed as BSSC candidates in marine sediments: ethanol, 2-[2-(4-methylphenoxy) ethoxy]-, octanoic anhydride, ethanol, 2,2'-(octylimino)bis-, bis(2-propylheptyl) phthalate, ibuprofenol acetate, trideceth-3 carboxylate, glyceryl oleate and 3-hydroxyibuprofen. The chromatograms have been digitally archived in the NORMAN Database System (DSFP) and are available for retrospective screening of ever-increasing number of substances added to the suspect list.

Gaps

- Lack of capacities (sampling, analysis, trained personnel) to analyse majority of WFD priority substances, Watch list compounds and emerging (future Black Sea Specific) contaminants.
- Lack of funds to carry out MSFD- and WFD-compliant monitoring at the national level.

Recommendations

- Introduction of substances identified as relevant during the JBSS 2016, 2017 and 2019 into national investigative monitoring programmes of Ukraine, Georgia and Russian Federation in order to collect a critical mass of pollution baseline data for their risk assessment.
- Provision of the state-of-the-art analytical techniques including large volume sampling, automated sample preparation, LC-MS/MS, GC-MS/MS and ICP-MS in the key Black Sea laboratories responsible for national monitoring.
- On-site development of new methods for analysis of Black Sea Specific Contaminants and related training of personnel in laboratories in order to obtain reliable monitoring data.
- Supporting the accreditation of key Black Sea laboratories for analysis of Black Sea Specific Contaminants.
- Ensuring funds for regular MSFD- and WFD-compliant monitoring and reporting at the national level.
- Refined risk assessment of BSSCs including verification of PNECs and adding hazard (PBT) properties.
- Addition of new Black Sea relevant suspect chemicals and their transformation products in the suspect screening list and retrospective screening of already available digitally stored chromatograms.

2.14 PASSIVE SAMPLING

The passive sampling data collected during the JBSS 2016 provided useful information on the occurrence and typical levels of several classes of trace organic pollutants in surface seawater of the Black Sea. Passive sampling has been confirmed as a robust technique that enables to detect pollutants at sub-ng L^{-1} level without the need of complex sampling equipment or laborious sampling operations (e.g. active extraction of large volumes of seawater).

This first investigative monitoring campaign served as a good starting point for future sampling campaigns. In future, data with an increased spatial resolution could be obtained by deploying passive samplers at multiple static sites or along defined transects using available infrastructure (e.g., buoys, offshore installations, opportunity ships). It was strongly recommended to use partitioning passive sampling (e.g. those based on SR samplers) as a tool for various monitoring purposes. A scientifically sound procedures can be derived for the conversion of passive sampling measurements to water quality assessment criteria (EQS) on the basis of C_{free}. In such case, future monitoring of bioaccumulative substances in marine biota may be replaced by passive sampling.

With regard to the sampling of polar compounds, conversion of chemical uptake by adsorption passive samplers to concentrations in water is so far associated with a relatively large uncertainty (Vrana et al., 2016). Nevertheless, the time integrative character of sampling, in combination with the simplicity of sampling operations and sample logistics, make passive samplers the method of choice in initial screening campaigns for identification of contaminants presence, patterns and their relative occurrence in monitored areas.

2.15 CONTAMINANTS IN SEA FOOD

A relatively high number of biota samples (20) were analyzed within EMBLAS-Plus, including fish samples and molluscs from Georgia, Ukraine and the Russian Federation. Wide-scope target screening of more than 2,400 substances resulted in detection of tens of priority pollutants and emerging contaminants in biota samples. The concentration levels of WFD priority substance mercury exceeded the EQS value in 12 (60%) of the analyzed samples. The overall concentrations of flame retardants PBDEs exceeded the EQS value of 8.5 ng/Kg in 17 out of 18 tested samples. In total, 35 emerging contaminants were detected and could be grouped into seven main categories (2 industrial chemicals, 8 pharmaceuticals & personal care products (PCPs), 4 pharmaceuticals TPs, 3 plant protection products (PPPs), 3 PPPs transformation products (TPs), 2 drugs of abuse & TPs and 3 stimulants' TPs). JBSS 2019 biota samples were dominated by the presence of pharmaceuticals TPs (74.3 % of the overall pollution). In total, 14 emerging contaminants exceeded their ecotoxicological threshold values: dropropizine, metolachlor, anabasin, gentamycin, nicotine, atrazine-desisopropyl, bioallethrin, caffeine, propoxur, dicrotophos, ethiofencarb-sulfone, dioxacarb, 4-formyl-antipyrine and dinoterb. These compounds, along with the WFD compounds mercury and PBDEs and the most frequently detected emerging contaminants (methylparaben, DEET (diethyltoluamide) and myclobutanil could be considered as candidates to be included in the list of the "Black Sea Specific Contaminants".

According to the risk assessment of suspect screening results (65,691 substances), additional nine substances were proposed as the Black Sea Specific Contaminants candidates in biota: methyl 3-oxooctadecanoate, didodecyl phosphate, ethanol, 2,2'-(octylimino)bis-, 4-ethylaniline, 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, erucamide, octadecyl hydrogen phthalate, 4-piperidone and diisooctyl isophthalate. The chromatograms have been digitally archived in the NORMAN Database System (DSFP) and are available for retrospective screening of ever-increasing number of substances added to the suspect list.

Gaps and recommendations

Similar recommendation as for the chemical contaminants in sea water and sediments are valid for biota.

The analysis of stranded dolphins in EMBLAS-II project conducted in 2017, revealed their excessive pollution by numerous pollutants. Therefore, apex predators could be included in regular monitoring studies for the investigation of biomagnification and bioaccumulation phenomena of priority pollutants and emerging contaminants within the food web.

2.16 PRIORITISATION OF BLACK SEA SPECIFIC CONTAMINANTS

A prioritisation scheme applied on the results obtained by wide-scope target screening of Black Sea water, biota and sediments samples has proven to be practicable and feasible. Using the NORMAN Prioritisation Framework and NORMAN Database System infrastructure, it was possible to propose first lists of Black Sea Specific Contaminants (BSSCs) in each of the matrices with tentative ecotoxicity threshold values. Most of the results were available from the sea water analyses and it was possible to draft a provisional list of ten BSSCs fulfilling the criteria for future 'regulatory monitoring'. Less samples were available for biota and sediments, therefore all substances, which exceeded their ecotoxicity threshold values in at least one sample/site were suggested to be monitored until a critical mass of data is available. The provisional lists of biota and sediment BSSCs consisted of 75 and 22 substances, respectively. The lists differ significantly; sea water BSSCs suggests that none of the 45 WFD PS (required to be monitored in coastal waters 12 times per year) should be included into the regional monitoring programme. A wider list of 51 substances, which exceeded their toxicity threshold values in at least one sea water sample, included eight WFD PS and also three surface water Watch List substances. The suggested biota BSSCs were strongly dominated by the presence of BDEs, PCBs, metals, dioxins and dioxin-like compounds, OCPs, PAHs and perfluorinated compound PFOS. The top eight sediment BSSCs were metals, followed by OCPs and PAHs. Similar to other European sea conventions, there is an obvious need to establish background concentrations for metals in the Black Sea region.

Suspect screening revealed presence of additional ca. 2,000 substances in water, biota and sediment samples, out of which 41, 203 and 117 were present in more than 50% of the samples in the respective matrices. The results were used to create a 'universe' of Black Sea contaminants and the obtained mass spectrometry information (digitally stored samples) is available for retrospective screening. The future work should aim at risk assessment of these substances by comparing their semi-quantitatively assessed concentrations against the toxicity threshold values and deriving their persistence and bioaccumulation properties.

Gaps and recommendations

Laboratories in the three countries participating in EMBLAS do not have a capacity to carry out monitoring of the full range of the proposed BSSCs. There is a need for technical assistance projects to purchase new modern laboratory equipment, establish methodologies for further update of the list and monitoring of BSSCs and train the laboratory staff.

The proposed BSSCs should be harmonised with the lists of Romania, Bulgaria and Turkey. The final combined list of BSSCs should be considered at the BSC level to be included into the revised BSIMAP. EQS values for BSSCs should be developed at the regional levels and harmonised with the other sea conventions at the EU MSFD level. Consequently, Programme of Measures to phase out BSSCs should be developed.

Despite the adoption of the EU water legislation, there is a serious lack of funding of the MSFD/WFD-compliant national monitoring programmes in Ukraine. Georgia will approximate its national water legislation with the EU legislation once the draft Law on Water Resources Management is adopted and the national monitoring programme will require further funding to be compliant with MSFD/WFD. The present legislation for the protection of marine environment in the Russian Federation does not explicitly require identification and subsequent phasing-out of the BSSCs. An agreement at the BSC level might be needed to agree on a common regional approach how to prevent the Black Sea environment from an excessive pollution by chemicals. This might require even closer cooperation with River Basin management organisations/commissions of the major rivers inflowing into the Black Sea.

Further regional surveys, including deep sea areas, should be carried out regularly (e.g., annually or bi-annually) in order to obtain representative picture of the chemical pollution of the entire sea and record pollution trends reflecting the success of the pollution reduction Programme of Measures. This cannot be organised at the national level and an assistance of the EC via funding follow up trans-national monitoring programmes might be required.

2.17 MARINE LITTER

The outcomes of the first multinational integrated assessment of marine litter pollution of the Black Sea covering sea surface, riverine, beach, sea floor litter and microplastics in water column and bottom sediments were presented. The results were obtained within massive scale international (JOSS) and national (NMS) surveys and monitoring studies using a harmonized MSFD approach for the first time. The surveys involved scientists from Georgia, Russia and Ukraine, while scientists from Romania, Bulgaria and Turkey participated to accompanying workshops and trainings and through cross-project collaboration with ANEMONE project. These pilot studies demonstrated the importance of harmonization of reported data for comparison of results between different surveys and areas. The observations confirmed that marine litter was present in all Black Sea areas and consists mostly of plastic. The concentrations varied widely. Average concentration of floating litter was estimated as 90 items/km^2, the median values of marine litter inflow with rivers varied from 4 to 72 items/hour, the average amount of beach litter was 474 item/100 m. There is still a need to improve spatial and time coverage of monitoring, identification of hot spots, source areas and monitoring organization on regional and national levels. Also, development and implementation of methodologies for targeted monitoring of the sea floor litter is required.

The first initial assessment of riverine floating macro litter input to the Black Sea showed that the median values of marine litter draining into the Black Sea with rivers contains 84% of plastic items where the most frequently found items consisted of identifiable Single Used Plastics (SUP). Black Sea beaches appeared as the most littered in comparison with other European seas with the highest rate of SUP with median value of 652 litter items/100 m.

There are still serious knowledge gaps in assessment of the level of marine litter pollution in the Black Sea and its hotspots, which are connected mostly with absence of any regular marine litter monitoring schemes in the project countries. At the same time, it is already clear that pollution by marine litter is a significant environmental problem which threatens the marine wildlife, coastal ecosystems, health status of seaside population and normal development of sea-oriented economics including tourist industry, fishery and shipping. This is a rapidly developing disaster which needs to be properly addressed with a global reduction of the production of plastic waste/products, strong regulation and policies, effective enforcement and adequate support infrastructure at all regional and national scales.

2.18 MICROPLASTICS

The abundance, extent and spatial distribution of microplastics contamination of sediments were determined across the Black Sea using samples obtained within the JBSS 2017. Microplastics (MPs) were determined in 83% of samples. The average abundance in all samples was 107,5 items/kg. The highest pollution occurred on the North-Western shelf where the abundance of MPs was 10 times higher than in sediments from the deep sea. The results showed the predominant abundance of PE/PP, polyamide and acrylate MPs in the samples. This correlates well with the global plastics production according to (Brien 2007), where PE (used in production of plastic bags, bottles, netting, drinking straws) amounts to 38% and PP (commonly used for ropes, bottle caps, netting) reaches 24% of plastics production in the world. Consequently, PE and PP in particular have high likelihood of ending up in the ocean environment (Andrady 2011). Geographical aspects of the Black Sea like high isolation from the World Ocean, the drainage area which exceeds its surface many times, intensive recreational and maritime uses of the sea, as well as continuously increasing population along the coastline make it specifically exposed for microplastics accumulation.

For more information on the extent and distribution of MPs in the Black Sea further monitoring activities with wider spatial coverage and improved sampling methodology are needed. It is recommended to enlarge the volume of sediment samples and also collect duplicate sample from each site to receive more complete datasets.

2.19 12-MONTHS NATIONAL PILOT MONITORING STUDIES (NPMS)

Longer-term systematic observations of selected parameters were carried out at several sites in Ukraine, Russian Federation and Georgia. The results are described in detail in a separate volume of the JBSS 12-months NPMS from 2017 and summarised in the EMBLAS Final Scientific Report. The NPMS were designed to better understand the processes determining pressures and impacts on marine ecosystems within the Black Sea riparian countries' exclusive marine zones.

Based on the data from the special pilot projects (12-months NPMS) carried out under the EMBLAS-II project, while keeping in mind the requirements of the WFD and MSFD Directives and national legislation, the following recommendations have been formulated for improvement and optimization of national systems and programmes of the Black Sea monitoring:

Gaps and recommendations

- All characteristics of marine ecosystems demonstrate cyclic variations during calendar year, therefore their dynamics should be followed during a year keeping in mind different variability of separate ecosystem constituents, i.e. similar requirements for frequency of sampling and observations should be formulated for all the countries and for separate ecosystem constituents.
- Guidelines should be elaborated for definition of core indicators with quantitative threshold values to evaluate progress towards the goal of achieving good environmental status in the Black Sea. The experience from the Baltic Sea Monitoring Programme (HELCOM https://helcom.fi/baltic-sea-trends/indicators/) should be considered. This will help to consider all the Black Sea peculiarities and ensure comparison of data with other European seas. The indicators should be divided into three categories characterising the state, pressure and impact.
- Keeping in mind high variability of physico-chemical (hydrological and hydrochemical) characteristics, in order to eliminate manual labour it is recommended to install automatic on-line recorders of the main physico-chemical parameters of the surface and bottom waters; it is also recommended to collect water samples of different origin for relevant parameters such as chemical contaminants, bacterial pollution, chlorophyll a, phytoplankton etc., which would help to determine representatively the influence and input of different types of water into forming of biocoenoses in the studied areas.
- The national monitoring programmes should cover all the areas of the national marine zones. Monitoring of the main hydrological and hydrobiological characteristics should be performed at least four times a year to trace annual cycles of marine ecosystems. Such surveys in the Black Sea should be harmonized and synchronized with the neighbouring countries in order to receive conjoined information for mapping (pseudophotography) of the entire Black Sea. The Black Sea Commission could have assumed the role of coordinator of these activities.
- For comprehensive analysis of data and preparation of Assessments of marine environment state, the following types of monitoring should be developed:
    - LTERM - long-term environmental (ecological) research monitoring for assessment of trends of core state indicators in separate typical marine areas or the areas of special value.
    - SM standard (routine) monitoring to fulfil the standard requirements of the WFD and MSFD.
    - RM - research monitoring for studying of emergency situations, as well as for screening and testing of new monitoring methods.
- The following should form an integral part of each Black Sea country's national monitoring system:
    - Establishment of 2-3 reference LTERM stations per country to perform all possible observations of marine ecosystem state; the reference stations should perform daily (in the worst case – every 10 days) observations of quickly changing characteristics, such as temperature, salinity, chlorophyll a, nutrients etc.). Those observations will provide countries with the possibility to develop national quality standards and indicators. All proven methods and indicators recommended from outside of the region could be tested on those stations.
    - Establishment of units for processing of data from satellite monitoring, which will help to operatively reveal the situations like blooming, hypoxia, oil spill etc.
- Based on analysis of the 12-months NPMS results, it is proposed to divide all types of observations, depending on their variability and influence on marine ecosystems, into three groups:
    - High frequency (physico-chemical characteristics, phytoplankton, zooplankton, microbiology) – observations should be performed daily or at least every 10 days.
    - Medium frequency (macrozoobenthos, macrophytobenthos, pollution of water, bottom sediments and hydrobionts) – observations should be performed monthly or at least quarterly taking into account annual cycles of river discharge and temperature.
    - Low frequency (fish, pollution of bottom sediments) – observations should be performed once in several years.

- For objective assessment of marine environment quality in the Ukrainian part of the sea, regular studies are required: at least quarterly in the open parts of the sea and monthly in the coastal and river estuary areas. Such typical **areas of studies ('polygons') sh**ould be established in advance depending on their significance and representativeness.
- For studies of benthic community and assessment of its state mapping of bottom landscapes and singling out of typical districts in the studies area is required. To do this, sounding bathymetric survey should be mandatory. Based on the sounding survey, bathymetric maps and bottom elevation maps should be made.

2.20 THE BLACK SEA WATER QUALITY DATABASE

A regional Black Sea Water Quality Database (BS WQD) has been developed within the EMBLAS project, in-line with the overall need to improve sharing of harmonized and interoperable monitoring data in the Black Sea region. The data stored in the database cover the period from 2016 to 2019 and comprise all the data collected within the JBSSs performed during EMBLAS and historical monitoring data obtained from the project partners. The database structure is based on the MSFD ecosystem approach and follows the hierarchy of MSFD Descriptors. It has been developed to be fully compatible with the European Commission's SeaDataNet, EMODNet and IPCHEM from the start.

The database comprises automated tool for integrated assessment of environmental (MSFD; Marine Assessment Units) and/or ecological (WFD; coastal water bodies) status based on MSFD indicators harmonized among the participating countries. Data Collection Templates for collection of the primary data and metadata have been shared with and tested by experts from the other three Black Sea countries (BG, RO, TK). BS WQD is at present the only marine environment database with the regional coverage in the Black Sea basin.

Following the initial setup, new activities were planned to further develop and upgrade Data Collection Templates (DCTs), database web-interface and Web-GIS.

2.21 MONITORING NETWORKS

The proposals of monitoring networks were prepared on the basis of methodological work and expedition surveys carried out in deep sea, coastal and territorial waters of Georgia, Russian Federation and Ukraine during 2016, 2017 and 2019. Network of stations, list of parameters and frequencies of sampling, indicators, assessment schemes were developed in the light of practical experience and following BSIMAP, MSFD and WFD principles. Economic assessment of costs for implementation of environmental monitoring in the project countries was made in 2018.

The monitoring network proposed in Ukraine has already became a part of the national Marine Strategy in 2020.

There is an overall need to improve national capacities for implementation of the monitoring programmes and secure related financing in the state budgets.

2.22 PUBLIC INVOLVEMENT

"The Black Sea is vulnerable, but very intriguing ecosystem, which needs to be protected" - this was a key message of the communication strategy of the project. The information products were developed for different stakeholder groups: from decision-makers to children. They included movies, infographics, books etc. Moreover, there was an opportunity to be actively involved in the Black Sea conservation through clean-up campaigns, dolphins monitoring campaigns and using application "Black Sea Savebook" for environmental monitoring of the coastal areas by citizens. Small scale actions were implemented by national NGOs, with focus on environmental protection and marine litter reduction.

Close cooperation with media through regular press-conferences and posts allowed sharing results widely created permanent interest to the project. All of this can be followed at the Facebook group "Fans of the Black Sea" and the project website.

There is a need to maintain the continuous involvement of all stakeholders and general public in activities of the follow up of the EMBLAS projects. The new communication strategy should include as a minimum promotion of outcomes of EMBLAS-II and EMBLAS-Plus projects, further upgrade of the project website, Facebook group 'Fans of the Black Sea', mobile application 'Black Sea SaveBook' and maintaining the traditions of 'Clean Beach Day'.