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Black Sea state of environment based on ANEMONE Joint Cruise

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Contents

Executive summary 13
1 Biodiversity 16
1.1 Pelagic Habitats 16
1.1.1 Phytoplankton 17
1.1.2 Microzooplankton 25
1.1.3 Mesozooplankton 30
1.1.4 Macrozooplankton 41
1.1.5 Ichthyoplankton 46
1.2 Benthic Habitats 48
1.2.1 Introduction 48
1.2.2 Assessment concept – a general framework 48
1.2.3 Material and methods 49
1.2.4 Results 52
1.2.5 Recommendations 59
1.3 Marine mammals (dolphins and porpoises) 60
1.3.1 Introduction 61
1.3.2 Material and methods 66
1.3.3 Results 67
1.3.4 Conclusions 71
2 Eutrophication 72
2.1 Introduction 72
2.2 Material and methods 73
2.3 Water column nutrients 77
2.4 Water column chlorophyll *a* 87
2.5 Photic limit (transparency) of the water column 91
2.6 Bottom dissolved oxygen 92
2.7 Assessment of trophic status and water quality with E - TRIX 93
2.8 Black Sea eutrophication integrated assessment – BEAST 95
2.9 Gaps and Recommendations 98
3 Contaminants 99
3.1 Introduction 99
3.2 Material and methods 100
3.3 Heavy metals 102
3.4 Total petroleum hydrocarbons (TPH) and Polycyclic aromatic hydrocarbons (PAH) 114
3.5 Organochlorine pesticides 124
3.6 Polychlorinated Biphenyls (PCBs) 131
3.7 Integrated assessment – all contaminants – CHASE tool 137
3.8 Conclusions 143
3.9 Gaps and recommendations 143
4 Marine litter 145

4.1 Overview of Marine litter at Black Sea basin level 145
4.1.1 Beach litter 146
4.1.2 Floating Litter 149
4.1.3 Sea Floor Litter 150
4.1.4 Microplastics 152
4.2 Marine Litter assessment at MSFD descriptor level 152
4.2.2 Floating litter 154
4.2.3 Bottom litter 157
5 Nested Environmental status Assessment Tool (NEAT) 158
6 Conclusions and future outlook 165
6.1 Key priorities for the Black Sea’s ecosystem protection 165
6.1.1 Climate change 165
6.1.2 Eutrophication 165
6.1.3 Pollution 166
6.1.4 Assessment Tools 166
7 REFERENCES 167
ANNEX Species list identified during the Joint Cruise 176

List of Figures

Figure 1.1 - Phytoplankton taxonomic composition during the warm season, 2019 (RO-BG-TR-UA transects) 19
Figure 1.2 - Spatial variation of average abundance and biomass during the warm season, 2019 19
Figure 1.3 - Distribution of phytoplankton average abundance (10^3 cells/L, left) and biomass (mg/m^3, right) during the warm season, 2019 20
Figure 1.4 - Vertical distribution of phytoplankton abundance (10^3 cells/L, left) and biomass (mg/m^3, right) during the warm season, 2019 21
Figure 1.5 - Phytoplankton taxonomic structure on the Ukrainian shelf based on average abundance and biomass during the warm season, 2019 22
Figure 1.6 - Phytoplankton taxonomic structure on the Romanian shelf based on average abundance and biomass during the warm season, 2019 22
Figure 1.7 - Phytoplankton taxonomic structure along the Bulgarian southern shelf based on average abundance and biomass during the warm season, 2019 22
Figure 1.8 - Phytoplankton taxonomic structure on the Turkish area based on average abundance and biomass during the warm season, 2019 23
Figure 1.9 - Map of the GES/nonGES area's spatial extent in the pelagic shelf habitats (UA, RO, BG, TR) based on SHL phytoplankton biomass in the warm season of 2019 24
Figure 1.10 - Map of the GES/nonGES area's spatial extent in the pelagic shelf habitats (UA, RO, BG, TR) based on SHL Shannon 95 index in the warm season of 2019 24
Figure 1.11 - Map of the GES/nonGES area's spatial extent in the pelagic shelf habitats (UA, RO, BG, TR) based on SHL Menhinick index in the warm season of 2019 25
Figure 1.12 – Vertical biodiversity of tintinnids species along the western part of the Black Sea 26
Figure 1.13 - Biodiversity of tintinnids community along the western part of Black Sea 27
Figure 1.14 - Community structure of tintinnids along western area of Black Sea – density (left) and biomass (right) 27
Figure 1.15 - Density (ind/L) of tintinnids from all three investigated sites (Romania, Bulgaria, and Turkey) of western Black Sea (for integrated layers) 28
Figure 1.16 - Density (ind/L) of tintinnids from the three investigated sites (Romania, Bulgaria, and Turkey) of the western Black Sea in the surface layer (left) and DCM layer (right) 28
Figure 1.17 - Bray-Curtis similarity of microzooplankton distribution according to the diversity and density of the species from each station 29
Figure 1.18 - nMDS analysed of microzooplankton in the western part of Black Sea 29
Figure 1.19 - Copepoda community structure in October 2019 along the UA-RO-BG-TR coast 31
Figure 1.20 - Shannon diversity index by abundance (left) and biomass (right) along the western Black Sea – GES/Non-GES assessment 32
Figure 1.21 - Box whiskers abundance (upper panel) and biomass (lower panel) presence in Ukrainian (UA), Romanian (RO), Bulgarian (BG) and Turkish (TR) areas 33
Figure 1.22 - Spatial distribution of mesozooplankton biomass in October 2019 34
Figure 1.23 - Noctiluca scintillans biomass spatial distribution in October 2019 34
Figure 1.24 - Mesozooplankton and N. scintillans density (up) and biomass (down) proportion dynamic in October 2019 35
Figure 1.25 - Fodder mesozooplankton community structure in abundance (up) and biomass (down) 36
Figure 1.26 - Bray-Curtis similarity matrix of mesozooplankton biomass 36
Figure 1.27 - Vertical mesozooplankton distribution pattern in abundance 37
Figure 1.28 - Spatial extent of the GES/nonGES area in the pelagic shelf habitats (UA, RO, BG, TR) based on mesozooplankton biomass index in October 2019 38
Figure 1.29 - Spatial extent of the GES/nonGES area in the pelagic shelf habitats (UA, RO, BG, TR) based on Copepoda index (%) in October 2019 39
Figure 1.30 - Macrozooplankton community structure of abundance and biomass in percentage 42
Figure 1.31 - Mean, Standard Deviation (SD), Standard error (SE) of A. aurita, M. leidyi, P. pileus, B. ovata density (left) and biomass (right) 42
Figure 1.32 - Mnemiopsis leidyi density (ind/m^3, left) and biomass (g/m^3, right) spatial distribution 42
Figure 1.33 - Beroe ovata density (ind/m^3, left) and biomass (g/m^3, right) spatial distribution 43
Figure 1.34 - Pleurobrachia pileus density (ind/m^3, left) and biomass (g/m^3, right) distribution 43
Figure 1.35 - Aurelia aurita density (ind/m^3, left) and biomass (g/m^3, right) spatial distribution 44
Figure 1.36 - Spatial extent of the GES/nonGES area in the pelagic shelf habitats (RO, BG, TR) based on M. leidyi biomass index (g/m^3) in October 2019 45
Figure 1.37 - Fish egg density in Romania, Bulgaria, and Turkey marine waters 47

Figure 1.38 - Fish larvae density in Romania, Bulgaria, and Turkey marine waters 47
Figure 1.39 - Map of the sampling network in the joint cruise superimposed on the broad habitat types identified in EUSeaMap project (EMODnet) 50
Figure 1.40 - Bray-Curtis similarity on fourth root transformed abundance data in the assessment area 53
Figure 1.41 - Assessment results of Circalittoral and Offshore circalittoral shelly mud with pelophitic bivalves and polychaetes based on EQRM-AMBI 54
Figure 1.42 - Assessment results of Offshore circalittoral mixed sediments with Modiolula phaseolina based on EQRM-AMBI 55
Figure 1.43 - Ecological status in the assessment area based on EQRM-AMBI*(n) 57
Figure 1.44 - Mean TUBI values in the assessment area 58
Figure 1.45 - Assessment of ecological status of benthic habitats in the assessment area based on EQRTUBI 58
Figure 1.46 - Percentage of ecological groups detected in the sampling stations....................... 59
Figure 1.47 - Single platform positioning used during the ANEMONE JC survey for marine mammal data collection 62
Figure 1.48 - Delphinus delphis ssp. ponticus Barabasch-Nikiforov, 1935 (@Mare Nostrum NGO)...... 63
Figure 1.49 - Tursiops truncatus ssp. ponticus Barabasch-Nikiforov, 1940 (@Mare Nostrum NGO) 63
Figure 1.50 - Phocena phocoena ssp. relicta Abel, 1905 (@Tonay/TUDAV) 64
Figure 1.51 - Line transect survey plan in the western Black Sea area 68
Figure 1.52 - Cetacean sightings distribution by specie recorded in the RO sector 69
Figure 1.53 - Cetacean sightings distribution by specie recorded in the BG-TR sector.................. 70
Figure 1.54 - Cetacean density heatmap for the ANEMONE JC. Red circles highlight the major hotspots 70
Figure 2.1 – Examples of ODV maps done by method 1 - original data (left) and method 2 – data products by weighted average gridding (right) 74
Figure 2.2 – Phosphate and Silicate concentrations - Horizontal distribution............................ 78
Figure 2.3 – Phosphate and Silicate concentrations - Horizontal distribution............................ 78
Figure 2.4 – Total oxidized inorganic nitrogen, ammonium and dissolved inorganic nitrogen - Horizontal distribution 79
Figure 2.5 – Total oxidised inorganic nitrogen and ammonium (SHL)...................................... 79
Figure 2.6 – Water column (0-100m) temperature 80
Figure 2.7 – Water column (0-100m) salinity.. 80
Figure 2.8 - Water column (0-100m) phosphate.. 81
Figure 2.9 – Box plots of DIP grouped by layers 81
Figure 2.10 – Water column (0-100m) silicate 82
Figure 2.11 – Box plots of SiO4 grouped by layers... 82
Figure 2.12 – Water column (0-100m) TNOx (nitrite+nitrate) 83
Figure 2.13 - – Box plots of TNOx grouped by layers... 83
Figure 2.14 – Water column (0-100m) ammonium.. 84
Figure 2.15 - Box plots of NH4 grouped by layers 84
Figure 2.16 – Total oxidised inorganic nitrogen and ammonium dominance at surface (up), in SHL (middle), and near bottom (down).. 85
Figure 2.17 – Nutrients ratio in the water column 86
Figure 2.18 - Chlorophyll a (µg/L) pattern of distribution: surface (L) and Surface Homogenous Layer (SHL)(R) 88
Figure 2.19 - Box-Whisker plots of chlorophyll a (µg/L) surface (L) and SHL (R) 88
Figure 2.20 – Cluster analysis of the sampling stations based on surface chlorophyll a (µg/L)......... 89
Figure 2.21 - Sentinel 3/OLCI pigment (chlorophyll a concentration), 01-06 October 2019 89
Figure 2.22 - Vertical distribution of chlorophyll a concentration (µg/L) by stations and pelagic habitats 90
Figure 2.23 - Cluster of the sampling stations based on SHL chlorophyll a (µg/L) 91
Figure 2.24 – Seawater transparency 91
Figure 2.25 – Dissolved oxygen water column content 92
Figure 2.26 – Dissolved oxygen saturation water column content 92
Figure 2.27 - Assessment of trophic status and water quality with the E-TRIX of the Black Sea shelf (surface) 94
Figure 2.28 - Assessment of trophic status and water quality with the E-TRIX of the Black Sea shelf (bottom) 94
Figure 2.29– BEAST surface distribution – ANEMONE JC 97

Figure 3.1 - Cu, Cd and Pb concentrations in seawater samples from NW, W and SW Black Sea, 2019 104
Figure 3.2 - Ni and Cr concentrations in seawater samples from NW, W and SW Black Sea, 2019 105
Figure 3.3 - Zn, Hg, Co and Fe concentrations in seawater samples from NW Black Sea, 2019 105
Figure 3.4 - Cu: Al and Cr:Al scatter plots for sediments from NW, W and SW Black Sea, 2019....... 107
Figure 3.5 - Cu and Cd concentrations in sediments samples from NW, W and SW Black Sea, 2019.. 107
Figure 3.6 - Pb, Ni and Cr concentrations in sediments samples from NW, W and SW Black Sea, 2019 108
Figure 3.7 - Mn, Al and Fe concentrations in sediments samples from NW, W and SW Black Sea, 2019 109
Figure 3.8 - Co, Hg, As and Zn concentrations in sediments samples from NW, W and SW Black Sea, 2019 110
Figure 3.9 - Classification of sediments from NW, W and SW Black Sea, 2019, based on Effects Range-Low (ERL) values 111
Figure 3.10 - Heavy metals concentrations in biota samples from NW, W and SW Black Sea, 2019 .. 113
Figure 3.11 - Distribution PAHs total, TEFs, Σ carcinogenic PAHs in surface water 115
Figure 3.12 - Gradient distribution of PAHs total, TEFs, in surface water 115
Figure 3.13 - Distribution PAHs total, TEFs, Σ carcinogenic PAHs in bottom sediments 117
Figure 3.14 - Gradient distribution PAHs total, TEFs, in bottom sediments 119
Figure 3.15 - Results of analysis of total petroleum hydrocarbons (TPH) in sediment samples 121
Figure 3.16 - Distribution of organochlorine pesticides in Black Sea surface waters, October 2019 . 126
Figure 3.17 - Share of DDT and its metabolites concentrations in NW, W and SW surface waters, 2019 126
Figure 3.18 - Distribution of organochlorine pesticides in surface sediments, October 2019.......... 129
Figure 3.19 - Distribution of organochlorine pesticides in Black Sea Mytilus galloprovincialis, October 2019........ 130
Figure 3.20 - HCB and heptachlor concentrations in Black Sea Mytilus galloprovincialis, in relation to threshold values stipulated by Directive 2013/39/CE, October 2019 130
Figure 3.21 - Dominant PCBs in the surface sediment and Comparison with threshold values. PCB dominances in sediment matrix (up), PCB's compared with the threshold values (down) 133
Figure 3.22 - Spatial distribution of total PCB (as sum of 7 congeners) concentrations in surface sediment. Total PCB distributions (up), Total PCB distribution map (down) 135
Figure 3.23 - Individual PCBs levels (ng/g wet weight tissue) in mollusks, October 2019.............. 137
Figure 3.24 - CHASE Overall Score, and CHASE Score per matrix, contaminants data, 2019 140
Figure 3.25 - Quality status of the assessment units/stations, based on CHASE Overall Score, contaminants data, 2019 141
Figure 3.26 - Quality status of the assessment units/stations, based on CHASE Matrix Score/Sediments, contaminants data, 2019 141
Figure 3.27 - Frequency of occurrence of hazardous substances with contamination ratio (CR) > 1 in seawater samples, 2019 142
Figure 3.28 - Frequency of occurrence of hazardous substances with contamination ratio (CR) > 1 in sediment samples, 2019 142
Figure 4.1 - Major currents of the upper layer circulation and main shipping routes (thick lines) in the Black Sea (after Topçu et al., 2013) 146
Figure 4.2 - Composition percentages (%) of identified waste along the Romanian Coast during the period 2011-2014 147
Figure 4.3 - Trends in number of litter items surveyed on 8 monitoring samples distributed from Vama Veche until Vadu, during 2016-2019........ 148
Figure 4.4 - Litter categories percentage distribution surveyed on 8 monitoring samples distributed from Vama Veche until Vadu, during 2016-2019........ 148
Figure 4.5 - Top 10 litter items (and G1 to N: corresponding TGML master list categories) on Mamaia (up, January 2015) and Vama veche (down, June 2015) beaches (Romania) (Golumbeanu et al., 2017) 149
Figure 4.6 - Typology (%) of sea floor litter along the Romanian Coast during the period 2011-2014 (Galgani et al., 2016). 150
Figure 4.7 - Typology (%) of sea floor litter along the Romanian Coast in 2019 (Source NIMRD) 151
Figure 4.8 - Number of items (%) of sea floor litter along the Romanian Coast in 2019 (Source NIMRD) 151
Figure 4.9 - Positions of the observational transects. In red, the direction of the vessel and passage sections 155

Figure 4.10 - Spatial distribution of floating litter densities for the 15 transects....................... 156
Figure 4.11 - Spatial distribution of floating litter densities per categories 156
Figure 4.12 - Percentage contribution of the 3 size classes of floating litter 157
Figure 5.1 - Spatial assessment units (SAUs) for Ukraine, Romania, Bulgaria and Turkey 159
Figure 5.2 - Area proportion for each SAU 160
Figure 5.3-SAU area proportion by country 160

List of Tables

Table 1.1 Code, boundaries and area of Marine Reporting Units (MRU) assessed during the ANEMONE Joint cruise 16
Table 1.2 Thresholds value for each indicators / Marine Reporting Units (MRU) / Country 18
Table 1.3 Dominant species in the study area during the warm season, 2019 23
Table 1.4 - Evaluation of plankton community ecological status indicators, based on the agreed indicators (mg/m^3) 40
Table 1.5 - M. leidyi biomass - applicability according to criteria (EU/2017/848) 44
Table 1.6 - Average biomass indicator limits - M. leidyi species (threshold values) for GES definition 44
Table 1.7 - Updated classification system used for assessment the ecological status of Circalittoral and Offshore circalittoral shelly mud with pelophitic bivalves and polychaetes 51
Table 1.8 - Updated classification system used for assessment the ecological status of Offshore circalittoral mixed sediments with Modiolula phaseolina 51
Table 1.9 - Results of the assessment of ecological status of Circalittoral and Offshore circalittoral shelly mud with pelophitic bivalves and polychaetes based on M-AMBI*(n) index and EQR 53
Table 1.10 - Results of the assessment of ecological status of Offshore circalittoral mixed sediments with Modiolula phaseolina based on M-AMBI*(n) index and EQR 54
Table 1.11 - Proportion of adversely affected broad habitat types in the marine reporting units based on the results of EQRM-AMBI*(n) 55
Table 1.12 - Integral values estimated for the three species of cetaceans in the W Black Sea area .. 64
Table 1.13 - General characteristics of the data collected during the ANEMONE joint cruise survey . 67
Table 1.14 - Integral values estimated for the three species of cetaceans in RO Sector 68
Table 1.15 - Integral values estimated for the three species of cetaceans in BG-TR Sector 69
Table 2.1 – D5 – Eutrophication - Criteria, Indicators, Targets and Threshold values 75
Table 2.2 - Threshold values for Chlorophyll a (mg/m^3) for broad pelagic habitat shelf by countries (summer) 87
Table 2.3 - Characteristics of waters according to TRIX value 93
Table 2.4 - Category 1 – Nutrients levels - BEAST 95
Table 2.5 - Glossary of terms used in BEAST 95
Table 2.6 - BEAST status – ANEMONE Joint Cruise 96
Table 3.1 - Stations network (UA, RO, BG and TR) for contaminants investigation, 2019 101
Table 3.2 - Heavy metals concentrations in seawater samples from NW, W and SW Black Sea, 2019 103
Table 3.3 - Heavy metals concentrations in sediments samples from NW, W and SW Black Sea, 2019106
Table 3.4 - Grain size composition of TR sediment samples, 2019 106
Table 3.5 - Variation ranges of heavy metals concentrations in sediments in NW, W and SW Black Sea during 2013 and 2019 Joint Scientific Cruises 112
Table 3.6 - Heavy metals concentrations in Mytilus galloprovincialis samples from NW, W and SW Black Sea, 2019 112
Table 3.7 - Minimum, average, maximum concentrations PAHs and PAHs total, TEFs, Σ carcinogenic PAHs in surface water 116
Table 3.8 - Minimum, average, maximum concentrations PAHs and PAHs total, TEFs, Σ carcinogenic PAHs in sediments 118
Table 3.9 - Nature of surfactant pollution and probable sources of pollution in the Black Sea in each station 120
Table 3.10 - Concentrations of PAHs, PAHs total, TEFs, Σ carcinogenic PAHs in biota 122
Table 3.11 - Concentrations of organochlorine pesticides in surface waters, October 2019 125
Table 3.12 - Percentage of observations exceeding threshold values stipulated by Directive 2013/39/CE, in surface waters, 2019 127
Table 3.13 - Concentrations of organochlorine pesticides in surface sediments, October 2019 128
Table 3.14 - Percentage of observations surpassing sediment quality criteria (EAC, ERL), in surface sediments, 2019 129
Table 3.15 - Concentrations of organochlorine pesticides in Black Sea Mytilus galloprovincialis, October 2019 129
Table 3.16 - Concentrations of individual PCBs (µg/L) in water samples from the Ukraine, Romanian, Bulgarian and Turkish area 132
Table 3.17 - Concentrations of individual and total PCBs (ng/g dry sed.) in surface sediment samples 134
Table 3.18 - Concentrations of individual and total PCBs (ΣPCBs) (ng/g wet tissue) in biota samples, October 2019 136

Table 3.19 - CHASE Overall Score, and CHASE Score per matrix, contaminants data, 2019 139
Table 4.1 - Plastic waste littered in the Black Sea 145
Table 4.2 - Criteria, including criteria elements, and methodological standards according to MSFD 153
Table 4.3 - Summary of floating litter survey 155
Table 4.4 - Summary of bottom litter survey, 2019 157
Table 5.1 - Surface of Spatial assessment units (SAUs) for Ukraine, Romania, Bulgaria and Turkey .. 159
Table 5.2 - NEAT assessment results for all SAUs filtering by MSFD Descriptors; weighting by SAU surface 162

Abbreviations

BEAST	**B**lack Sea **E**utrophication **As**sessment **T**ool
BG	Bulgaria
BG-TR	Bulgaria-Turkey
BOD	Biological Oxygen Demand
BSC	Black Sea Commission
Chl a	Chlorophyll *a*
CIL	Cold Intermediate Layer
COD	Chemical Oxygen Demand
DCM	Deep Chlorophyll Maximum Layer
DIN	Dissolved Inorganic Nitrogen – sum of nitrite, nitrate, and ammonium
DIP	Dissolved Inorganic Phosphorus - phosphate
EcoQ	Ecosystem Quality Objective
E-TRIX	Trophic Index
EU	European Union
GES	Good Environmental Status
HS	Hot Spot
IA	Initial Assessment
JC	Joint Cruise
K-W	Statistical test Kruskal-Wallis
L	Left
LBS	Land Based Sources
MRU	Marine Reporting Unit
MSFD	Marine Strategy Framework Directive
N	Number of samples
N/P	Nitrogen – Phosphorus ratio
NIMH	Institute of Hydrology and Meteorology
NSI	National Statistical Institute
NW	Northwest
ODV	Ocean Data View
OOAO	One-Out-All-Out-principle: the worst assessment of a quality element determines the overall assessment result
R	Right
RO	Romania
SHL	Surface Homogenous Layer
Si/N	Silicon - Nitrogen ratio
Si/P	Silicon - Phosphorus ratio
SigT	Measure of the density of seawater at a given temperature
Std.Dev.	Standard Deviation
TNOx	Sum of Nitrite and Nitrate
TOC	Total Organic Carbon
TR	Turkey
UA	Ukraine
WFD	Water Framework Directive
WHO	World Health Organization
WQ	Water Quality
WW	Wastewater
WWTP	Wastewater Treatment Plant

Executive summary

The “State of Environment Report of the western Black Sea based on Joint ANEMONE cruise” has been prepared under the ANEMONE Project – “Assessing the vulnerability of the Black Sea marine ecosystem to human pressures“, coordinated by the National Institute for Marine Research and Development “Grigore Antipa” (NIMRD), in partnership with Mare Nostrum NGO, the Institute of Oceanology – Bulgarian Academy of Sciences (IO-BAS), the Scientific and Technological Research Council of Turkey/Marmara Research Center (TUBITAK-MAM), the Turkish Marine Research Foundation (TUDAV), Turkey, and the Ukrainian Scientific Center of Ecology of the Sea (UkrSCES).

ANEMONE project is an integral part of the overall ongoing process of harmonization of Black Sea region policy, in compliance to relevant European policy in the field of marine environment protection.

The time/duration of the cruise, polygons and parameters, methodology of data acquisition and processing and indicators for good environmental status assessment were selected in compliance and relevance of Black Sea Monitoring and Assessment Guideline (BSMAG), aligned to the findings and recommendations from the BSIMAP (Black Sea Integrated Monitoring and Assessment Programme).

A team of 17 marine scientists from 4 countries (Bulgaria, Romania, Turkey, and Ukraine) on board the research vessel Mare Nigrum carried out sampling of water, sediments, and marine organisms in order to assess the health of the marine environment, in offshore as well as deep sea locations, including oceanographic measurements and environmental sampling, within the Romanian, Bulgarian and Turkish national waters of Black Sea. The total track line for this survey was around 700 nautical miles.

Mare Nigrum is a multipurpose research vessel, which carries out a wide variety of survey operations in offshore as well as deep sea locations, acoustic and oceanographic surveys, buoy handling operations, environmental sampling, geological, geophysical, and hydrographic surveying. RV Mare Nigrum provided a range of additional mobile equipment for various tasks including a variety of grabs and corers, and additional sampling equipment.

The Joint cruise, conducted between 30 September and 7 October 2019, comprised 3 sampling area / polygons and 21 stations selected to cover shelf and open sea pelagic habitats and similar benthic habitats of each partner country. An impressive number of water and sediment physical, chemical (including pollutants) and biological samples (~1500), related to 120 parameters were measured during the cruise, of relevance for indicator-based assessment of the western Black Sea environmental status.

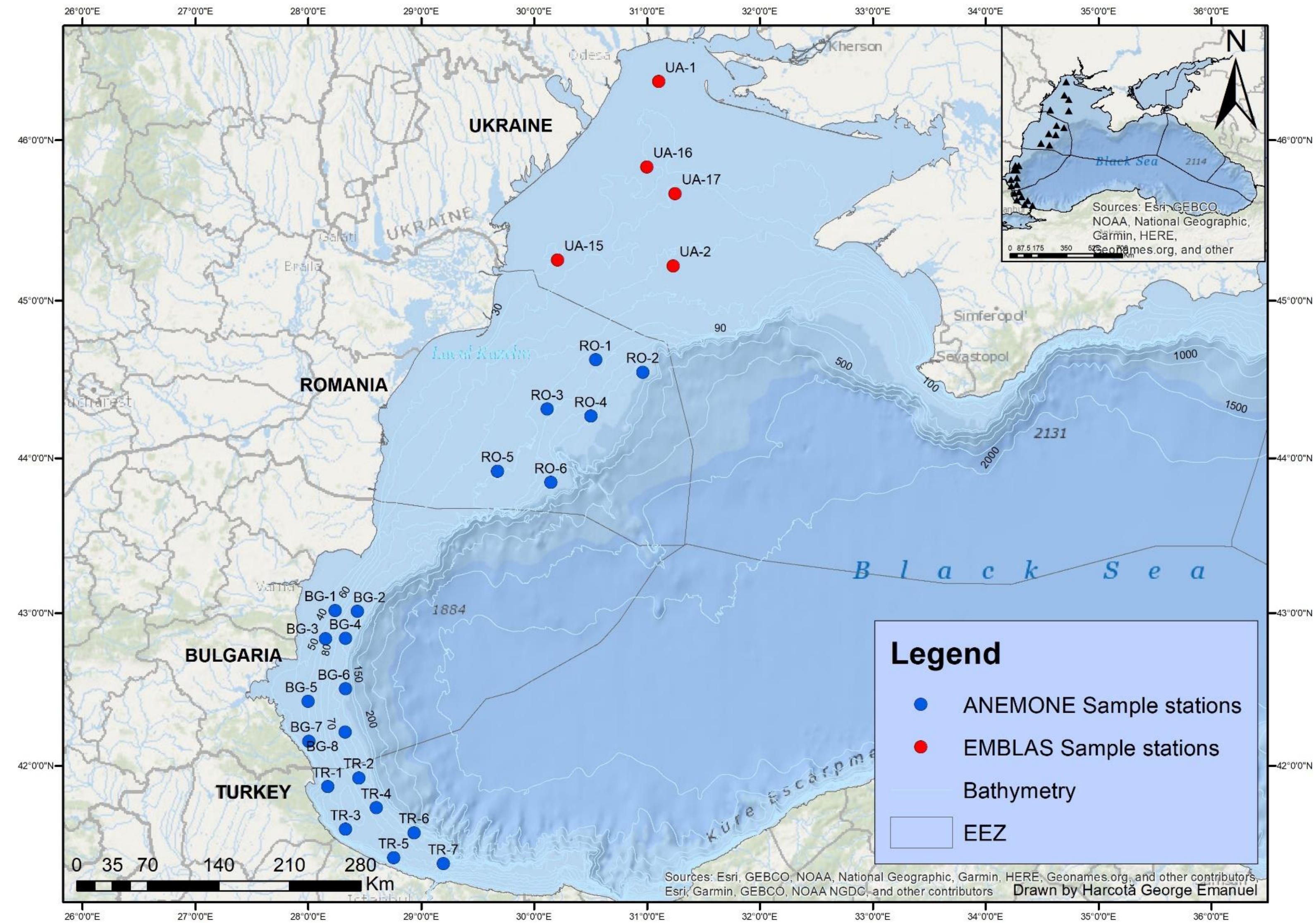
Samples were collected from the seabed of the Romanian, Bulgarian and Turkish Black Sea Shelf and extended measurements for dissolved oxygen, temperature, salinity, nutrients, pollutants, and biological parameter (chlorophyll, phytoplankton, ichthyoplankton, micro, meso and macrozooplankton) were performed in 21 stations. Additional results from the Ukrainian waters sampled during the EMBLAS project (“Improving Environmental Monitoring in the Black Sea”) during August-September 2019 were included in the report, as the permission to enter to Ukrainian waters was not given in due time.

List of samplings stations, coordinates and depths

Station name /code	Latitude (N)	Longitude (E)	Bottom depth (m)	Water sampling	Sediment sampling	Biota sampling	Marine Litter
UA-1	46.3596	31.1055	27.7	CTD, plankton nets	Van Veen grab		
UA-16	45.8325	31.0000	24.8	CTD, plankton nets	Van Veen grab		
UA-17	45.6667	31.2506	40.4	CTD, plankton nets	Van Veen grab		
UA-15	45.2533	30.2106	25	CTD, plankton nets	Van Veen grab		
UA-2	45.2159	31.2350	49.7	CTD, plankton nets	Van Veen grab		

RO-1	44.6253	30.5490	78	CTD, plankton nets	Van Veen grab	Dredge	Beam trawl
RO-2	44.5468	30.9641	106	CTD, plankton nets	Van Veen grab		
RO-3	44.3124	30.1173	76	CTD, plankton nets	Van Veen grab		
RO-4	44.2679	30.5072	103	CTD, plankton nets	Van Veen grab	Dredge	Beam trawl
RO-5	43.9162	29.6778	67	CTD, plankton nets	Van Veen grab	Dredge	Beam trawl
RO-6	43.8430	30.1526	103	CTD, plankton nets	Van Veen grab		
BG-1	43.0172	28.2403	50	CTD, plankton nets	Van Veen grab		Beam trawl
BG-2	43.0104	28.4359	86	CTD, plankton nets	Van Veen grab	Dredge	Beam trawl
BG-3	42.8336	28.1538	60	CTD, plankton nets	Van Veen grab	Dredge	Beam trawl
BG-4	42.8345	28.3323	91	CTD, plankton nets	Van Veen grab		
BG-5	42.4222	28.0001	49	CTD, plankton nets	Van Veen grab		
BG-6	42.5044	28.3329	98	CTD, plankton nets	Van Veen grab	Dredge	Beam trawl
BG-7	42.1601	28.0072	48	CTD, plankton nets	Van Veen grab	Dredge	Beam trawl
BG-8	42.2214	28.3296	105	CTD, plankton nets	Van Veen grab		
TR-1	41.8597	28.1753	75	CTD, plankton nets	Van Veen grab	Dredge	Beam trawl
TR-3	41.5778	28.3312	71	CTD, plankton nets	Van Veen grab		
TR-4	41.7198	28.6047	89	CTD, plankton nets	Van Veen grab	Dredge	Beam trawl
TR-5	41.3870	28.7584	77	CTD, plankton nets	Van Veen grab	Dredge	Beam trawl
TR-7	41.3479	29.1999	35	CTD, plankton nets	Van Veen grab	Dredge	Beam trawl
TR-6	41.5542	28.9395	88	CTD, plankton nets	Van Veen grab		
TR-2	41.9180	28.4486	90	CTD, plankton nets	Van Veen grab		

Special Chapter is dedicated to the holistic Nested Environmental status Assessment Tool (NEAT) which evaluates the ecological status based on five classes adopted from the assessment scheme of the Directive 2000/60/EC of the European Parliament and the Council, establishing a framework for the Community action in the field of water policy Water Framework Directive (WFD).
The SoE-ANEMONE is expected to contribute to the improvement of national monitoring programs in Bulgaria, Romania, Turkey and Ukraine in compliance to MSFD implementation, as well as assist the Black Sea Commission in the effort to develop integrated monitoring system for the Black Sea at basin-wide scale.



Map of study area

1 Biodiversity

"The quality and occurrence of habitats and the distribution and abundance of species are in line with prevailing physiographic, geographic and climatic conditions."

1.1 Pelagic Habitats

Biological diversity describes the variety of life, operates at various scales, from genes, species to entire ecosystems. Biodiversity, therefore, refers to all life-forms and their behaviors, the environments or habitats in which they live, and the complex system of relationships between organisms, such as food webs and competition for resources. A rich ecosystem has many available habitat niches, and many different organisms, which fill those niches. Such a system containing a wide variety of life-forms generally is more resilient to environmental change than one with either a more restricted range of species or where the species present have a narrower range of lifestyles. Oceans cover more than 70% of the earth's surface, and therefore marine biodiversity is an essential part of the global system.
The pelagic realm spans through the whole water column and it is the largest ecosystem on Earth (Kaiser et al., 2011). These habitats are dependent on the movements of water masses and the complex interactions between biological and physical processes. Plankton communities as phytoplankton and zooplankton constitute an important component of such habitats. Plankton species have fast turn-over rates and therefore respond quickly to changes in the environment. Moreover, plankton plays an important role in the functioning of marine ecosystems and in biogeochemical cycles because they are a key component of the trophodynamics of pelagic ecosystems. The communities' composition provides a good indication of the status of pelagic ecosystems, responding to a variety of pressures, in particular nutrient enrichment, NIS, alteration in hydrographical conditions and contaminants.
Commission Decision 2017/848/EU identified the following types of pelagic broad habitat types:

- Variable salinity
- Coastal
- Shelf
- Oceanic/beyond the shelf

Out of these four habitat types, the shelf waters are relevant to the ANEMONE Joint cruise. Shelf waters are marine systems away from coastal influence, down to the shelf break. They experience more stable temperature and salinity regimes than coastal systems, and their seabed is below wave disturbance (Table 1.1).

Table 1.1 Code, boundaries and area of Marine Reporting Units (MRU) assessed during the ANEMONE Joint cruise

Country Code	(Sub)Region	Description	MRU code	Depth range (m)	MRU total area (km^2)	MRU assessed area (km^2)	MRU assessed area (%)
UA		Coastal	ShW_UA_1	0-30	2511	2511	100
			ShW_UA_3		2423	2423	100
		Shelf	ShW_UA_5	30-200	3119	3119	100
			ShW_UA_7		7311	7311	100
RO	BLK	Shelf	BLK_RO_RG_MT01	30-200	20140	20140	100
BG	BLK	Shelf	BLK_BG_AA_ Shelf_South	30-200	5521	5521	100
TR		Shelf	TR_KARD1_BS1_ Shelf_West	40-200	4022	4022	100

Commission Decision 2017/848/EU sets one primary criterion (D1C6) for pelagic broad habitat types, which should be assessed in terms of "the extent of habitat adversely affected (km^2) as a proportion (%) of the total extent of the habitat type". For the criterion, the plankton composition, abundance and biomass measured in the Joint Cruise will be used to the extent possible to assess the structure and functions of pelagic broad habitat types.

ANEMONE Joint Cruise was carried out from 30 September to 7 October 2019 along 21 stations distributed over the shelf pelagic habitats of each country by Romanian (6 stations), Bulgarian (8 stations), Turkish (7 stations).
The Ukrainian team contributed to the SoE report adding data for phytoplankton and zooplankton communities derived from EMBLAS project ("Improving Environmental Monitoring in the Black Sea") during August-September 2019, along with five stations over coastal-shelf pelagic habitats.

1.1.1 Phytoplankton

Anthropogenic activities carried out around the Black Sea basin, and the influence of tributary rivers can enrich marine waters in nutrients and their eutrophication. Nutrient enrichment can lead to increased phytoplankton biomass, increased frequency and duration of microalgal blooms, and increased primary productivity. Measurements of phytoplankton biomass are included in the Black Sea countries monitoring program, quantitative estimates of phytoplankton, representing a good indicator of annual and seasonal variability of phytoplankton communities.
According to the Commission Decision 2017/848/EU, a primary criterion is D1C6 – the condition of the habitat type, including its biotic and abiotic structure and its functions (e.g. its typical species composition and their relative abundance, absence of particularly sensitive or fragile species or species providing a key function, size structure of species), is not adversely affected due to anthropogenic pressures. This criterion is assessed in terms of the extent of habitat adversely affected in square kilometers (km^2) as a proportion (percentage) of the habitat type's total extent. Based on the pelagic plankton communities, indicators were developed to evaluate the status of the Black Sea. Between these indicators, the phytoplankton biomass is one of the most common and agreed to be applied among Black Sea countries.

Material and methods

A total of 68 samples have been collected during the 30.09 – 07.10.2019 expedition along 21 stations distributed over the shelf pelagic habitats of each country by Romanian (23 samples), Bulgarian (26 samples), Turkish (19 samples) teams. The Ukrainian team added 18 samples collected during August-September 2019 along 5 stations over their pelagic shelf habitat.
Samples were collected by 5L Teflon Niskin bottles attached to CTD - SBE 25 - Rosette System equipped with in situ fluorometer (Chelsea Minitraca). The sampling depths were selected according to the CTD profile and the in situ fluorometer readings: surface, temperature/salinity gradient (thermocline), fluorescence max (deep-sea chlorophyll) and 1 m above the station depth.
The individual cell biovolume (V, μm^3) was derived by measurements by approximating the cell shape of each species to the most similar regular solid, calculated by the respective formulas used routinely in the respective lab. Cell bio-volume was converted to weight (W, ng) following Hatchinson (1967). The Romanian, Bulgarian, and Turkish biovolume calculation was according to MISIS project intercalibration exercise (Moncheva et al., 2014). The Ukrainian biovolume calculation was according to Zotov, 2018 and I.S. EN 16695:2015.
Species identification was mainly after Schiller (1937), Kisselew (1950), Proshkina-Lavrenko (1955), Carmelo (1997), Fukuyo (2000) and the taxonomic nomenclature according to the on-line database of World Register of Marine Species (WoRMS).
The most adequate indicator to assess the area's ecological status covered by the Joint Cruise was the phytoplankton biomass. Even though phytoplankton species diversity assessment needs further clarification, we agreed to apply the Menhinick and Shannon 95 indexes. For all these indexes it was calculated an average value for the Surface Homogenous Layer (SHL), e.g. integrated data from the surface to the thermocline or the deep chlorophyll *a* maximum (if available) to assess homogenous habitats.
For the **Ukrainian** shelf pelagic habitat, a linear model of transition from recent phytoplankton biomass values to historical values, according to the ratio of the averages of historical and last year phytoplankton biomass for northwestern Black Sea, was applied. Comparing the long-term data set was noted that phytoplankton biomass in 50-60th ranged from 0.7 to 1 g/m^3 at the sea's central parts. The average seasonal values of phytoplankton biomass in the last period are approximately equal and sometimes significantly lower than historical ones. Therefore, RefCon values were adopted as 75% of the average seasonal values of AcStat, which were then specified, considering the usual for the region values of phytoplankton biomass, which are observed in the absence of "blooms". Target concentrations were calculated as Target = RefCon +0.5*RefCon, with the rounding of the

corresponding values to tens and hundreds, depending on the value's order.
For the **Romanian** shelf pelagic habitat, the GES thresholds were set following the concept of 37% deviation from the baseline conditions where appropriate (BG IAR, 2013; RO IAR, 2013).
For the revision of the thresholds in **Bulgaria**, several statistical methods were used that have been applied in other marine regions (USEPA, 2001) based on the Sygnal detection theory (SDT) - ROC curves and combined methodology used by EPA (USEPA, 2001) – SDT, Regime Shift (Rodionov, 2005) and CUSUM (IBM SPSS Statistics) on data for the period 1961-2017 (Mavrodieva et al., 2017, Moncheva, Doncheva, 2017).
In **Turkey,** it was agreed to be used the thresholds for the pelagic shelf habitat from Bulgaria.
The Menhinick index, a mathematically simple index that expresses the diversity, was calculated from species richness and total abundance. The threshold was set based on the classification system as proposed by Spatharis, Tsirtsis (2010).
Shannon95 index is a derivative of the classical Shannon's diversity index (Shannon, 1948) and was computed using the Shannon index equation on the taxa's biomass data that together constitute 95% of the total recorded biomass. The threshold for GEnS was assumed at values > 2 (MARMONI Project Report, 2012).
The thresholds for the MSFD related indicators are presented in Table 1.2.

Table 1.2 Thresholds value for each indicators / Marine Reporting Units (MRU) / Country

Indicator	Ukraine (MRU)	GES	Romania (MRU)	GES	Bulgaria (MRU)	GES	Turkey (MRU)	GES
Biomass (mg/m^3)	ShW_UA_3	<1400	BLK_RO_RG_MT01 (30 - 200m)	<800	BLK-BG-AA-Shelf-South (30-200m)	<600	TR-KARD1 (40-200m) west BS1 shelf	<600
	ShW_UA_5	<800						
	ShW_UA_1	<1400						
	ShW_UA_7	<650						
Shannon '95	ShW_UA_3	>2	BLK_RO_RG_MT01 (30 - 200m)	>2	BLK-BG-AA-Shelf-South (30-200m)	>2	TR-KARD1 (40-200m) west BS1 shelf	>2
	ShW_UA_5							
	ShW_UA_1							
	ShW_UA_7							
Menhinick	ShW_UA_3	>0.12	BLK_RO_RG_MT01 (30 - 200m)	>0.12	BLK-BG-AA-Shelf-South (30-200m)	>0.12	TR-KARD1 (40-200m) west BS1 shelf	>0.12
	ShW_UA_5							
	ShW_UA_1							
	ShW_UA_7							

The extent of the habitat surface area is in square kilometres (km^2) and as a proportion (percentage) of the total extent of the MRU. It was accepted that a MRU achieved GES if 90% of the area (volume) is assessed in Good Environmental Status by each of the indicators.

Results

In the warm season of 2019, a total of 301 species, varieties and forms were identified in the study area from 20 taxonomic classes. The phytoplankton was composed of Dinoflagellates - 151 species (50 % of the total number of species), among which the genera Gymnodinium (20 species), Protoperidinium (20 species) and Gyrodinium (12 species) were the most diverse. Among diatoms (65 species), genera Chaetoceros (14 species) and Thalassiosira (7 species), along with genera Coscinodiscus, Thalassionema, Pseudo-nitzschia, and Nitzschia, showed the highest species richness. A relatively high number of species were identified for Chlorophyceae (15 species), Prymnesiophyceae (15 species), Cryptophyceae (15 species) and Cyanophyceae (8 species), while the classes Chrysophyceae (2), Dictyochophyceae (3) and Trebouxiophyceae (4) represented few species only (Figure 1.1).

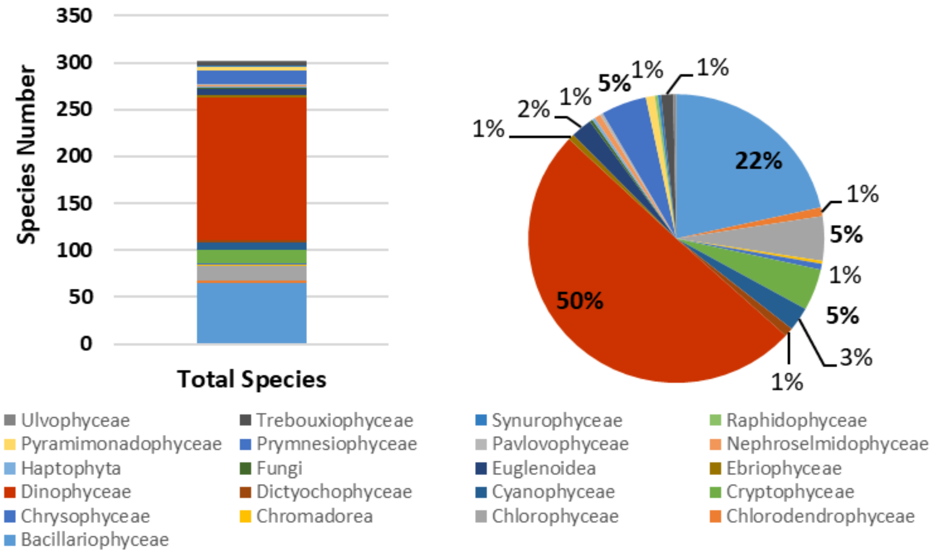


Figure 1.1 - Phytoplankton taxonomic composition during the warm season, 2019 (RO-BG-TR-UA transects)

The average abundance of phytoplankton for the entire area varied between 26.49·10^3 cells/L and 666.42·10^3 cells/L and the average biomass, between 89 mg/m^3 and 1966 mg/m^3 (Figure 1.2). Phytoplankton **average abundance** recorded in Bulgarian shelf waters (BG-7, 666.42·10^3 cells/L) was approx. three times higher than in Ukrainian shelf waters (UA-1, 201.42·10^3 cells/L), while in Romanian and Turkish shelf habitats, it was very low (RO-2, 82.28·10^3 cells/L, TR-6, 58.55·10^3 cells/L). In **average biomass**, the values recorded in Romanian waters (RO-2, 1966 mg/m^3) were more than two times higher than in Ukrainian waters (UA-4, 860 mg/m^3), while in Bulgarian and Turkish shelf habitats, the values were lower (BG-7, 388 mg/m^3, TR-5, 625 mg/m^3) (Figure 1.3).

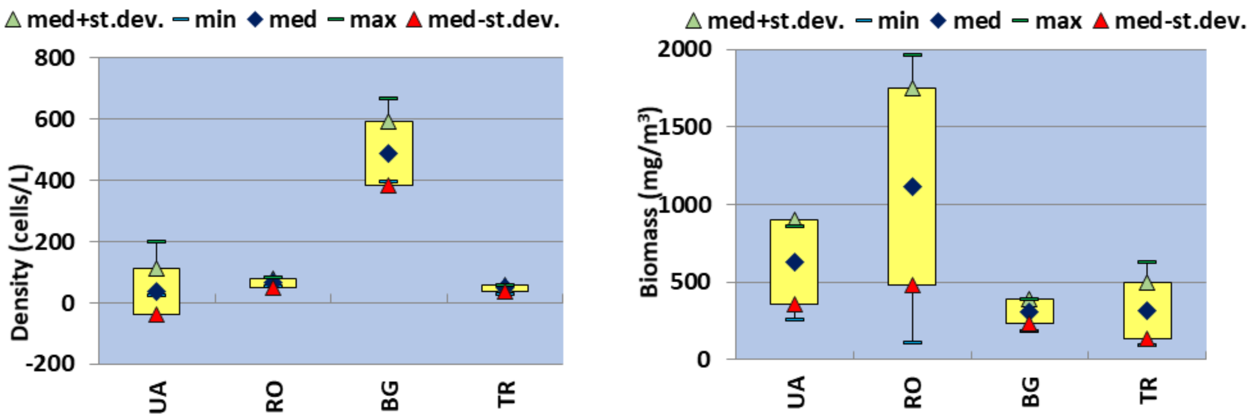


Figure 1.2 - Spatial variation of average abundance and biomass during the warm season, 2019

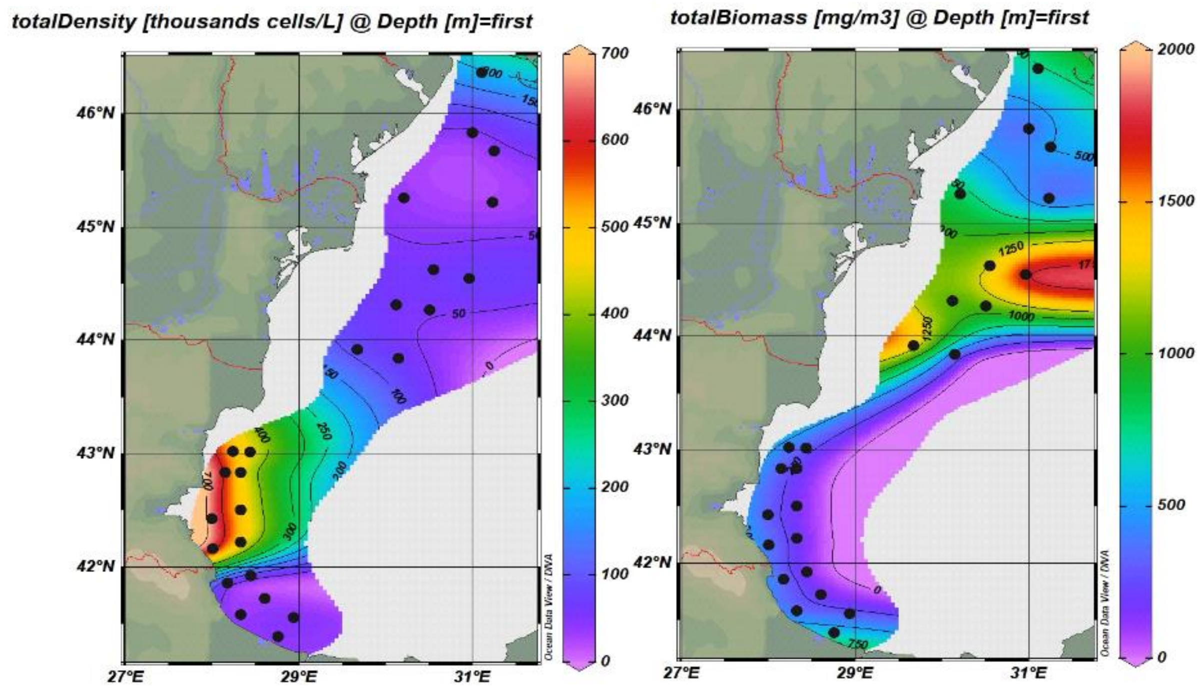


Figure 1.3 - Distribution of phytoplankton average abundance (10^3 cells/L, left) and biomass (mg/m^3, right) during the warm season, 2019

The phytoplankton abundance for the study area varied between 530 cells/L (TR-2, 85 m) and 978.48·10^3 cells/L (BG-8, 0 m) and the biomass, between 1.30 mg/m^3 (BG-8, 95 m) and 5193.16 mg/m^3 (RO-2, 29 m). The highest values of abundance and biomass were distributed in the surface layer down to the thermocline (0-30 m), where conditions were favourable for phytoplankton growth, while a pronounced decrease with depth was observed (Figure 1.4).

The values ranged between 5.4·10^3 cells/L and 462·10^3 cells/L in Ukrainian waters, respectively, 3 mg/m^3 and 2876 mg/m^3. *Emiliania huxleyi* (432·10^3 cells/L) accumulated in the deep chlorophyll maximum layer on UA-1 station (18 m depth). The highest biomasses occurred in the lower range of thermocline (27 m depth, 9.6°C), where the dinoflagellates of the genus Neoceratium reached 2600 mg/m^3.

In Romanian waters, the phytoplankton varied between 1.9·10^3 cells/L and 165·10^3 cells/L, respectively, 2 mg/m^3 and 5 200 mg/m^3. The highest development was registered in the thermocline layer (station RO-2), both in density and biomass. The phytoplankton community was dominated by the coccolithophore, *Emiliania huxleyi* (105·10^3 cells/L), the cryptophyte, *Hillea fusiformis* (18·10^3 cells/L) and the dinoflagellates, *Tripos fusus* (57·10^3 cells/L) and *Mesoporos perforatus* (19·10^3 cells/L). In biomass, the dinoflagellates, *Tripos fusus* and *T. muelleri* represented the majority with 3240 mg/m^3, respectively 1521 mg/m^3.

In Bulgarian waters, the values ranged between 8.5·10^3 cells/L and 978·10^3 cells/L, respectively, 1668 mg/m^3 and 668 mg/m^3. In terms of density, a relatively homogenous structure of the surface, thermocline, down to the near-bottom layers characterized stations 1, 3, 5 and 7, mainly with the dominance of the coccolithophore, *Emiliania huxleyi*, the diatom, *Cyclotella caspia*, cryptophytes (microflagellates, *Hemiselmis* sp., *Hillea fusiformis*) and chlorophytes (*Pyramimonas* sp. and *Nephroselmis astigmatica*). The stations with greater depths (2, 4, 6 and 8) presented a clear difference with higher values in the thermocline and above it and reduced ones below it. The highest abundance was accumulated in the surface layer, on BG-8 where the community was mainly formed by *Emiliania huxleyi* (327·10^3 cells/L), microflagellates (188·10^3 cells/L), *Pyramimonas* sp. (88·10^3 cells/L), *Nephroselmis astigmatica* (50·10^3 cells/L) and *Hillea fusiformis* (38·10^3 cells/L). The phytoplankton accumulated in the surface and thermocline layers and sharply decreased to depths in terms of biomass. The highest biomass showed on station BG-2, in the thermocline layer. The community was mainly dominated by the dinoflagellate, *Tripos fusus* (383 mg/m^3) along with the diatoms, *Proboscia alata* (66 mg/m^3) and *Pleurosigma elongatum* (24 mg/m^3) and the coccolithophore, *Emiliania huxleyi* (46 mg/m^3).

In Turkish waters, the phytoplankton varied between 530 cells/L and 130·10^3 cells/L, respectively, 12 mg/m^3 and 2205 mg/m^3. The phytoplankton reached the maximum development in the surface layer, both in density and biomass. The maximum density was recorded on TR-4, mostly by *Emiliania huxleyi* ($67 \cdot 10^3$ cells/L), along with the diatoms *Proboscia alata* ($43 \cdot 10^3$ cells/L) and *Cylindrotheca closterium* ($14 \cdot 10^3$ cells/L). The values decreased through the thermocline and near-bottom layers. In term of biomass, the diatom, *Pseudosolenia calcar-avis* (2 070 mg/m^3), reached its maximum on TR-5.

Phytoplankton communities' taxonomic structure along-shelf habitat was featured by the dominance of species from "other groups" (as Prymnesiophyceae, Cryptophyceae, Prasinophyceae) in abundance (contributing up to 88 %). At the same time, for the **biomass,** dinoflagellates represented the bulk of the assembly (~99 %), diatoms ranked second (~98 %) and "other groups" accounting for ~25 %.

The average abundance was between $26.50 \cdot 10^3$ cells/L and $201.42 \cdot 10^3$ cells/L in the Ukrainian shelf, the minimum in UA-5 and the maximum in UA-1. Up to 88 % of the total was constituted by species from "other groups", while diatoms and dinoflagellates represented only 12 %, respectively, 38 % (Figure 1.5). The average biomass in the Ukrainian shelf varied between 254 mg/m^3 (UA-2) - 860 mg/m^3 (UA-4). Both diatoms and dinoflagellates had significant contributions (up to 98 %) due to large species' development, such as *Pseudosolenia calcar-avis* and *Neoceratium* sp., *Tripos muelleri*, *T. furca*, *Dinophysis acuta*.

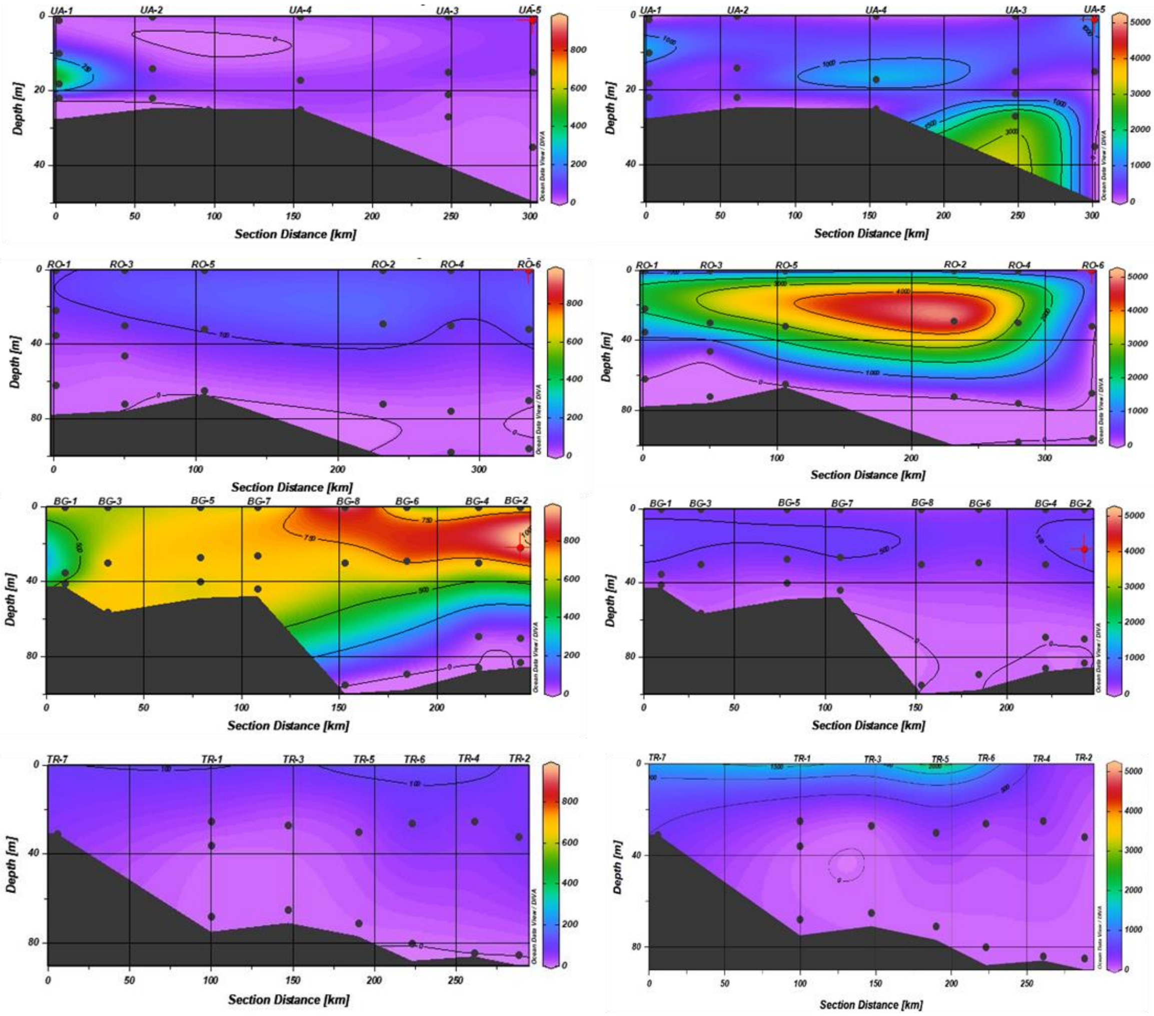


Figure 1.4 - Vertical distribution of phytoplankton abundance (10^3 cells/L, left) and biomass (mg/m^3, right) during the warm season, 2019

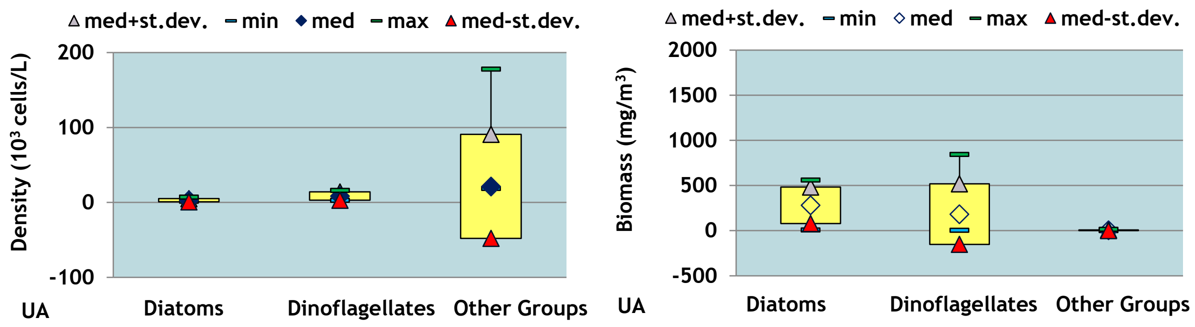


Figure 1.5 - Phytoplankton taxonomic structure on the Ukrainian shelf based on average abundance and biomass during the warm season, 2019

Along the Romanian shelf (Figure 1.6) similar pattern was observed with species from “other groups” contributing to ~82 % in abundance and with the dominance of dinoflagellates (up to 99 %) in biomass. Although the total average density (52.38-82.30·10^3 cells/L) was lower than in the Ukrainian shelf, the average biomass was up 1965.82 mg/m^3 on RO-2, due to a higher development of large size dinoflagellates from the genus Tripos.

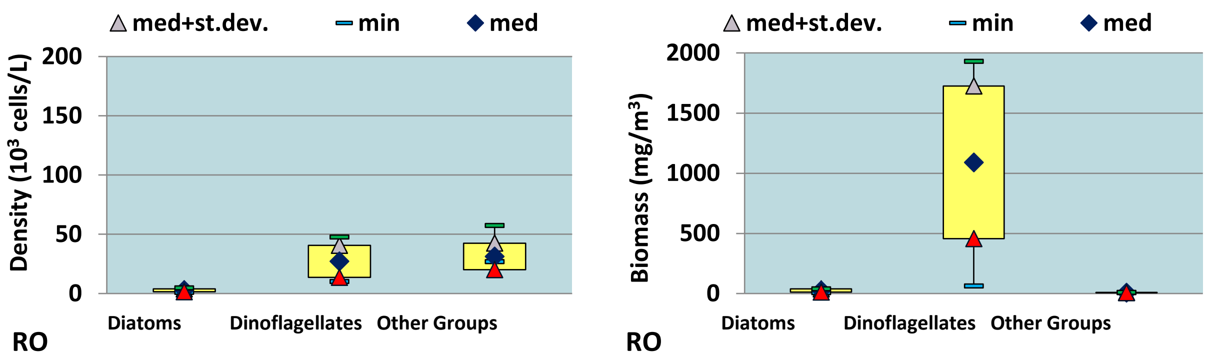


Figure 1.6 - Phytoplankton taxonomic structure on the Romanian shelf based on average abundance and biomass during the warm season, 2019

In the **Bulgarian** shelf, the average abundance reached the highest values, 396.80·10^3 (BG-4) - 666.42·10^3 cells/L (BG-7), and followed the same pattern with up to 78 % being represented by species from “other groups” (Figure 1.7). The average biomass was lower than in Romanian and Ukrainian shelves, with values between 180.83 mg/m^3 (BG-6) - 387.64 mg/m^3 (BG-7). The dinoflagellates accounted for a maximum of 71 % of the total average biomass on BG-2 (230.47 mg/m^3).

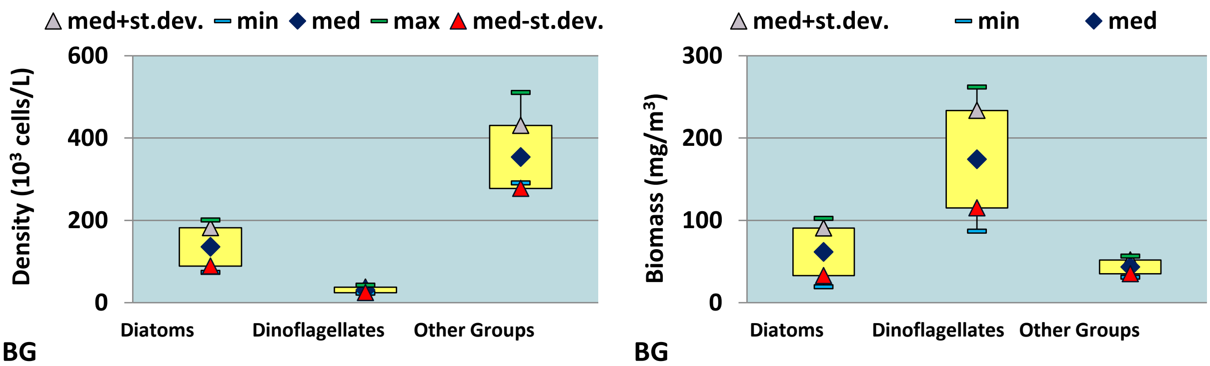


Figure 1.7 - Phytoplankton taxonomic structure along the Bulgarian southern shelf based on average abundance and biomass during the warm season, 2019

The average abundance reached values slightly lower in the Turkish shelf than in the Romanian shelf, between 28.58·10^3 (TR-3) – 58.55·10^3 cells/L (TR-6). It was followed the pattern with the dominance of species from other groups in abundance (up to 84 %), but the diatoms had a higher contribution (up to 63 %) than in the other shelves. In average biomass, the diatoms contributed with over 60% on all the stations, dinoflagellates ranked second with a maximum of 34 % of the total (Figure 1.8).

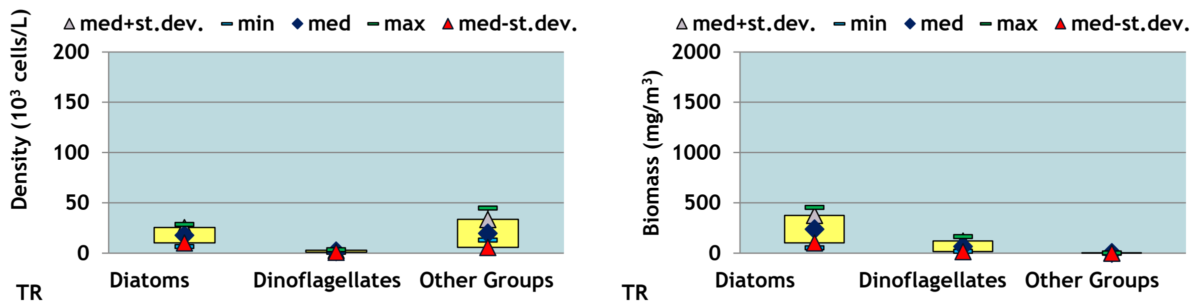


Figure 1.8 - Phytoplankton taxonomic structure on the Turkish area based on average abundance and biomass during the warm season, 2019

In the Ukrainian shelf waters, *Emiliania huxleyi* dominated in abundance, accounting for about 70 % of the total, while in biomass, three species represented together 85 % of the total (*Pseudosolenia calcar-avis, Neoceratium* sp. and *Tripos muelleri*).
E. huxleyi maintained its dominance in **abundance** in the other shelves, but in a lower proportion (41 % in RO, 21 % in BG and 48 % in TR). On the Romanian shelf, it was accompanied by other species such as *Tripos fusus* (12.8 %), *Hillea fusiformis* (8.65 %) and *Gymnodinium* sp. (3.31 %), while in the Bulgarian shelf, the microflagellates, *Cyclotella caspia, Pyramimonas* sp. and *Nephroselmis astigmatica* reached together a proportion of 45 % from the total. In the Turkish shelf waters, besides *E. huxleyi*, species such as *Cylindrotheca closterium, P. calcar-avis, Proboscia alata* dominated in a proportion of 42 % of the total (Table 1.3).
P. calcar-avis dominated in **biomass** along Ukrainian and Turkish shelves in a proportion of 47 %, respectively, 72 % of the total, while in Romanian and Bulgarian shelves, *T. fusus* reached the highest contribution (52 %, respectively, 33 % of the total). Other dominant species in biomass were: *Neoceratium* sp. (25 % in UA), *T. muelleri* (12 % in UA, 32 % in RO), *P. alata* (9 % in BG and 7 % in TR), *E. huxleyi* (5 % in BG), *T. furca* (10 % in TR) and *Prorocentrum micans* (4 % in BG and 2 % in TR).
No phytoplankton blooms were observed in the study period, the highest density (978.48·10^3 cells/L in BG-8, surface layer) being below 1 million cells/L. Even though they did not reach an abundance higher than 57·10^3 cells/L, large-sized species such as *T. fusus*, *T. muelleri*, *Neoceratium* sp. and *P. calcar-avis* achieved high biomass values (2009-3239 mg/m^3) in Ukrainian, Romanian and Turkish shelf waters.

Table 1.3 Dominant species in the study area during the warm season, 2019

	Ukraine	Romania	Bulgaria	Turkey
In abundance	*Emiliania huxleyi*	*Emiliania huxleyi* *Tripos fusus* *Hillea fusiformis* *Gymnodinium* sp.	*Emiliania huxleyi* Microflagellates *Cyclotella caspia* *Pyramimonas* sp. *Nephroselmis astigmatica*	*Emiliania huxleyi* *Cylindrotheca closterium* *Pseudosolenia calcar-avis* *Proboscia alata*
In biomass	*Pseudosolenia calcar-avis* *Neoceratium* sp. *Tripos muelleri*	*Tripos fusus* *Tripos muelleri*	*Tripos fusus* *Proboscia alata* *Emiliania huxleyi* *Prorocentrum micans*	*Pseudosolenia calcar-avis* *Tripos furca* *Proboscia alata* *Prorocentrum micans*

The result of GES assessment based on the SHL phytoplankton biomass data shows that in UA the two out of the 4 identified MRU achieved GES, in BG 100 % of the MRU achieved GES while in TR and RO, the MRU's under consideration did not achieve GES as < 90 % of the MRU surface area/volume was in GES (Figure 1.9).

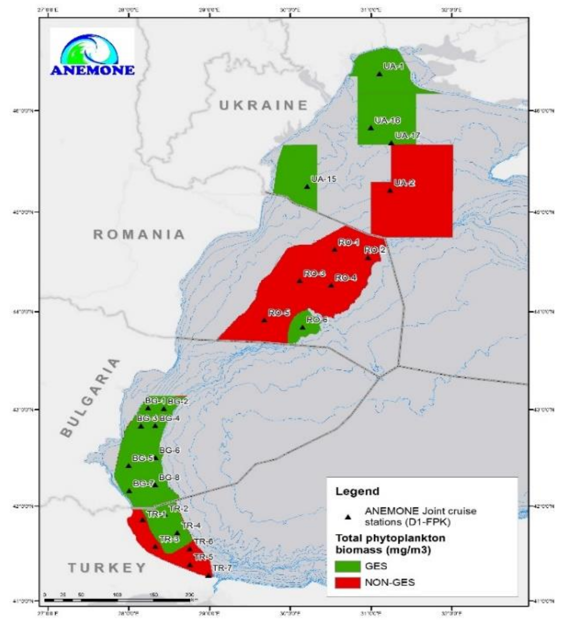


Figure 1.9 - Map of the GES/nonGES area's spatial extent in the pelagic shelf habitats (UA, RO, BG, TR) based on SHL phytoplankton biomass in the warm season of 2019

The result of GES assessment based on the SHL Shannon95 index shows that only in BG the MRU achieved GES while the other MRU’s identified in RO, UA and TR did not achieve GES as < 90% of the MRU surface area/volume was in GES (Figure 1.10).

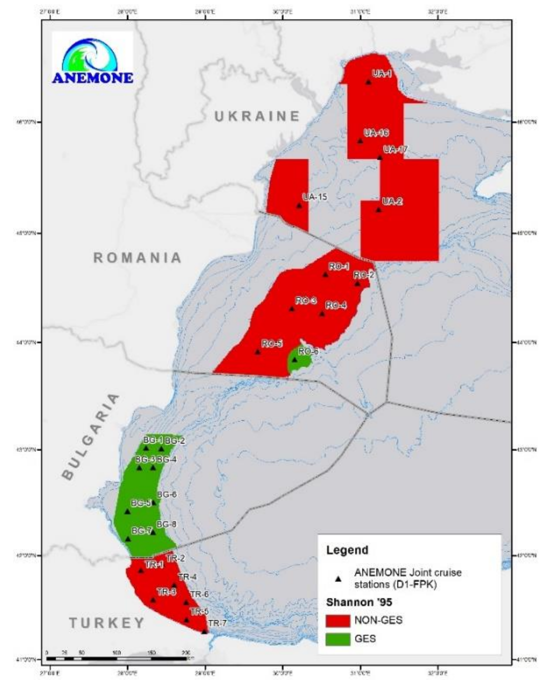


Figure 1.10 - Map of the GES/nonGES area's spatial extent in the pelagic shelf habitats (UA, RO, BG, TR) based on SHL Shannon 95 index in the warm season of 2019

The result of the GES assessment based on the SHL Menhinick index shows that in RO the MRU achieved GES. Also, in UA, one out of four identified MRU’s achieved GES while in BG and TR the MRU’s did not achieve GES as < 90 % of the MRU’s surface area/volume was in GES (Figure 1.11).

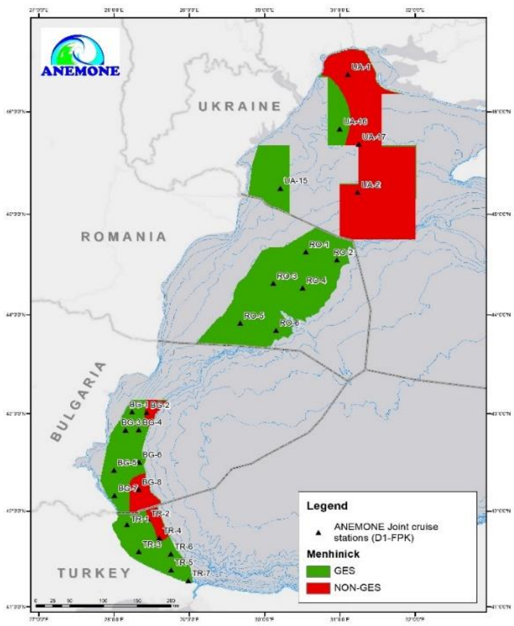


Figure 1.11 - Map of the GES/nonGES area's spatial extent in the pelagic shelf habitats (UA, RO, BG, TR) based on SHL Menhinick index in the warm season of 2019

Conclusions

The most important classes representing the phytoplankton community in the warm season of 2019 were Dinophyceae and Bacillariophyceae, accounting for 72 % of the total number of identified species (301 species).

The phytoplankton abundance for the study area varied between 530 cells/L (TR-2, 85 m) and $978.48 \cdot 10^3$ cells/L (BG-8, 0 m) and the biomass, between 1.30 mg/m^3 (BG-8, 95 m) and 5193.16 mg/m^3 (RO-2, 29 m).

The highest values of abundance and biomass were distributed in the surface layer down to the thermocline (0-30 m), where conditions were favourable for phytoplankton growth, while a pronounced decrease with depth was observed.

In terms of density, the phytoplankton community was dominated by the coccolithophorid, *Emiliania huxleyi*, along the entire study area. Other species such as *Tripos fusus, Hillea fusiformis, Gymnodinium* sp. (in Romania), Microflagellates, *Cyclotella caspia, Pyramimonas* sp., *Nephroselmis astigmatica* (in Bulgaria), *Cylindrotheca closterium, Pseudosolenia calcar-avis, Proboscia alata* (in Turkey) were observed. In terms of biomass, *Pseudosolenia calcar-avis, Prorocentrum micans, Proboscia alata,* and *Tripos genus* species dominated the phytoplankton community.

The assessment of GES was made based on SHL phytoplankton biomass, Shannon95 index and Menhinick index, based on the concept that a MRU has achieved GES if 90 % of its area is assessed in GES. In Ukraine, only the phytoplankton biomass indicator showed that two out of four MRU's achieved GES and the Menhinick index showed that one MRU achieved GES. In Romanian shelf waters, GES was achieved only based on the Menhinick index results. The assessment of Bulgarian shelf waters by applying all three indicators showed that the MRU achieved GES in two situations. In Turkey, none of the indicators' results showed a good environmental status of the MRU assessed.

1.1.2Microzooplankton

Material and methods

To analyse the microzooplankton component, particularly the loricate ciliate community, the samples were taken from the 0 m and deep chlorophyll maximum (DCM) layers of the Black Sea shelf zone of Romania, Bulgaria, and Turkey. Samples were collected in 500 ml labelled plastic containers from Niskin bottles and preserved with formalin 4%. In the laboratory, the samples were concentrated to a final volume of 10 ml by repeated sedimentation. The final volume was analysed by the inverted microscope (Olympus XI 51) with magnification factors of 200x and 400x. The taxonomic identification of tintinnids was made according to the lorica's shape and dimensions, indicated by literature (Petran, 1958 (b), Abboud-Abi Saab, 2008). For qualitative and quantitative analysis, both empty tintinnids and those with protoplasm were considered because mechanical and chemical disturbances associated with collection and fixation procedures have been demonstrated to cause cell detachment (Thompson & Alder, 2005). The density of organisms was expressed as individual species/litre (ind/L). The lorica volume was calculated according to the lorica's total length and aboral diameter and to the geometric form assumed for each species, respectively. Biomass was expressed as carbon biomass (µgC/L) using the specific biovolume conversion formula for formalin conserved biological material (Verity & Langdon, 1984).

The species richness (R), Shannon-Wiener index (H) and Simpson Index (D) indices of diversity were computed to investigate the various aspects of microzooplankton biodiversity and assemblages. Non-metric multidimensional scaling (NMDS) and Bray-Curtis similarity were also used. Microsoft Excel and PRIMER 7 were used to calculate and interpret the data.

Results

Following the samples' analysis, a total number of 10 species of tintinnids belonging to five families were identified along with the Romanian, Bulgarian and Turkish Black Sea shelf area (Annex).

The lowest biodiversity was recorded in the Turkish shelf area (5 species), while the Bulgarian area recorded the highest diversity (8 species) fact, which is also confirmed by the results of Shannon-Wiener (1.06 and 1.32) and Simpson (2.46 and 2.84) indexes.

From all ten species identified in the western part of the Black Sea, *Tintinnopsis minuta* and *Metacylis mediterranea* were present only in the Romanian site, while the species *Tintinnopsis campanula* and *T. cylindrica* were present only in the Bulgarian site. The species common to the three investigated sites (Romania, Bulgaria, and Turkey) are *Amphorellopsis acuta*, *Eutintinnus tubulosus* and *Salpingella decurtata*.

Regarding the vertical distribution of species richness, it can be noted that the biodiversity decreases with depth in the Romanian and Bulgarian sites while in the Turkey site, the biodiversity increases with depth (Figure 1.12). Vertical differentiation was made by the species *Tintinnopsis minuta*, *Eutintinnus tubulosus* and *Salpingella decurtata* in surface layer (0M) of Romanian site, by *Tintinnopsis cylindrica* and *Eutintinnus tubulosus* in surface layer of Bulgarian site and by *Rhizodomus tagatzi* present in DCM layer of Turkish site.

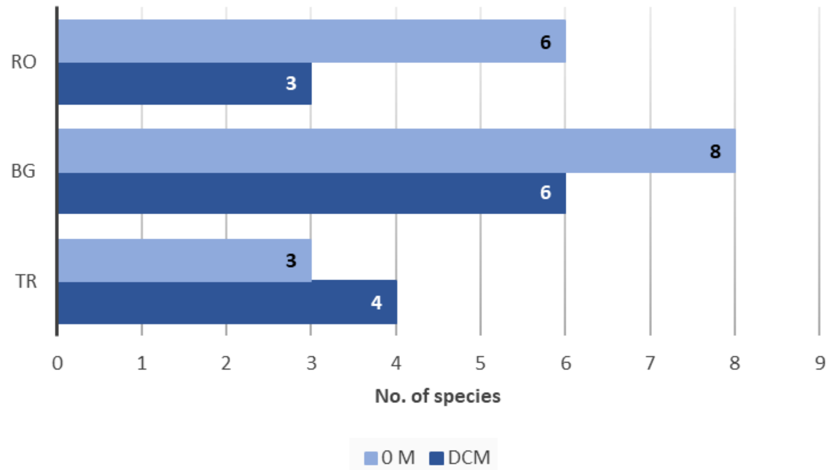


Figure 1.12 - Vertical biodiversity of tintinnids species along the western part of the Black Sea

The microzooplankton community has been enriched in the last two decades with newly introduced species in the Black Sea basin (Gavrilova, 2001, Gavrilova & Dovgal, 2016, Selifonova & Makarevich, 2018, Boicenco et al., 2019). Being cosmopolitan species, they have acclimatized to the new conditions so that they are currently part of the microzooplankton community in the Black Sea basin. This fact is also reflected by the present study in which both indigenous and non-indigenous species were identified. Their number registered variations in the western Black Sea both from one site to another and from one horizon to another (Figure 1.13). The higher diversity and abundance of non-indigenous species were observed in the stations with small depth, more common in the Bulgarian and Turkish sites.

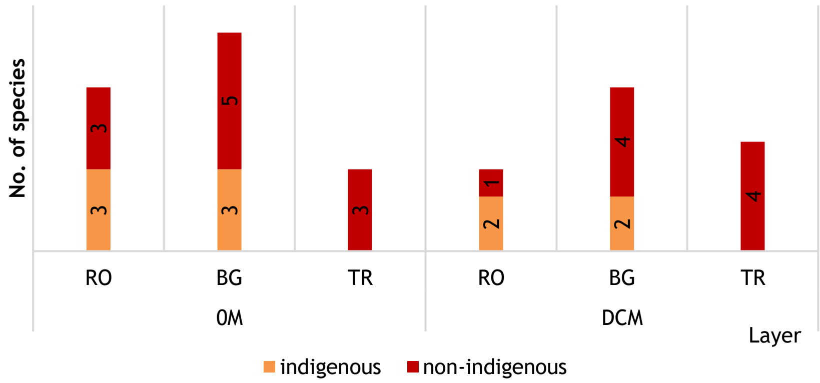


Figure 1.13 - Biodiversity of tintinnids community along the western part of Black Sea

The Romanian site was characterised by the mean density (58 ind/L) and biomass (0.41 µgC/L) of the microzooplankton component. Analysing the biotic and environmental data, a correlation of low abundances with higher salinities (17.9-18.4 PSU) was highlighted.
The highest density of tintinnids was recorded in the RO-5 station where the species *Amphorellopsis acuta* dominates. Most likely, the highest diversity in this station is due to the smaller depth respectively to the salinity which is under 18 PSU. In RO-4 and RO-6 stations the lowest abundances were recorded, situation explained by the fact that the stations depths are over 100 m and salinities between 8.2-8.4 PSU.

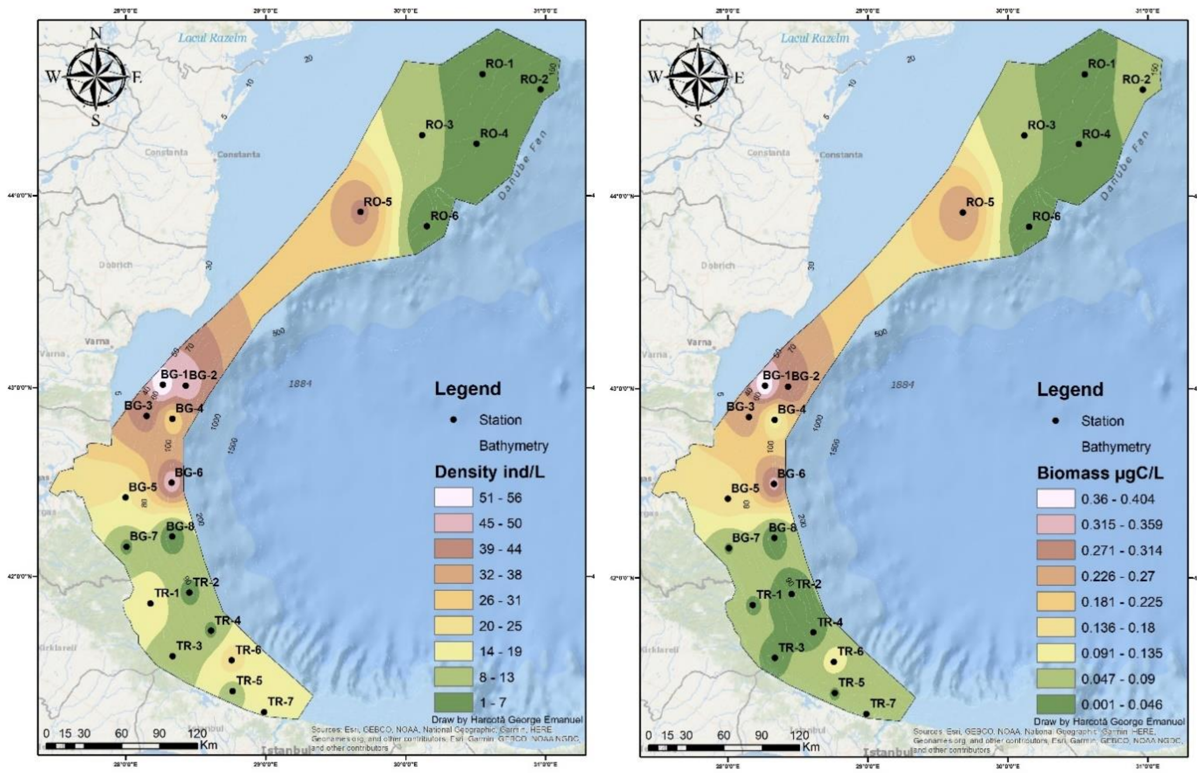


Figure 1.14 - Community structure of tintinnids along western area of Black Sea - density (left) and biomass (right)

The Bulgarian shelf area was characterized by the mean density - 245 ind/L and biomass - 1.61 µgC/L of the tintinnids community. These values can be explained by the fact that a larger number of stations were located at small depths but also probably due to lower salinities (8.2 – 17.5 PSU) recorded on this site.
The dominant species in both layers are *Amphorellopsis acuta* followed by *Salpingella decurtata*. *A.*

acuta recorded mean density and biomass values of 119 ind/L and 0.98 µgC/L while S. *decurtata* recorded mean density and biomass values of 84 ind/L and 0.05 µgC/L (Figure 1.13, Figure 1.14).
The station with the highest density (56 ind/L) and biomass (0.41 µgC/L) of tintinnids was BG-1. BG-1 is the station with the smallest depth (43 m).
In the Turkish site, the mean density of the tintinnids community was 88 ind/L and the mean biomass was 0.32 µgC/L. In terms of density, the dominant species in both surface layer and DCM is *Salpingella decurtate*, the estimated values being 51 ind/L. The highest biomass was recorded by *Amphorellopsis acuta*, mean biomass values being 0.25 µgC/L. This situation is due to the larger biovolume of the *A. acuta* species. The highest density of tintinnids was recorded in the TR-6 station (24 ind/L).
Analysing the quantitative distribution of microzooplankton from the entire investigated area, it is observed that the highest tintinnids mean density was registered in the Bulgaria site, followed by the Turkish and Romanian sites (Figure 1.14). Differences were also highlighted in terms of quantity between the two layers analysed (0 m and DCM) (Figure 1.15, Figure 1.16), in the sense that in the 0 m layer were recorded higher density values than in the DCM layer.

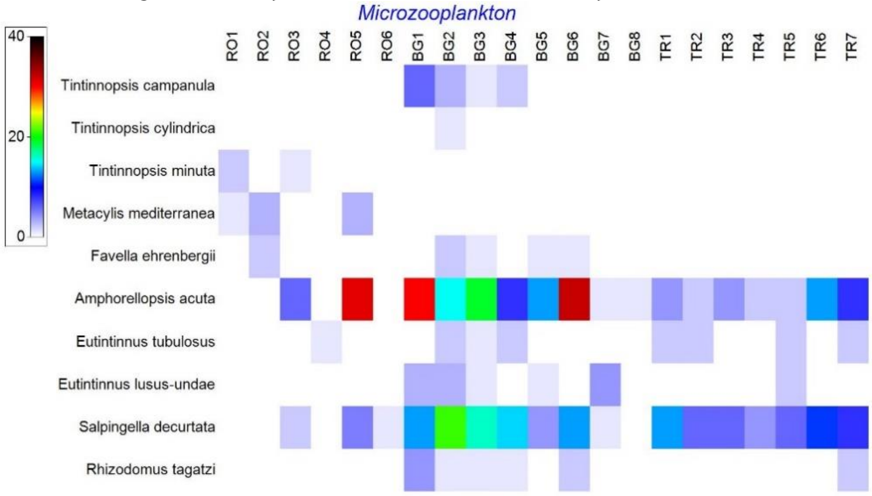


Figure 1.15 - Density (ind/L) of tintinnids from all three investigated sites (Romania, Bulgaria, and Turkey) of western Black Sea (for integrated layers)

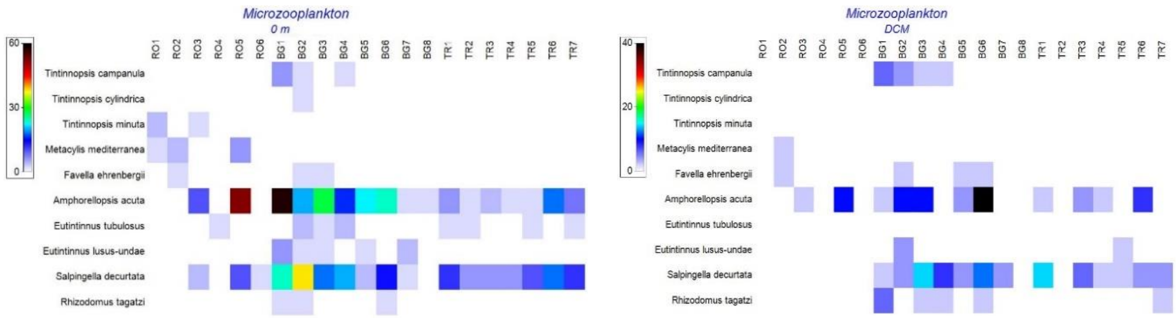


Figure 1.16 - Density (ind/L) of tintinnids from the three investigated sites (Romania, Bulgaria, and Turkey) of the western Black Sea in the surface layer (left) and DCM layer (right)

Bray-Curtis similarity and non-Metric multidimensional scaling (nMDS) were used to visualize the similarity level of the stations from the whole area investigated. Both analyses indicate high variability in the Figure 1.17Figure 1.18). This may be due in the first instance because of the environmental conditions in this area of the Black Sea (greater distance from shore, greater depths on stations, higher salinity). The highest similarity between the stations is found in the Turkish site where the environmental conditions were more uniform.

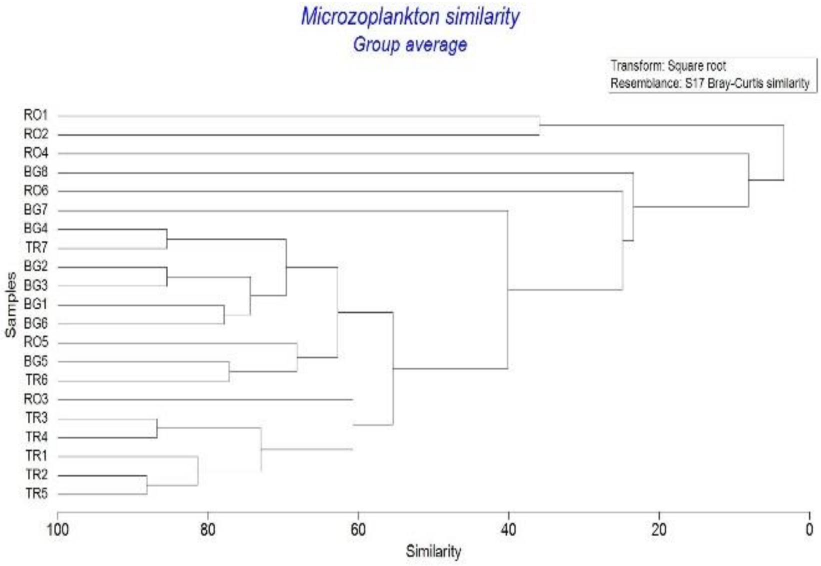


Figure 1.17 - Bray-Curtis similarity of microzooplankton distribution according to the diversity and density of the species from each station

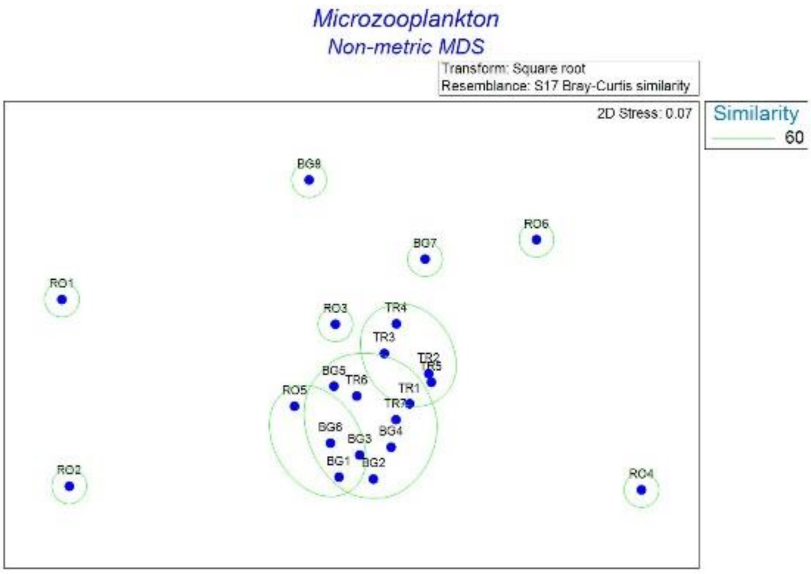


Figure 1.18 - nMDS analysed of microzooplankton in the western part of Black Sea

The presence of 5 species of tintinnids newly introduced in the Black Sea basin (*Amphorellopsis acuta*, *Eutintinnus tubulosus*, *E. lusus-undae*, *Salpingella decurtate* and *Rhizodomus tagatzi*) but also the tendency to enrich the microzooplankton component from the last decades with new non-indigene species (Gavrilova & Dolan, 2007, Gavrilova & Dovgal, 2016, Gavrilova, 2017, Selifonova & Makarevich, 2018), can make this component an indicator of the assessment of the marine environmental status, that corresponds to the descriptor D2 (D2C1 criteria).

Conclusions

Qualitatively, in the western area of the Black Sea were identified 10 species of tintinnids. Their distribution varied in wide limits both from one site to another and on stations and horizons. The only species common to the three sites were *Amphorellopsis acuta*, *Eutintinnus tubulosus* and *Salpingella decurtata*. It is worth mentioning that in the Turkish area, the tintinnids community was made up exclusively of non-indigenous species. In comparison, the Romanian and Bulgarian tintinnids community included both indigenous and non-indigenous species in various proportions with the mention that they were inferior in quantity.

Quantitatively, big differences were noticed between the three analysed sites. Thus, in the Bulgarian

site, the abundance values were 3 and 4 times higher, respectively, than those registered in the Turkish and Romanian sites. This situation can be correlated with the lower salinities in the Bulgarian site and with the lower depth of the stations in this area.
The dominant species were *Amphorellopsis acuta* in the Romanian and Bulgarian sites and *Salpingella decurtata* in the Turkey site, respectively. The dominance of these species is following their ecology, reaching the maximum abundance in the summer-autumn season (Selifonova & Makarevich, 2018, Monti-Birkenmeier et al., 2019).

1.1.3 Mesozooplankton

Zooplankton species are one of the most important biotic components influencing all the functional aspects of an aquatic ecosystem, such as food chains, food webs, energy flow and cycling of matter. They play an important role in the conservation of energy from the primary to secondary level. The biomass abundance and species diversity of zooplankton are used to determine the conditions of the aquatic environment (Sharmila et al., 2017).
There is a considerable scientific and practical interest in understanding how the biological components of marine systems respond to both single and multiple stressors. The response of zooplankton to environmental conditions is of particular interest due to the central and mediating role that this group occupies as a trophic link between planktonic primary producers and larger consumers. Consequently, any variation in zooplanktonic biomass and species composition has implications on biogeochemical cycling, trophic dynamics, fisheries and other ecosystem services (Varkitzi et al., 2018).

Material and methods

A total of 62 mesozooplankton samples were collected along the Romanian, Bulgarian and Turkish Black Sea coasts from discrete layers, depending on water stratification and thermocline depth, during ANEMONE JOINT CRUISE onboard of RV Mare Nigrum in October 2019 (Figure 1.19). A sampling of mesozooplankton in front of Ukraine waters was carried out at 5 stations during the EMBLAS project. A number of 11 mesozooplankton samples were collected.
Sampling was performed using a Juday net (0.1 m^2 mouth opening area, 150 µm mesh size) by vertical hauls. The samples were stored in 500 ml plastic jars and preserved with 4% buffered formaldehyde solution for further identification and enumeration of plankton species.
In the laboratories the samples were concentrated to 100-150 cm^3, homogenised, quantitative and qualitative processing was performed in the Bogorov chamber. In the subsample(s) all plankters were counted until each of the three dominant taxonomic groups reached 100 individuals. For estimation of large animals' numbers, the whole sample was observed. All species were identified taxonomically to the species level except for the meroplankton larvae. The number of individuals and mean individual weights were used for estimating the density as ind/m^3, respectively the biomasses as mg/m^3 wet weight (Alexandrov et al., 2011).
Species identification was made mainly after Morduhay-Boltovskoy (1968, 1969 and 1972) and the taxonomic nomenclature according to the online database of the World Register of Marine Species (WoRMS). The structure of the zooplankton community has been analysed in terms of taxonomic composition and key groups, total and average abundance and biomass. Statistical analyses were performed by applying PRIMER 5 of PRIMER-E Ltd, Plymouth (2001).
Among the various zooplankton indicators generally proposed so far at European scale, only Mesozooplankton biomass (mg/m^3), Copepoda biomass (%), Shannon-Wiener index are used due mainly to available data. The threshold values are set at seasonal bases for the identified Marine Reporting units (MRU) in the coastal, shelf and open broad habitat types in each country. Various statistical methods for threshold setting have been applied in different countries - based on the Sygnal detection theory (SDT) - ROC curves and combined methodology used by EPA (USEPA, 2001) – SDT, Regime Shift (Rodionov, 2005) and CUSUM (IBM SPSS Statistics), 90 percentile (Deliverable T 1.3).
Units of measurement: extent of the habitat surface area in square kilometres (km^2) and as a proportion (percentage) of the total extent of the MRU. It was accepted that a MRU has achieved GES if 90 % of its area (volume) is assessed in Good Environmental status by each of the indicators. For the assessment of the proportions in the Good/Not Good environmental state, it was applied the Inverse Distance Weighted (IDW) interpolation in GIS and the area in GES is estimated as the number of pixels below the assigned threshold for the indicator. No integration tool has been applied. For the Turkey areas, Bulgarian thresholds for indicators were agreed.
The indicators are partially operational (legally accepted) but not validated against relevant

pressures.
Biomass of copepods (CB %) - contribution of copepods biomass to total mesozooplankton biomass. Copepods are a key group that contributes significantly to the diet of planktivorous fish (sprat and anchovy, partly horse mackerel), reflect the composition of the zooplankton community and food availability for zooplanktivorous fish. Copepods are mostly herbivores or omnivores, therefore, this indicator would be indirectly impacted by eutrophication (via changes in primary productivity and phytoplankton composition), whereas direct impacts are expected from climatic changes, predation, the introduction of synthetic compounds (at point sources).
Mesozooplankton biomass - Biomass is calculated using the abundance of species/taxa present in the mesozooplankton community and their individual weights. The indicator reflects the composition of the zooplankton community. Mesozooplankton indirectly exposed to the eutrophication process (in case of the amount of food composition and size) and catches of commercially exploited fish (through changes in the pelagic food chain), while the direct impact is shaped by climate change (temperature and salt mode), predation on fish and gelatinous plankton.
Shannon-Wiener index - reflects the number of species in a dataset, taking into account how evenly the basic entities (such as individuals) are distributed among species. The values of a diversity index depend on both the number of species and evenness increase. For a given number of species, the value of a diversity index is maximized when all species are equally abundant.
***Noctiluca scintillans* biomass (N. sci %)** - contribution of *N.scintillans* biomass to total mesozooplankton biomass. The wide feeding spectrum (phytoplankton, zooplankton and detritus) of the species, development in high bloom concentrations, usually after the mass development of phytoplankton, determines its ecological importance for the pelagic ecosystem (Kiørboe&Titelman 1998; Dela-Cruz et al., 2003). The indicator is relevant to eutrophication (D5).

Results

In October 2019 a total of 46 species/taxa were identified in the study area, from 18 taxonomic classes. The bulk of the species pool was composed of Hexanauplia (subclass Copepoda) – 13 species/taxa (28 % of the total number of species) among which the order Calanoida (8 species), Cyclopoida (3 species) were the most diverse (Annex 2). Among benthic larvae (10 taxa) phylum Arthropoda (3), Mollusca (2), Annelida (2 taxa) and along with Platyhelminthes, Nemertea, Phoronida, Bryozoa showed the highest taxa richness. A relatively high number of species were identified for class Branchiopoda (6 species) - *Penilia avirostris, Pseudevadne tergestina, Podonevadne trigona, Podon leuckartii, Pleopis polyphemoides, Evadne spinifera*. Phylum Chordata (4 species/taxa), Ctenophora (3), Cnidaria (2), Chaetognatha (1), Myzozoa (1) were represented by a few species/taxa only (Annex). A common feature for the entire investigated area was the dominance of Copepoda species from 65 % (Bulgaria) to 84 % (Romania) with the identical percentage in front of the Ukrainian and Turkish coast (TR – 75 %, UA- 73 %). Although Copepoda is the most abundant group, community composition varied between countries (Figure 1.19) *O. similis* (33 ± 10 %), *A. clausi* (28 ± 4 %) and *P. elongatus* (24 ± 11 %) are typical for Romanian shelf, *O. davisae (33 ± 13), A. tonsa* (20 ± 6) and *P. elongatus (10* ± 7) characterized Bulgarian shelf, while in Turkey *O. davisae* (28 % ± 17), *A. clausi (33* ± 7 %) co-shared the dominant zooplankton assemblage with the almost equivalent presence of *P. elongatus* (9%) and *P. parvus*.- 12 %. *A. clausi* and *O. davisae* contributed 79 % in the Ukrainian shelf area.

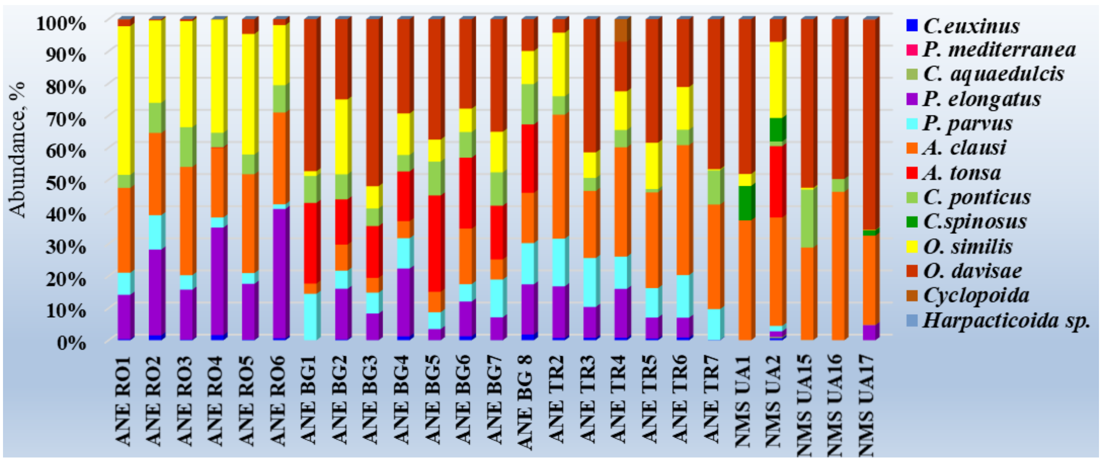


Figure 1.19 - Copepoda community structure in October 2019 along the UA-RO-BG-TR coast

Species diversity itself has two separate components: 1) the number of species present (species richness), and 2) their relative abundances/biomass (termed dominance or evenness) (Magurran, 2004). Diversity index comes from the information theory in ecology, a theoretical measure of relative abundance/biomass of each species if their share in the community would be equalized. The Shannon diversity index (H') is an index that is commonly used to characterize species diversity, richness and evenness of the species present in a community.
In biological communities, the Shannon-Wiener diversity index varies from 0 to 5 and mainly falls between 1.5 and 3.5. According to this index, values less than 1 characterize heavily polluted condition, and values in the range of 1 to 2 are characteristics of moderate polluted condition, while the value above 3 signifies stable environmental conditions (Shah et al.,2013). We accepted >3 bit/ind as a threshold for coastal and >2 for shelf habitats.
Taking into consideration the Shannon index calculated for the samples from Ukraine to Turkey, an increase is observed. The values ranging from 1.93 (UA – 15) to 3.9 (BG-8) in abundance structure and from 0.67 (BG-4) to 3.12 (BG-1) in biomass (Figure 1.20). Lower index value reflected certain species dominance (*A. clausi, O. davisae, P. polyphemoides, P. setosa*) within the zooplankton community. As a rule, stations from north to south in the whole study area were characterized with index values above the thresholds defined, with small exceptions (BG-4, BG-5 in biomass).

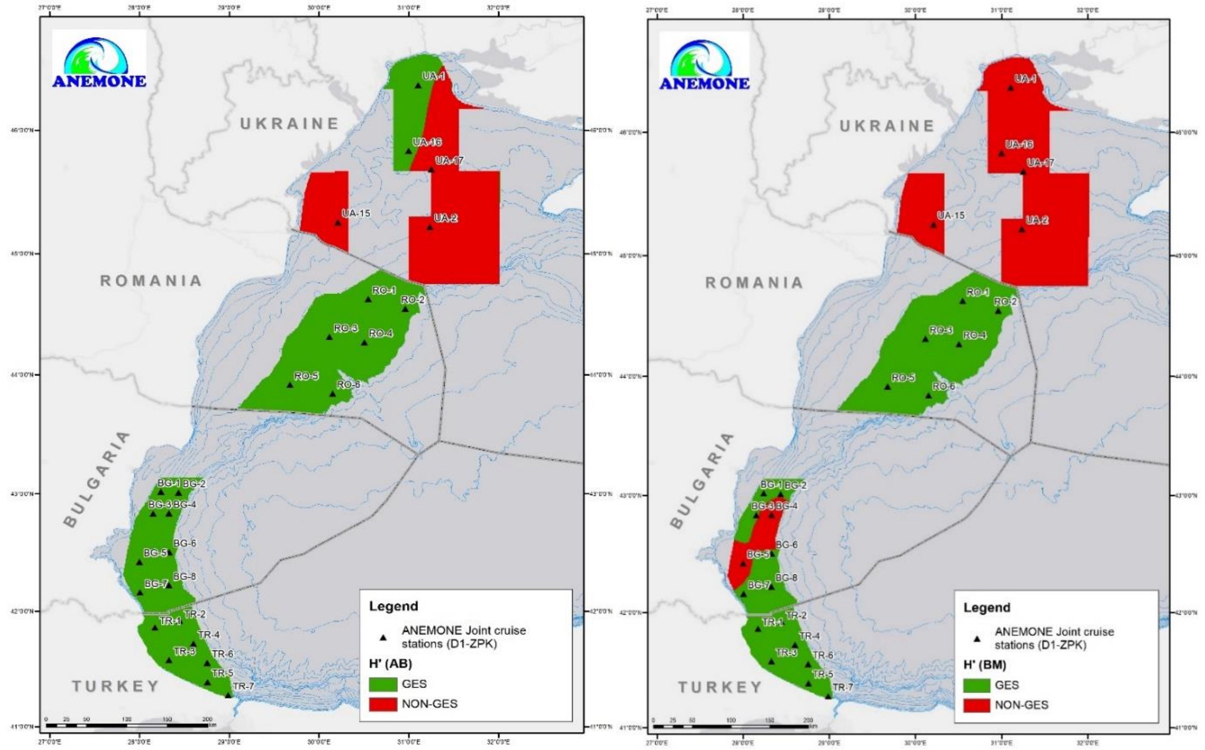


Figure 1.20 - Shannon diversity index by abundance (left) and biomass (right) along the western Black Sea – GES/Non-GES assessment

Mesozooplankton metrics manifested huge variability in the study area, with almost the same range - the abundance ranged from 1783 ind/m^3 to 15900 ind/m^3 (about 9 times) and the biomass varied 8 folds (min=32.39 mg/m^3; max=265.98 mg/m^3, within an average of 128.57 mg/m^3, SD 55.34 mg/m^3). Remarkable consistency in the density (Romanian shelf) and biomass (Bulgarian shelf) was evident with negligible differences among stations (Figure 1.21).

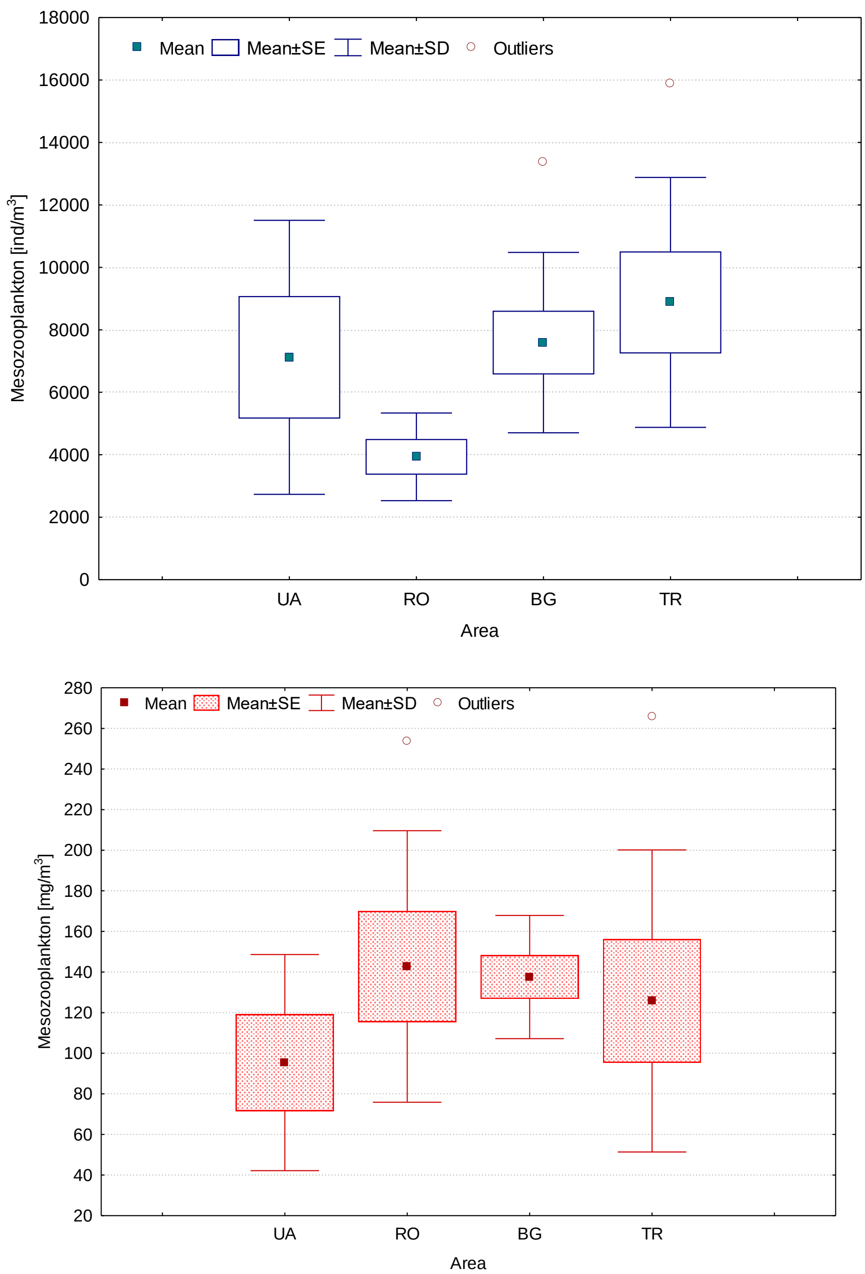


Figure 1.21 - Box whiskers abundance (upper panel) and biomass (lower panel) presence in Ukrainian (UA), Romanian (RO), Bulgarian (BG) and Turkish (TR) areas

A specific pattern distinguished the mesozooplankton abundance spatial distribution along the MRUs – a decrease from the North (UA) to South (RO) and from inner to outer shelf along the UA - RO coasts, relatively high values measured in inner shelf stations along with the BG and TR pelagic habitats, whereas no specific trend in biomass figure was observed. Maximum abundances were registered in Turkey (TR-7) – 15900 ind/m^3, Bulgaria (BG3) – 13361 ind/m^3 and Ukraine (UA-1) – 12961 ind/m^3 and associated with mass development of *A. clausi*, *O. davisae*, *P. avirostris*, *O. doica* and Bivalvia larvae. In contrast, maximum biomass was measured in Romanian (253 mg/m^3) and Turkish (265 mg/m^3) shelf habitat due to *P. setosa* dominance (Figure 1.22).
Regarding the mesozooplankton's community structure, the fodder component was dominant in UA and BG, partly in RO but in TR *N. scintillans* prevailed with over 60 %. The non-fodder component recorded low densities in all stations but it influenced the biomass community structure (Figure 1.23). In 40 % of cases, indicator values surpassed the threshold of 30 %. The highest share was associated mainly with four stations in the Romanian shelf habitat (RO-1 to RO-4) (Figure 1.24) and all Turkey stations excluding TR-7.

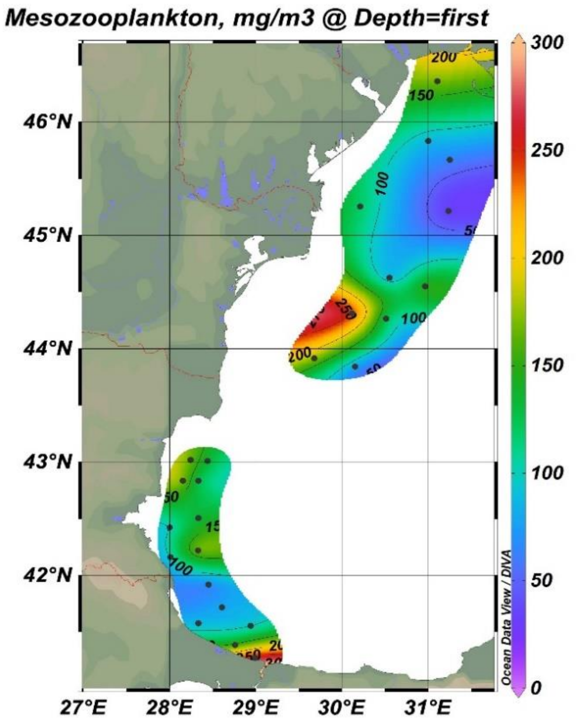


Figure 1.22 - Spatial distribution of mesozooplankton biomass in October 2019

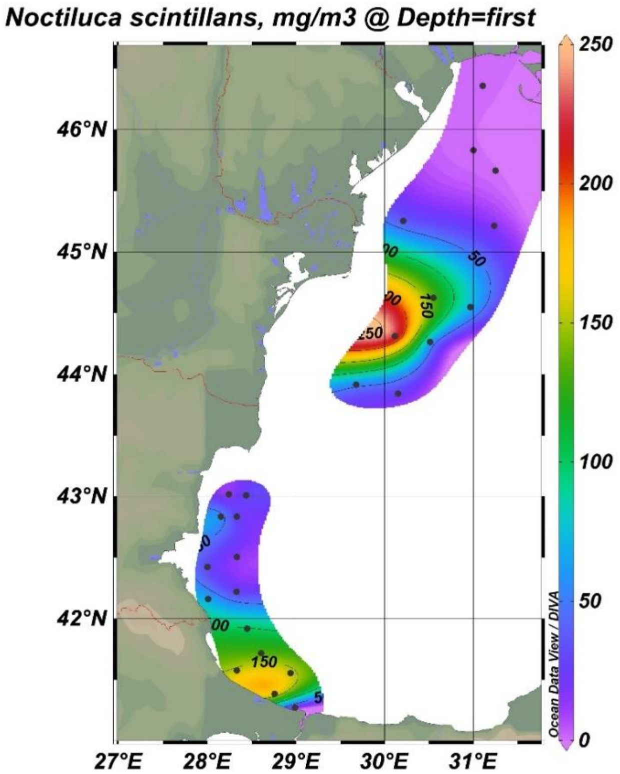
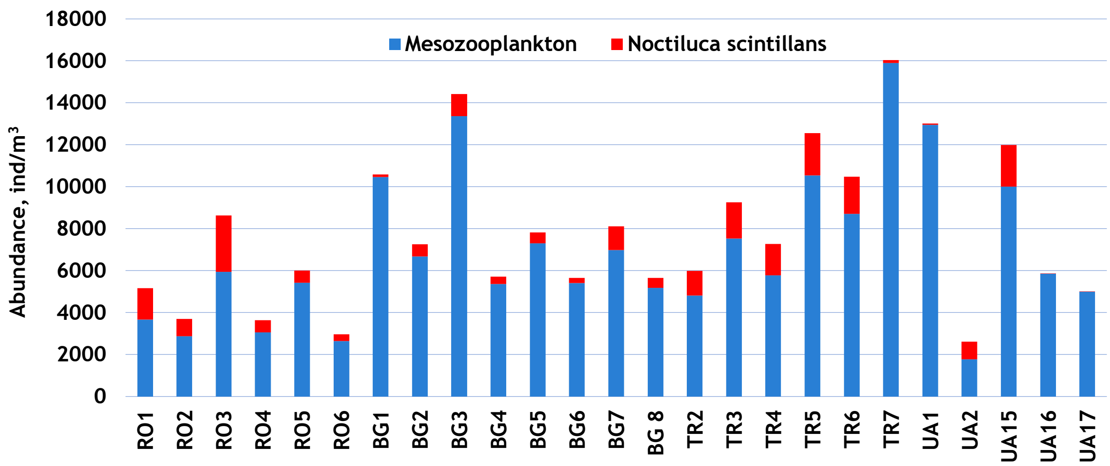


Figure 1.23 - Noctiluca scintillans biomass spatial distribution in October 2019



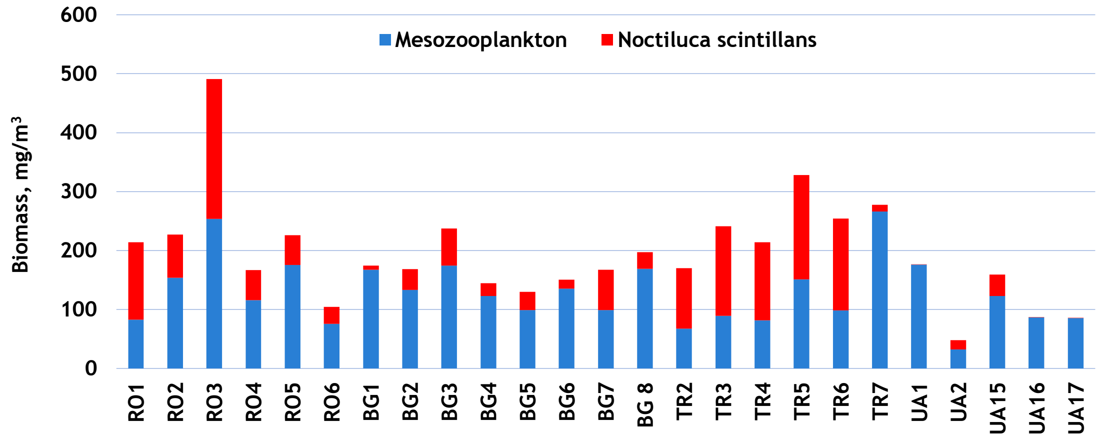


Figure 1.24 - Mesozooplankton and N. scintillans density (up) and biomass (down) proportion dynamic in October 2019

As far as the fodder component is concerned, Copepoda represented the bulk of the community with the maximum value of density (9552 ind/m^3 in TR7) and biomass (109.9 mg/m^3 in BG8), being followed by Ukraine and Romania (Figure 1.25). Cladocera and Meroplankton are well and mainly presented in Bulgarian (28 % - Cladocera, 4 % benthic larvae), Ukraine (22 % - Cladocera) and Turkey (10 %-Cladocera, 5 % Meroplankton) pelagic habitat. *P. setosa* and *O.dioica* included in the group Others recorded higher values of density in TR (2247 ind/m^3) and biomass in RO - 149.6 mg/m^3 (Figure 1.25). Bray-Curtis similarity matrix show evident discrimination of Ukrainian MRUs with other MRUs located in RO, BG and TR. Most probably higher abundance and biomass was based on the freshwater impact (Figure 1.26).

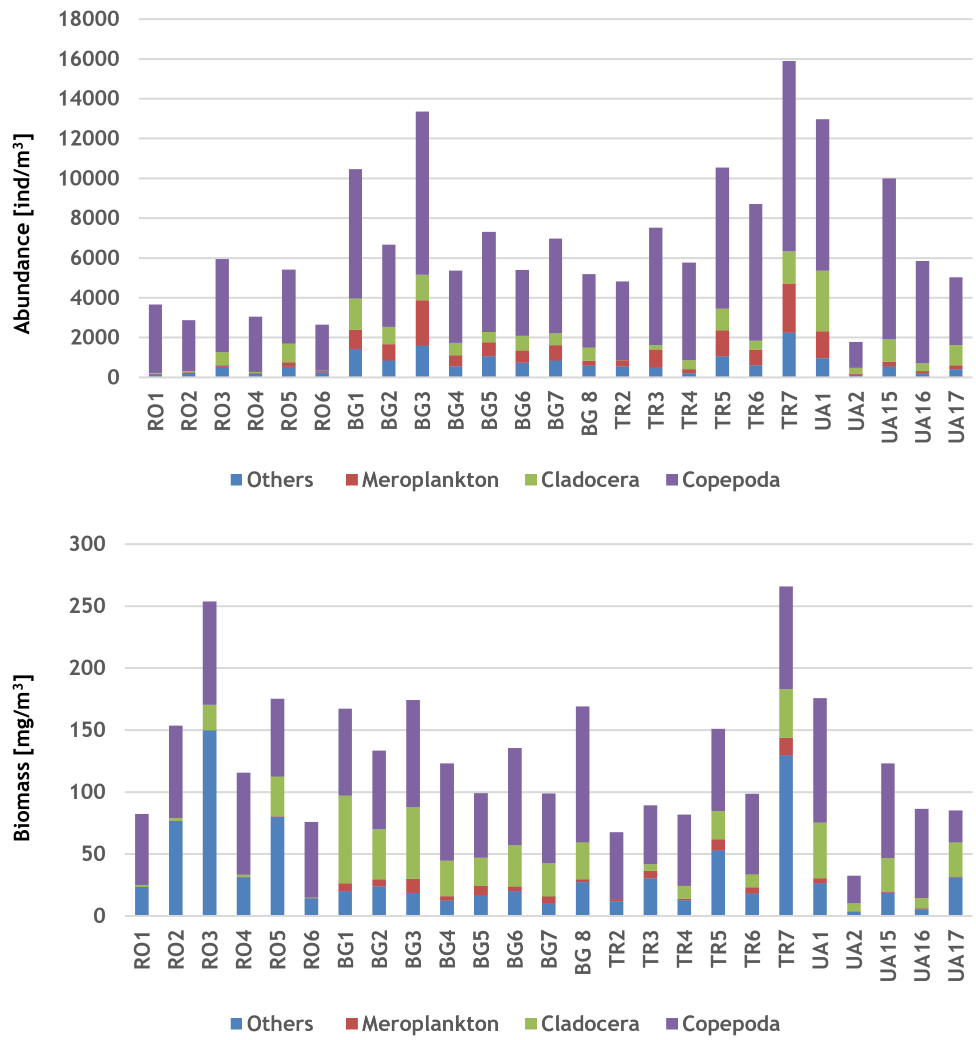


Figure 1.25 - Fodder mesozooplankton community structure in abundance (up) and biomass (down)

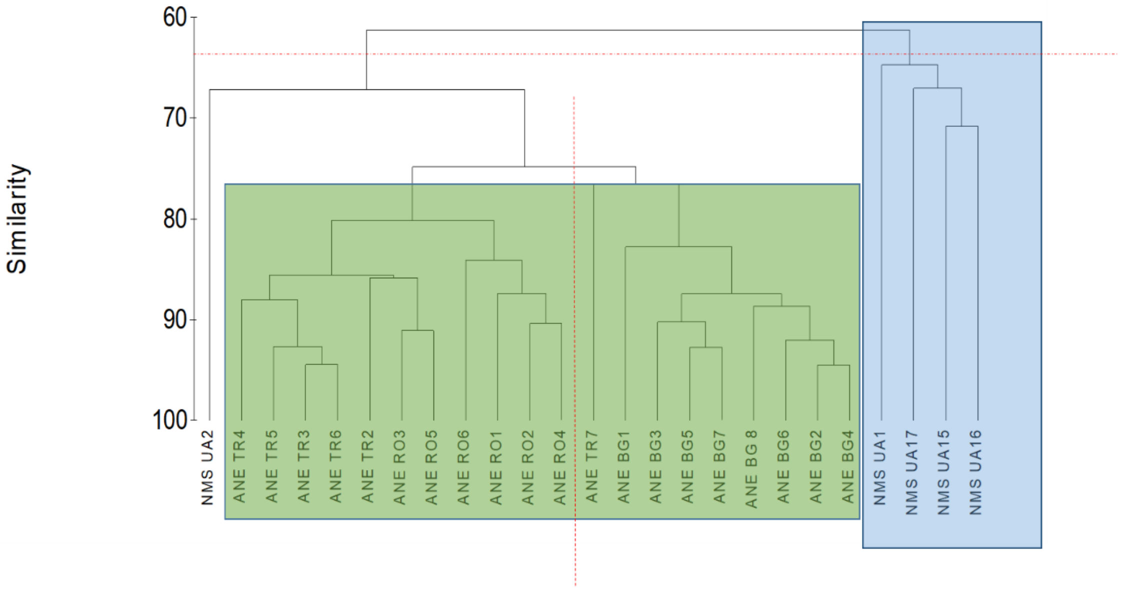


Figure 1.26 - Bray-Curtis similarity matrix of mesozooplankton biomass

Mesozooplankton biomass vertical distribution

Zooplankton has limited ability to control their horizontal position, but they can, to a large extent, regulate their vertical distribution by their mobility (Price, 1989; Pearre, 2003; Hirche et al., 2006). Hence, zooplankton can be expected to select a habitat that optimizes feeding conditions and minimizes their perceived predation risks (Fiksen & Giske, 1995; Aksnes et al., 2004; Basedow et al., 2010). The zooplankton in front of Ukraine was concentrated mainly in the upper layers (according to the density) and decreased in abundance gradually with depth (Figure 1.27). In the Romanian shelf, mesozooplankton were distributed quite uniformly within Surface Homogeneous Layer (SHL) and TC (Thermocline). Mesozooplankton contribution to the total abundance in the SHL and TC was almost identical for BG and TR shelf with a mean of 48 % for upper and 38 % in lower (TC) layers. The relative abundance of *C. euxinus* was higher in the deeper layers (> 60 % of *C. euxinus* and *P. elongatus* populations at the depth >40 m). *N. scintillans* contributed markedly to the zooplankton community at the Bulgarian stations in the thermocline and under surface homogenous layer (USHL) - lower depth strata (20-40 m, 30-40 m, 30-50m, 50-100 m).

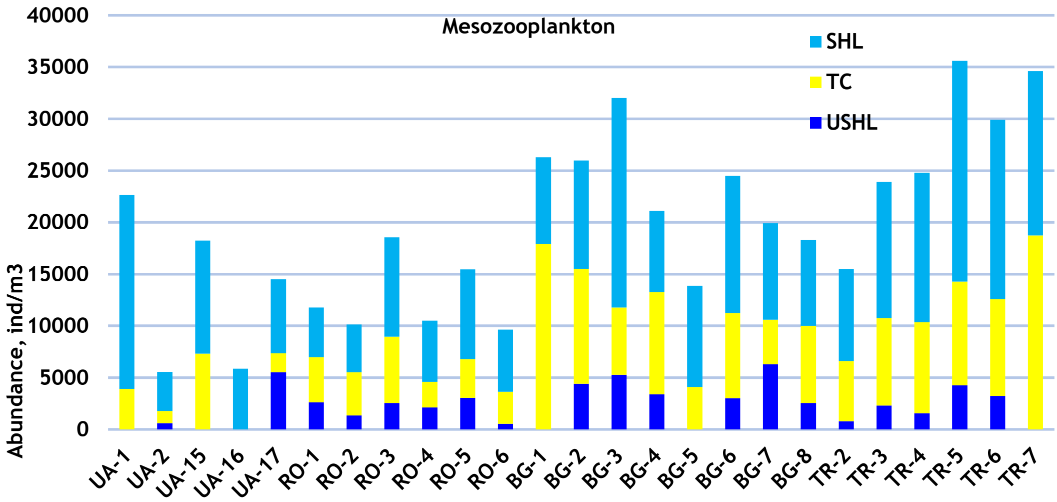


Figure 1.27 - Vertical mesozooplankton distribution pattern in abundance

Mesozooplankton indicator based ecological status assessment

The response of zooplankton to environmental conditions is of particular interest due to the central and mediating role that this group occupies as a trophic link between planktonic primary producers and larger consumers (Varkitzi et al., 2018).

The indicators agreed used for the mesozooplanktonic component assessment are:

- ✓ Mesozooplankton biomass
- ✓ Copepoda biomass
- ✓ Shannon- Wiener index

Mesozooplankton biomass

Biomass of mesozooplankton includes information for major key groups, forming the structure of the planktonic fauna, being represented by Copepoda, Cladocera, Meroplankton, and Other groups represented by *Oikopleura dioica* and *Parasagitta setosa*.

Zooplankton metrics exhibit strong variability in time and space under the influence of natural and anthropogenic factors, which reflects the total mesozooplankton biomass.

High values for mesozooplankton biomass suggest a higher trophic environment and increasing concentrations of the planktonic fauna, being an indirect indicator of the food ability in the water column, respectively eutrophic conditions. On the other hand, mesozooplankton biomass reduction indicates enhanced predator pressure in the food chain (jellyfish, ctenophores and small pelagic fish) (HELCOM, 2012).

Figure 1.28 demonstrated the 100 % extent of Assessed Marine Reporting Units (MRU) in Ukraine, Bulgaria and Turkey shelf was covered in 100 %, while the Romanian MRU with 20140 km^2 extent was assessed in 56 % only. A uniform threshold value for Romania, Bulgaria and Turkey was applied (> 70 mg/m^3), whereas in Ukraine four different thresholds were defined due to strong environmental gradients in the pelagic communities. The evaluation of zooplankton biomass indicates that in the RO, BG and TR MRUs the values are above the threshold indicating good status with negligible exclusion in Romania (15 %) and Turkey (1 %). By contrast, in four MRUs in Ukraine registered values are significantly below the thresholds, which implies that good status has not been achieved (Figure 1.28).

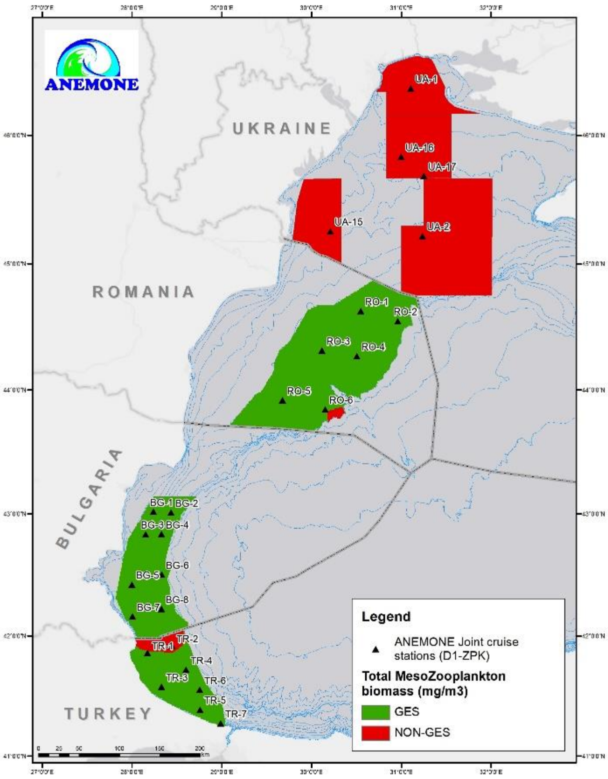


Figure 1.28 - Spatial extent of the GES/nonGES area in the pelagic shelf habitats (UA, RO, BG, TR) based on mesozooplankton biomass index in October 2019

Copepoda biomass

The indicator reflects changes in the zooplankton community. These changes are indirectly related to changes in nutrient composition and directly related to fish communities, climate and phytoplankton community composition, and have a direct impact on both phytoplankton communities and fish growth. The zooplankton community, and its dominant member, the copepods, have a crucial role in the pelagic food web dynamics in transferring energy from primary producers to a form utilizable by fish.

Zooplankton is affected by changes in primary production, indicative of eutrophication, and by changes in the structure and abundance of the fish community, indicative of overfishing. Therefore, zooplankton lives between top-down and bottom-up dynamics and can potentially yield a lot of information on the state and dynamics of the aquatic ecosystem (Jeppesen et al., 2011).

The copepods species composition affects directly both the phytoplankton and zooplankton species composition and has the potential to affect the biodiversity in these communities (HELCOM, 2012).

Good status during the assessment period was found in the three of MRUs in front of Ukraine (ShW_UA_1; ShW_UA_5, ShW_UA_3) – 100 %, Romania with 69 % of extent, 94 % and 97 % in front of Turkey and Bulgaria (Figure 1.29).

Analysing the samples collected in October 2019, the good environmental status was not achieved in all the sampling stations, respectively MRUs, both for Copepoda and Mesozooplankton biomass, the recorded values being lower than the threshold (Table 1.4).

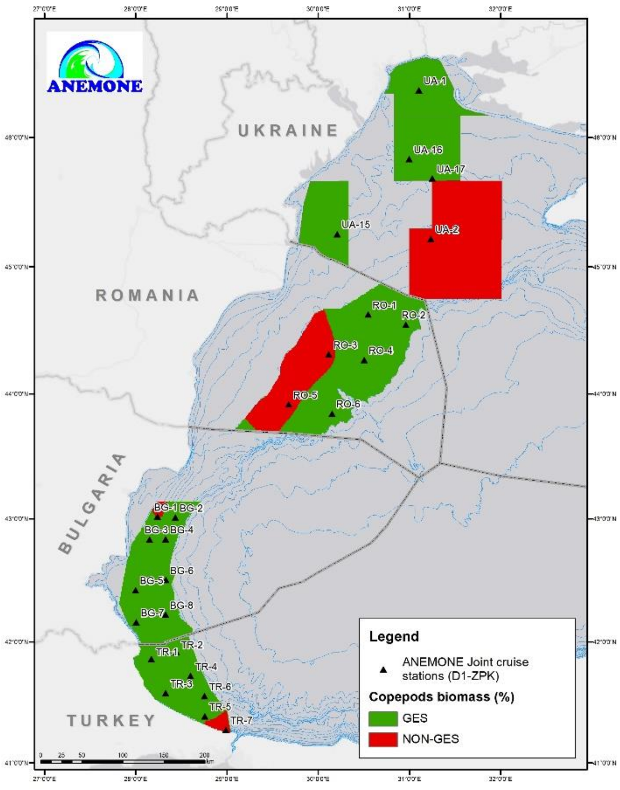


Figure 1.29 - Spatial extent of the GES/nonGES area in the pelagic shelf habitats (UA, RO, BG, TR) based on Copepoda index (%) in October 2019

Conclusions

The confidence of the evaluation of the indicators is low since the time (once per year) and area (only one broad habitat type – shelf) used cover fairly the required frequency and space.
As far as the ecological status assessment is concerned, GES status was not achieved in all MRUs.
Development of new additional indicators and parameters, setting of, validation and revision of GES thresholds for plankton indicators is required.

Table 1.4 - Evaluation of plankton community ecological status indicators, based on the agreed indicators (mg/m³)

Description		D1 Biodiversity - Pelagic habitat							
Criteria code		D1C6							
Element		Phytoplankton			Mesozooplankton				GES achieved
Indicator (parameter)		Biomass	Shannon 95	Menhinick	Biomass	Copepoda	Shannon Wiener Biomass	Shannon Wiener Abundance	
Unit of the value		mg/m³	ind/bit		mg/m³	%	ind/bit	ind/bit	
MRU/Season		Spatial extend in GES (%) Summer 2019							
Coastal	ShW_UA_1	100	0	100	0	100	0	0	Not in GES
	ShW_UA_3	100	0	7	0	100	0	71	Not in GES
	Coastal GES achieved in %	100	0	54	0	100	0	35	Not in GES
Shelf	ShW_UA_5	100	0	37	0	100	0	60	Not in GES
	ShW_UA_7	0	0	100	0	0	0	0	Not in GES
	BLK_RO_RG_MT01	7	4	100	99	69	100	100	Not in GES
	BLK_BG_AA_Shelf_South	100	100	78	100	97	64	100	Not in GES
	TR_KARD1_BS1_Shelf_West	47	0	88	85	94	100	100	Not in GES
	Shelf GES achieved in %	36	19	88	64	64	60	71	Not in GES

1.1.4Macrozooplankton

Macrozooplankton, i.e. zooplankton larger than 2 cm, encompass organisms characterized by very different size, features, and behaviour such as jellyfish. Swarms of gelatinous macrozooplankton have been recurrently reported worldwide, causing concern due to their negative impact on different societal activities, such as tourism and fishery (Wiebe & Brotz, 2016). Jellyfish on the marine ecosystem have been considered a stress factor, or as a disturbing indicator, their appearance indicating bad environmental status. However, gelatinous species also have an important role on the environment, the phenomenon of jelly-falls, meaning the transport of particles and carbon from the sea surface through the water column to the seabed (Doyle et al., 2014). As such, increased gelatinous biomass may translate into increased transfer of this material to the ocean floor and thus enhancing the magnitude and importance of the biogeochemical and ecological processes associated with jelly blooms (Lebrato, 2012). Thus, investigations on the ecology and spatio-temporal distribution of jellyfish, their biogeochemical role, life cycles, ecophysiology, and behaviours, are important in order to further our understanding of their role in a changing ocean.

Material and methods

Macrozooplankton sampling is performed by Hansen-type net with a diameter of 70 cm and aperture of mesh size - 300µm. The macrozooplankton was taken onboard of the research vessel “Mare Nigrum”, which allowed the proper and safe handling of the net, but at the same time provided the stability conditions necessary for the analysis of the samples immediately after sampling.
The biological material is obtained by towing the net vertically in the water mass (from 2m above the seabed to the surface), at low speed (0.5-1 m/s), in order to prevent damage to gelatinous organisms or clogging of the net. After collection, the net is washed with a seawater hose to remove organisms or mucus from them.
The organisms collected in the net are carefully moved to a bucket and immediately identified, counted and measured. The large specimens are washed with sea water, above the container in which the sample was extracted from the net. All organisms in the sample are measured (depending on the species: width, aboral length, respectively total length). The measurements shall be carried out by means of a ruler, by positioning them directly on the laboratory table or on graph paper (in the case of large organisms of the species *A. aurita*). In the case of small specimens, a checkered Petri dish, filled with water, in which the bodies are suspended, is used to allow their measurement without the occurrence of body deformation.
The density and wet biomass of gelatinous organisms were expressed as ind/m^3 and mg/m^3, respectively. The calculation of these parameters was performed according to the recommendations of the Black Sea Monitoring Guidelines Macroplankton (Gelatinous plankton) (Shiganova T.A., 2005).

Results

Four gelatinous species were identified during the Joint cruise and monitoring campaign of Ukraine: Scyphozoa - *Aurelia aurita* (Linnaeus, 1758), Ctenophora – aborigine *Pleurobrachia pileus* (O. F. Müller, 1776), non-native *Mnemiopsis leidyi* A. Agassiz, 1865 and *Beroe ovata* Bruguière, 1789. The mean macrozooplankton abundance and biomass at whole investigated area from Romania to Turkey reached 0.6 ind/m^3 (±1.12 SD), 1.58 g/m^3 (±3.6 SD) respectively. Maximum of average gelatinous density and biomass was recorded in Bulgaria shelf (3.1 ± 2.4 ind/m^3 and 9.4 ± 11.4 g/m^3) while in front of Romania (2.34 ± 1.97 ind/m^3, 4.69 ± 5 g/m^3) and Turkey (1.5 ± 0.87 ind/m^3, 4.16 ± 3.93 g/m^3) they were almost equal. *P. pileus* (6 ind/m^3) and *A. aurita* (23.85 g/m^3) dominated the gelatinous structure in density (*P. pileus* – 82%) and biomass (*A. aurita* – 68%) respectively, with maximum in Bulgarian waters (Figure 1.30, Figure 1.31).
As a result of the mosaic distribution usually observed in gelatinous species, *M. leidyi* metrics varied between 0.03 to 0.57 ind/m^3 and 0.02 – 8.7 g/m^3. The frequency of occurrence covered 100% in Romanian and Turkey waters but 80% in Bulgarian. The spatial distribution of abundance and biomass revealed Mnemiopsis maximum at st. BG-2, BG-4 (Bulgaria) and TR-7 (Turkey). On the surface of the Romanian continental shelf, the species *M. leidyi* reached the maximum density value of 0.36 ind/m^3 in RO-6 station - depth 95 m, 0.55 ind/m^3 in a Bulgarian station with 80m depth and in Turkey - 0.57 ind/m^3 (TR-7, depth 32 m) (Figure 1.32). The respective biomass figure shows maximum value of 8.7

g/m³ in BG-4 station, followed by 4. 88 g/m³ in Romanian shelf (RO-4), and in Turkish area, with the maximum density value - 1.4 mg/m³ in TR-3 (depth 65 m) (Figure 1.32).

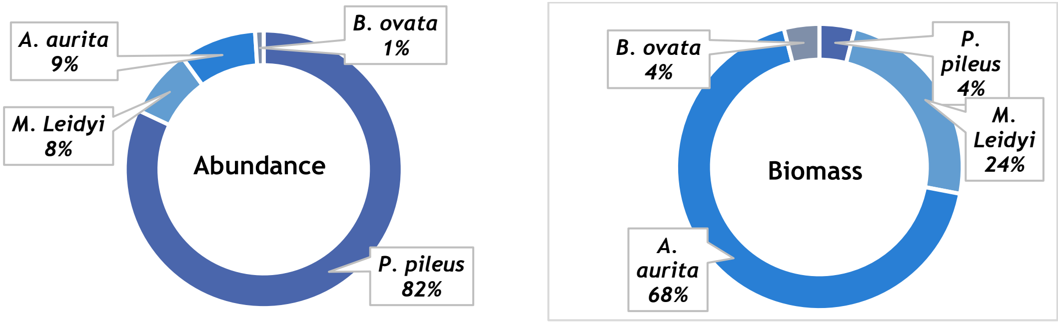


Figure 1.30 - Macrozooplankton community structure of abundance and biomass in percentage

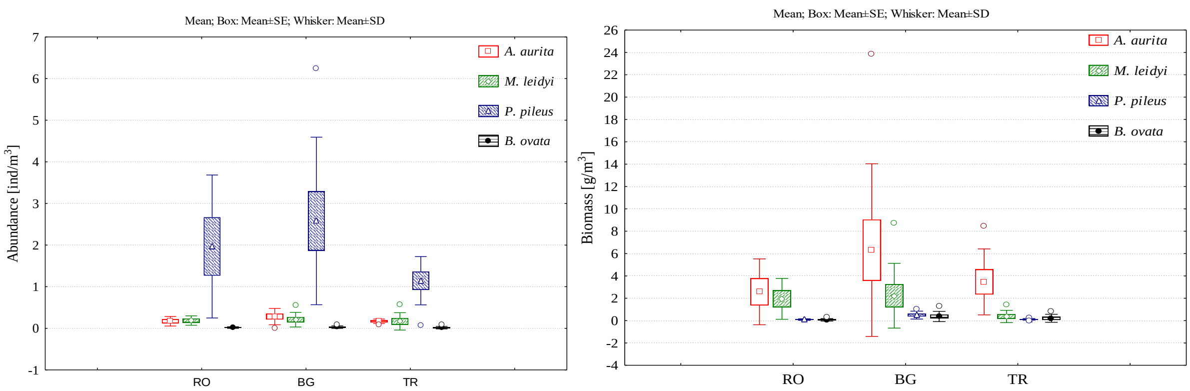


Figure 1.31 - Mean, Standard Deviation (SD), Standard error (SE) of A. aurita, M. leidyi, P. pileus, B. ovata density (left) and biomass (right)

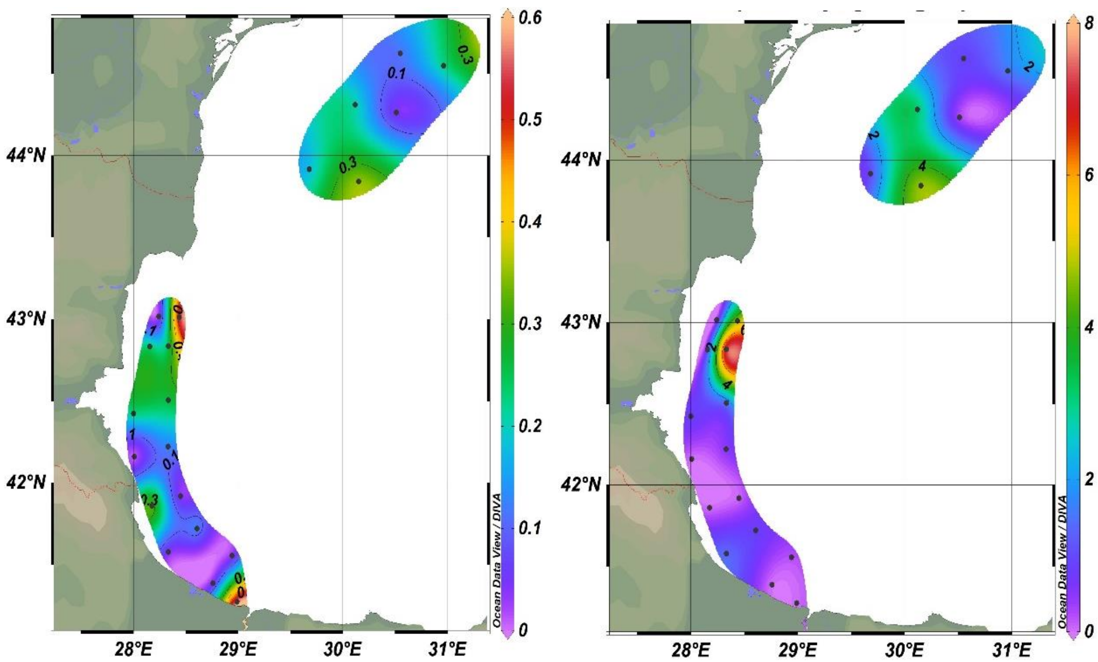


Figure 1.32 - Mnemiopsis leidyi density (ind/m³, left) and biomass (g/m³, right) spatial distribution

The presence of another non-indigenous species - *B. ovata* in the macrozooplankton community structure is negligible – 55 % frequency of occurrence and range from 0.03 to 0.09 ind/m³ and between 0.03 to 1.129 g/m³. The species demonstrated almost the same spatial distribution as *Mnemiopsis* with higher quantity in the outer shelf of Bulgarian waters (Figure 1.33). During the late summer early autumn *B. ovata* reached the maximum density value for Romania (RO-5 station) - 0.04 ind/m^3 and in Turkish waters the maximum abundance value was 0.8 ind/m^3 in TR-7 station (Figure 1.33). The biomass figure almost repeated the density spatial distribution (Figure 1.33).

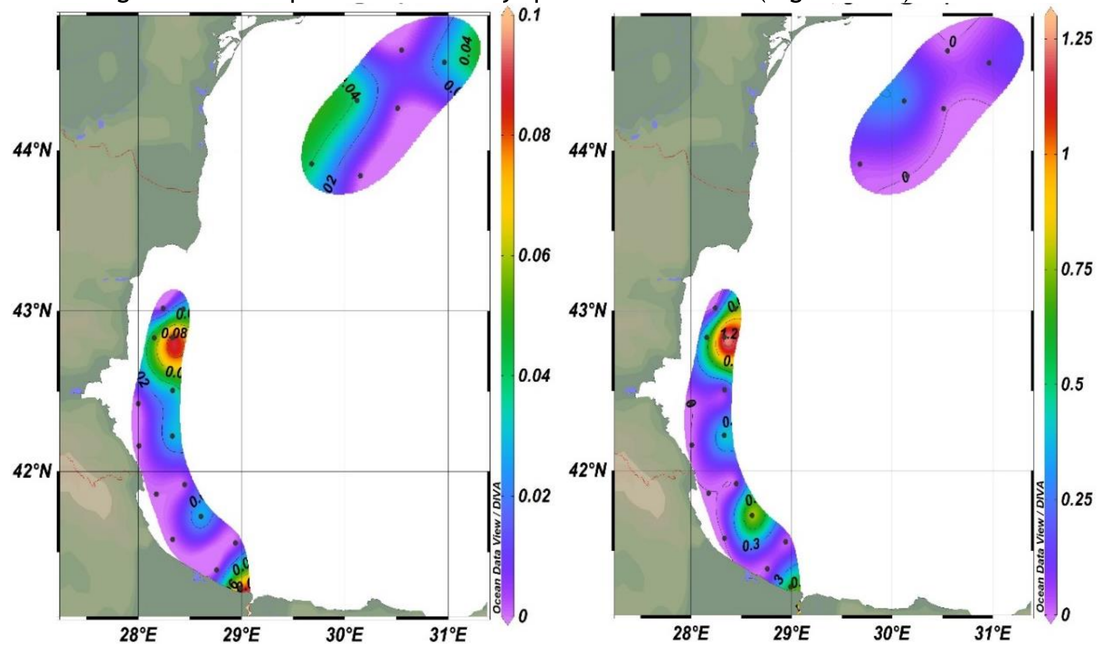


Figure 1.33 - Beroe ovata density (ind/m^3, left) and biomass (g/m^3, right) spatial distribution

The most abundant native Ctenophora species *P. pileus* reached the maximum density value of 4.61 ind/m^3 in RO-5 and Bulgaria (BG-3 – 6 ind/m^3, BG-5 – 4 ind/m^3). Turkish area, characterized with identical density spatial distribution (mean 1.14 ind/m^3 SD ± 0.6) (Figure 1.34). From the biomass point of view, the species *P. pileus* reached the maximum value of 1.04 g/m^3 in BG-3 where the highest abundance was recorded and BG5 as well. Lower *P. pileus* biomasses in front of Romania (0.1 g/m^3 SD ± 0.07) and Turkey (0.12 g/m^3 SD ± 0.06) reflected small size of specimens (Figure 1.34).

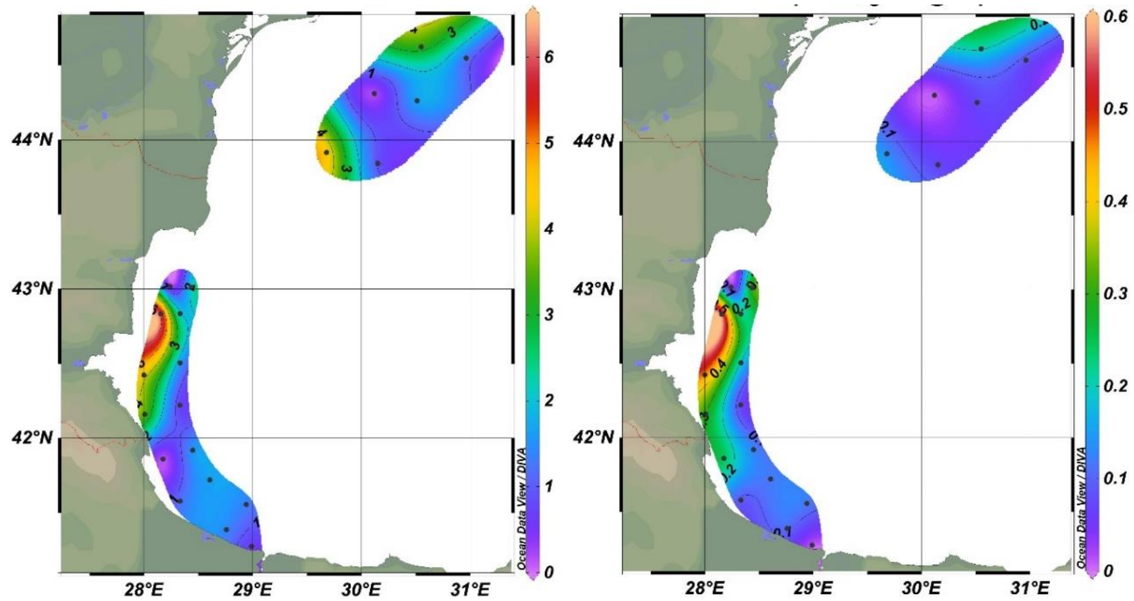


Figure 1.34 - Pleurobrachia pileus density (ind/m^3, left) and biomass (g/m^3, right) distribution

A. aurita dominated Jellyfish community structure in biomass reaching 23g/m^3 in BG-5 (inner shelf) with average 6.31 g/m^3, SD ±4.7 (Bulgarian South shelf) (Figure 1.35). The species was well pronounced in Turkish shelf where the frequency of occurrence was 100% and average 3.47 g/m^3, SD

± 2.95 while in Romania it ranged between 0.01 to 7.21 g/m^3 (Figure 1.35). The contribution of registered individuals to total abundance was negligible during late summer from 0.03 to 0.55 ind/m^3.

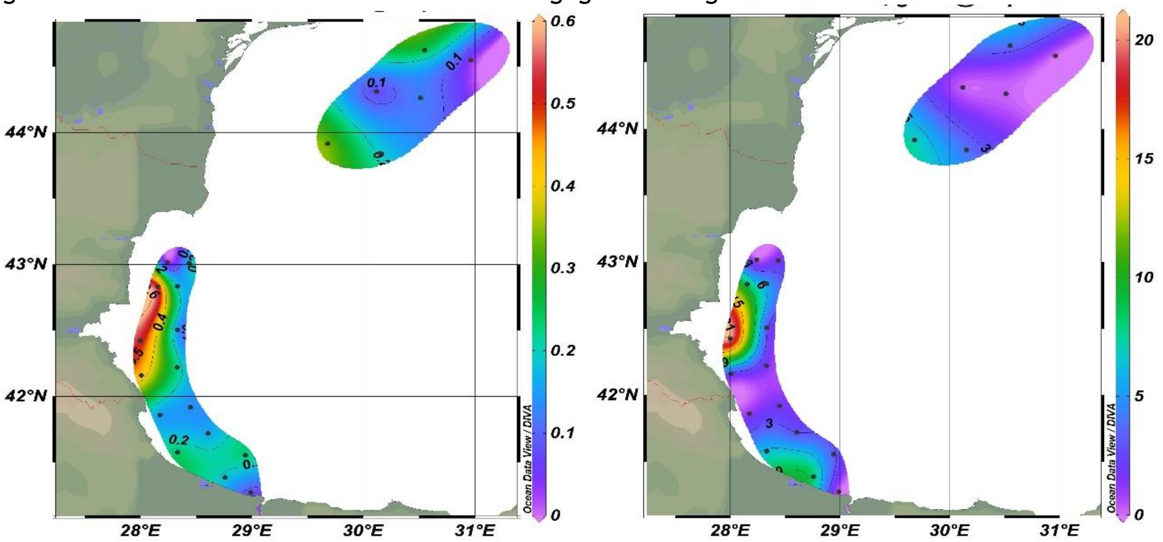


Figure 1.35 - Aurelia aurita density (ind/m^3, left) and biomass (g/m^3, right) spatial distribution

Macrozooplankton indicator based ecological status assessment

Although the species *Mnemiopsis leidyi* is considered an indicator of the species diversity and ratio within the zooplankton community regarding to D1,4 its role as an indicator of D2 non-indigenous species is extremely important. Some of alien species are identified as invasive (what is the situation with *M. leidyi*) and their abundance or biomass, trends in population, temporal occurrence and spatial distribution are an important indicator to determine the status of the species and respectively the probability to achieve good status (Table 1.5).

Table 1.5 - M. leidyi biomass - applicability according to criteria (EU/2017/848)

	Primary criterion	Secondary criterion	Criterion, indicator, GES and Objectives according to 2010/477 / EU
Impact		D2C2 - The abundance and spatial distribution of non-native species, especially invasive species, which contribute significantly to adverse effects on certain groups of species or large habitat types.	2.1.1. Trends in the abundance, temporal incidence and spatial distribution in the natural environment of alien species, especially invasive alien species, especially in risk areas, in relation to the main vectors and the main routes of spread of these species. Indicator: Biomass of *M. leidyi* GES: Average biomass value ≤ 4 g/m^3 or 120 g/m^2 (Vinogradov et al., 2005) Objective: To assess the abundance and distribution of *M.leidyi* to determine whether or not it has a negative influence on the population of native species and natural habitats.

On the base of calculated critical biomass of ctenophore *M. leidyi* that does not affect mesozooplankton abundance, 4 g/m^3 or 120 g/m^2 (Vinogradov et al., 2005) was identified as a threshold for GES (Table 1.6). The *M. leidyi* biomass was reduced approximately 6 folds from north (Romania and Bulgaria) to Turkey. Follow the same water masses (RO-6) the highest biomass was registered in Bulgarian shelf (BG-4) (Figure 1.36).
Obviously, recorded concentrations exceeded the GEnS threshold in two stations only leading to 94 % extent of GEnS in Romanian shelf, 100 % in Turkey but 87% in Bulgaria.

Table 1.6 - Average biomass indicator limits - M. leidyi species (threshold values) for GES definition

Indicator	Limits (threshold values) Vinogradov et al., 2005	Defining GES
Biomass M. leidyi	≤ 4 g/m^3 (4000 mg/m^3)	GES
	≥ 4 g/m^3 (4000 mg/m^3)	Non-GES

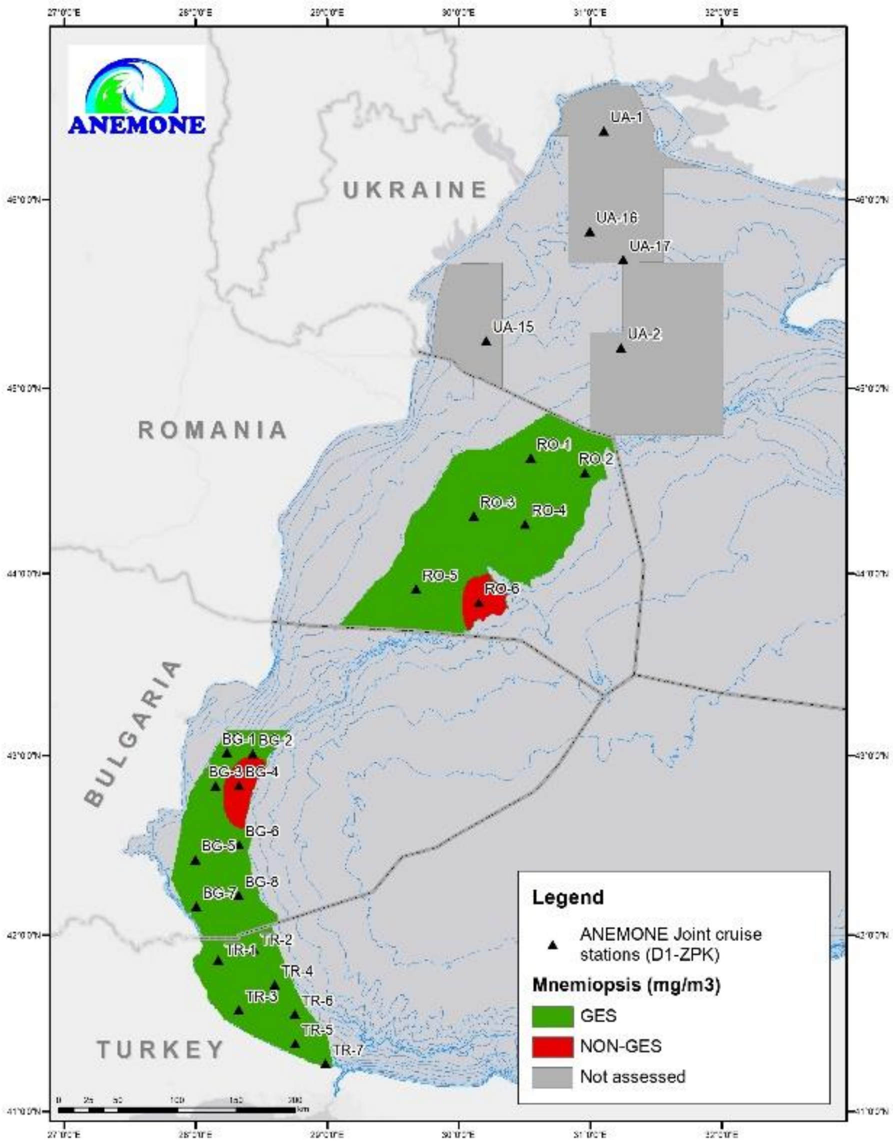


Figure 1.36 - Spatial extent of the GES/nonGES area in the pelagic shelf habitats (RO, BG, TR) based on M. leidyi biomass index (g/m^3) in October 2019

Conclusions

M. leidyi dominated outer shelf stations with high values of density and biomass compared to stations in Turkey where low values were identified.

The frequency of *B. ovata* (natural predator of *M. leidyi*) was much higher compared to Turkish station, this was influenced by the high densities of *M. leidyi* in the Romanian marine area.

Unlike the other species, A. *aurita* had a uniform frequency of occurrence in the analyzed stations, but with higher density and biomass values in the Turkish stations.

P. pileus had an approximately uniform distribution in all the analyzed stations.

M. leidyi species exceeded the GES limit index (4000 mg/m^3) in the RO-6 station, which indicates bad environmental status.

1.1.5 Ichthyoplankton

Material and methods

An international expedition took place on the continental shelf of the Black Sea (Romania, Bulgaria, Turkey) in which 21 ichthyoplankton samples were collected (6 samples in Romanian marine waters, 8 samples at the Bulgarian waters and 7 samples from the Turkish marine platform).

The biological material was obtained by towing the net vertically in the mass of water (from 2 m above the seabed to the surface), at low speed (0.5-1 m/s). After collection, the net was lifted on the ship deck and gently washed with seawater in order to release organisms that were trapped in the net mesh.

The ichthyoplankton samples were stored in 500 ml plastic bottles and preserved in 4% buffered formaldehyde solution. The processing of ichthyoplankton samples consisted of complete sorting under stereomicroscope of each collected sample, establishing the qualitative and quantitative structure of ichthyoplankton.

The qualitative and quantitative composition of ichthyoplankton was determined taking into account the main distinguishing features for fish eggs: their shape and diameter, the presence or absence of the fat drop, the diameter and appearance of the fat drop, the homogeneity or segmentation of the calf, the size of the perivitelline space. To determine the larvae stage, meristic, morphometric characteristics, pigmentation, body shape and position of the anal orifice were considered (Dehnic, 1973).

Qualitative analysis of fish eggs and larvae consisted in identifying them up to species level. From the quantitative point of view, the results obtained were expressed in individuals per cubic meter (ind/m^3). The spatial distribution of ichthyoplankton and juveniles was achieved by marking on the distribution map, the densities values obtained from the analysis of ichthyoplankton samples.

Results

The qualitative and quantitative structure of fish eggs and larvae was influenced by the net used for sampling and by the collection direction (vertical), the period of the expedition, the environmental conditions, the number of stations and their location. For ichthyoplankton sampling, Bongo net is proper for collection but in this case Hansen net was used. From the 21 samples analyzed, the ichthyoplanktonic component was present in 13 stations.

From the qualitative point of view, ichthyoplankton was represented by three species: *Sprattus sprattus* (sprat), *Merlangius merlangus* (whiting), *Mullus barbatus ponticus* (red mullet). The three species were identified in the egg and larval stage.

The coastal pelagic sprat forms important concentrations and makes large migrations between feeding places and breeding areas. The Black Sea sprat prefers cold waters and performs irregular migrations due to temperature conditions.

Whiting is a demersal and gregarious species, with a widespread in the waters of the Atlantic Ocean, the Mediterranean Sea, the Black Sea. In summer it prefers deep waters, approaching the shore with the help of cold currents (10-15˚C). In spring appears on the shore at a temperature of 7-8˚C; when the water is heated to 15-16 ˚C, the species retreats to depths.

A bento-pelagic species, red mullet is found in coastal waters, from 30 to 100 m, on stony, sandy, muddy bottoms, being spread in all Black Sea regions.

Sprat eggs were reported in Romanian waters with a density value of 0.164 ind/m^3 in RO-6 station with a depth of 95 m, and in Bulgarian waters with a density value of 0.144 ind/m^3 in station BG-6 with a depth of 90 m. Whiting eggs were present in 3 stations in Romanian waters (RO-1 with a depth of 70 m, RO-3 with a depth of 70 m and RO-4 with a depth of 98 m) with 0.292 ind/m^3, the highest density (Figure 1.37).

On the Bulgarian continental shelf, whiting eggs were present in two stations (BG-3 and BG-4). The maximum density value of 0.122 ind/m^3 was registered in BG 4 station, with a depth of 85 m (Figure 1.37).

In Turkish marine waters, whiting eggs were present in a single station, TR-4 with a depth of 80 m, recording a density of 0.130 ind/m^3 (Figure 1.37).

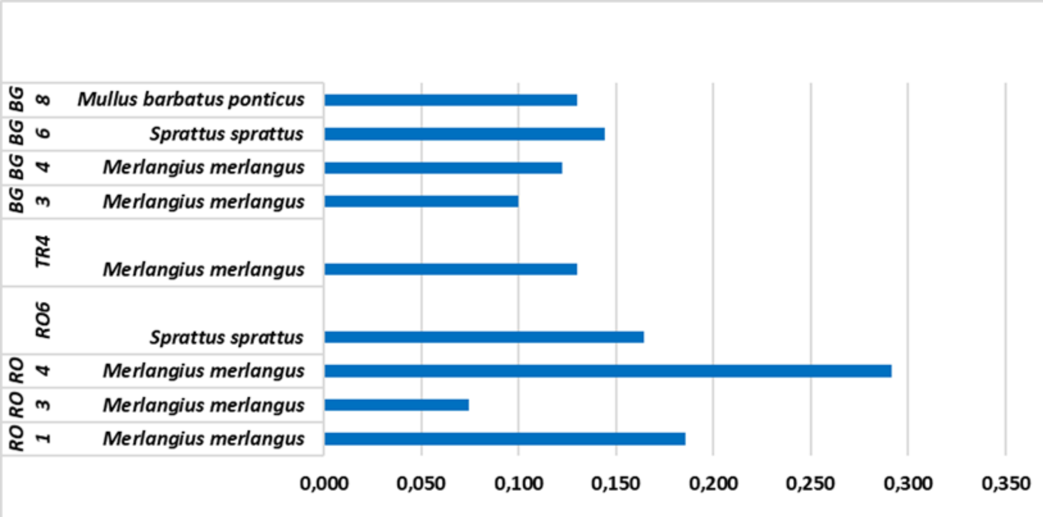


Figure 1.37 - Fish egg density in Romania, Bulgaria, and Turkey marine waters

Ichthyoplankton was present in 8 stations in the larval stage, the dominant species being whiting. On the surface of the continental platform of Romania it was reported in 3 stations, with the maximum density of 0.148 ind/ m^3 in RO-1 station, with a depth of 70 m (Figure 1.38).
In the Bulgarian marine area, only red mullet larvae were identified in the analyzed samples, recording a maximum density of 0.250 ind/m^3, in the BG-3 station with a depth of 52 m (Figure 1.38).
Two species of fish larvae being represented by whiting and red mullet were identified in Turkish marine waters. From all the analyzed samples, whiting larvae recorded a maximum density value of 0.309 ind/m^3 (Figure 1.38).

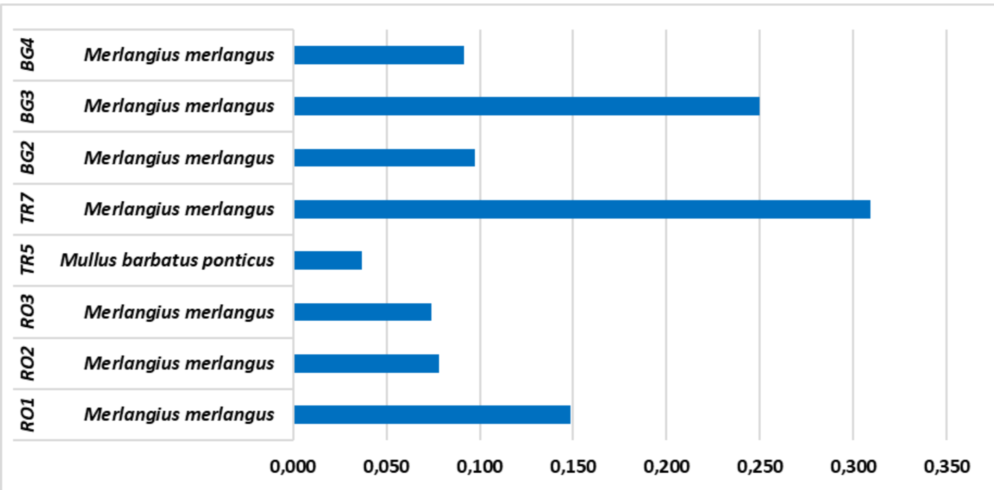


Figure 1.38 - Fish larvae density in Romania, Bulgaria, and Turkey marine waters

Conclusions

The qualitative structure of ichthyoplankton in samples collected in October 2019 included eggs and larvae of *Merlangius merlangus*, and *Mullus barbatus ponticus* and a low number of *Sprattus sprattus* eggs, the dominant species being the whiting.
The maximum value of the eggs density was 0.292 ind/m^3 (whiting), in RO-4 station, and the minimum value was 0.074 ind / m^3 (red mullet), in RO-3 station.
The density of fish larvae reached the maximum value in TR-7 station, 0.309 ind / m^3 (whiting), and the minimum value was 0.037 ind/m^3 (red mullet), in TR-5 station.
The qualitative and quantitative structure of eggs and larvae was determined by the net used for collection and the direction of collection (vertical), the period of the expedition, the environmental conditions of this period, the number of stations and their locations.

1.2 Benthic Habitats

1.2.1 Introduction

Considering the marine environment as a precious heritage that must be protected, preserved and where possible, restored to maintain biodiversity and ensure that oceans and seas are clean, healthy and productive, the European Parliament and Council adopted in 2008 the Marine Strategy Framework Directive (Directive 2008/56/EC). The results of the initial assessment carried out by the EU Member States in 2012 showed the necessity to significantly improve the quality and coherence of the determination of good environmental status (GES) by the Member States and at the same time the importance of regional cooperation to this end. Therefore, in 2017 EC adopted a new decision (COM DEC 2017/848) laying down the criteria and methodological standards on GES of marine waters.
The establishment and strengthening of sustainable networks and cooperation platforms between regional partners, capable of providing a real contribution in addressing priorities of common concern related to marine environment monitoring and protection represent the main objective of the ANEMONE project. In this context, joint marine monitoring initiative aims at the exchange of best practices and use of harmonized new methodologies, with the final goals of filling the knowledge gaps and improving the availability of cross-border compatible environmental monitoring data and information within the Black Sea Basin among scientists, general public and relevant stakeholders.
Concerning benthic habitats, the joint monitoring initiative tested the common indicator M-AMBI*(n) and identified the needs for further development of classification systems for different habitat sub-types occurring in the marine waters of the Black Sea countries, and the elaboration of pressure indicators, allowing better assessment of GES in the region.

1.2.2 Assessment concept – a general framework

The European criteria and methodological standards for assessing the marine environmental status, including of benthic broad habitat types, are provided by COM DEC 2017/848. The Decision sets out the following criteria to be used for benthic habitats:

- D6C1 Physical loss
- D6C2 Physical disturbance
- D6C3 Adverse effects of physical disturbance on habitats
- D6C4 Extent of habitat loss
- D6C5 Extent of adverse effects on the condition of a habitat

The COM DEC EU/2017/848 criteria and standards were transposed to the Black Sea Monitoring and Assessment Guideline (BSMAG), developed under ANEMONE project to outline a regionally harmonized framework for assessing the Black Sea environmental status, including its benthic habitats.
The current investigation addresses the assessment of benthic habitats overall extent of adverse effects under criterion D6C5. The evaluation of the pressures and the extent of habitat loss are not in the scope of this study. The assessment under criterion D6C5 has two aspects: (1) evaluation of the quality (adverse effects) of the habitat, and (2) evaluation of the spatial extent over which the habitat is in good or degraded quality.
The adverse effects on benthic habitats and the associated typical invertebrate communities from the impact of overall pressures in a given location (sampling point) are assessed using the common indicator M-AMBI*(n). The index is proven as indicative of the impact of the combined predominant pressures (eutrophication and pollution from point and diffuse sources) occurring in the Bulgarian and Romanian coastal waters (Todorova et.al, 2018). However, it still needs validation against the physical disturbance pressure over the Black Sea shelf. The common good status threshold for AMBI*(n) was derived in the intercalibration exercise carried out under the Water Framework Directive (WFD) and accepted with Commission Decision EU/2018/229.
The extent to which good environmental status is achieved is expressed as an estimate of the proportion of adverse effects per broad habitat type and whether this has achieved the extent threshold value. The regionally agreed threshold proposed in BSMAG was set at 15 % maximum allowable extent of adverse effects as a proportion of the total natural extent of the habitat type in a particular marine reporting unit (MRU).

Assessments of seabed habitats require the use of maps of habitat types as a prerequisite to estimate the extent of each habitat, which is adversely affected. A predictive map of seabed habitats, covering all MSFD regions, including the Black Sea, is provided by the European project EMODnet Seabed Habitats according to the EUNIS typology, and also aggregated to MSFD broad habitat types (Figure 1.39).

The extent of each habitat in good or not good status was estimated using GIS approaches described in more detail further in the methods.

The MRU for which the evaluation is made includes the joint survey area over the shelf of Romania, Bulgaria and Turkey enclosed between the 40-110m isobaths (Figure 1.40). Additionally, each country assessed the benthic habitats within their respective national MRUs covered by the joint cruise sampling network: southern shelf in the Bulgarian EEZ, shelf waters in the Romanian shelf and shelf waters in the Turkish marine area.

The Bulgarian southern shelf is delimited by the 30-200 m isobaths in West-East direction, the EEZ boundary with Turkey and a line transversal to the Bulgarian coastline south of c. Galata (Figure 1.39). This assessment area is distinguished from the northern Bulgarian shelf by smaller width and decreasing influence by the Danube inflow. There are four benthic broad habitat types present on southern Bulgarian shelf seabed: the predominant is offshore circalittoral mud, which occupies over 54 % of the total seabed, followed by circalittoral mud and offshore circalittoral mixed sediments, while circalittoral mixed sediments are negligible (Table 1.11). The mixed sediments contain significant biogenic fraction of shell rubble, hash and sand of different size.

The Romanian MRU includes marine waters between 30 and 200m isobaths on continental and outside continental shelf along the Romanian coast delimited by the average seasonal and annual salinity in the range of 16-17.5 PSU. Similar to the Bulgarian shelf, there are four benthic broad habitat types present on the Romanian shelf seabed: the predominant are offshore circalittoral mud and offshore circalittoral mixed sediments, while circalittoral mud and circalittoral mixed sediments are negligible (Table 1.11). The mixed sediments contain mainly biogenic fraction represented by broken mollusc shells.

The Turkish shelf MRU (TR-KARD1 west BS1 shelf) is delimited by the 10 and 200 m isobaths in west direction. According to EMODnet habitats map, there are two benthic broad habitat types present on the Turkish shelf seabed: circalittoral mud and offshore circalittoral mud.

The broad habitat types under D1, 6 are integrated at MRU scale by estimating the proportion of broad habitat types in good status from the number of all habitats present. Proportion thresholds is recommended by BSMAG to be set at 100 % (all habitats present in given MRU shall be in good status). As an exception, one of the broad habitat types is allowable to be in "not good" status in a particular MRU on the conditions that < 15 % of its national extent is in "not good" status and < 15 % of overall seabed in the MRU is in "not good" status.

1.2.3 Material and methods

To test the framework for assessing benthic habitats provided by BSMAG, a joint survey was carried out in the Romanian, Bulgarian and Turkish shelf areas at designated sampling stations for macrozoobenthos, selected to allow the collection of representative data to assist the Black Sea environment assessment at sub-regional scale.

In total, 21 stations were established on Romanian, Bulgarian and Turkish shelf, distributed as follows: 6 in Romania, 8 in Bulgaria and 7 in Turkey. Most of the stations were located at depths between 48 and 106m, except for one station on the Turkish shelf located at 35m depth (Figure 1.39). From each station, the teams from the three countries collected and processed a replicate sample using the Van Veen grab with 0.1 m^2 surface, according to the common protocol. For all sampling stations, photos of benthos samples have been taken to show the sediment structure.

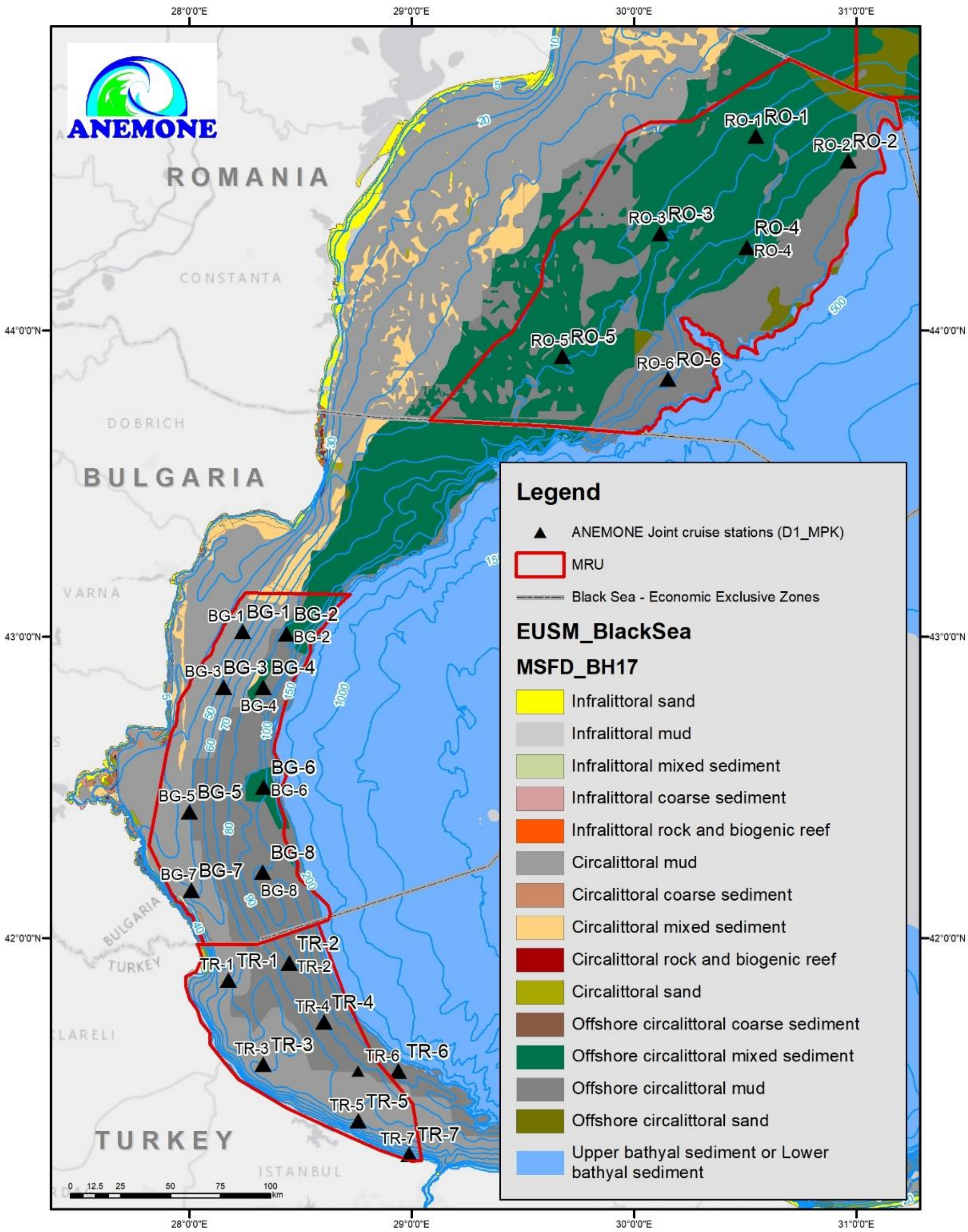


Figure 1.39 - Map of the sampling network in the joint cruise superimposed on the broad habitat types identified in EUSeaMap project (EMODnet)

Collected samples were pre-washed onboard through 0.5mm mesh size for removal of the excess sediment and subsequently preserved with formaldehyde 4% buffered in seawater and stored in plastic containers for further processing in the laboratory. A visual description of each sample was done to identify the predominant sediment types and possible main benthic communities.
In laboratories, all benthic organisms were identified to the lowest possible taxonomic level, according to the agreed regional methodology (Todorova & Konsulova, 2005) and the resulted data were included in a common database built on the EMODnet format.
These data were used to assess the environmental status of both circalittoral and offshore circalittoral broad habitat types in the sampled area. To this end, despite the ecological differences between shelf areas of the three countries (biological, substrate and depth range), hypothetically it was considered as one assessment area.
The structure of macrozoobenthic communities was analyzed in terms of species composition,

density, dominance, diversity and biomass. The AZTI Marine Biotic Index, AMBI (Borja et al., 2000) was calculated using the version 2019 of the free software available at www.azti.es. Same software automatically performed the calculation of the diversity index Shannon-Weaver (H') and species richness (N). The results of the three indices described above were imported in excel and multivariate M-AMBI*(n) index (Sigovini et al., 2013) was calculated by averaging their normalized values.
PRIMER version 7 was used to identify the main benthic communities in the assessment area by running Bray-Curtis similarity analysis on the fourth root transformed abundance data. Prior to the analysis, data were averaged between the three replicate samples collected in all 21 stations, for better representation of the benthic communities. Also, SIMPER analysis was performed to identify the percentage contribution of each species to the overall similarity (dissimilarity) within each of the two major groups identified from the cluster analysis.
The ecological status of the circalittoral and offshore circalittoral identified in the assessment area was evaluated using M-AMBI*(n) index based on the classification systems developed for Romania (Abaza et al., 2018) with respective thresholds, which were re-calculated using the data gained in the period 2014-2019, including the data from this joint cruise. The classification systems were developed following the approach agreed by the member states Bulgaria and Romania in the intercalibration exercise under the WFD (Todorova et. al., 2018) and the GES threshold was defined in accordance with Commission Decision EU/2018/229.
The classification systems developed for two widespread habitats on the Romanian shelf were applied to calculate the ecological status of the main communities identified in our assessment area, namely:

- Circalittoral muds and shelly muds with *Mytilus galloprovincialis* (Table 1.7) to the habitat Circalittoral and Offshore circalittoral shelly mud with pelophitic bivalves and polychaetes;
- Offshore circalittoral mixed sediments with *Modiolula phaseolina* (Table 1.8) to the second habitat.

Table 1.7 - Updated classification system used for assessment the ecological status of Circalittoral and Offshore circalittoral shelly mud with pelophitic bivalves and polychaetes

	AMBI	**H**	**S**	**M-AMBI*(n)**	
RefCond (Baseline)	0.9	3.6	30	0.92	
	EQR AMBI	EQR H	EQR S	EQR M-AMBI*(n)	MSFD
High	0.9	0.9	0.9	0.9	good
Good	0.68	0.68	0.68	0.68	
Moderate	0.45	0.45	0.45	0.45	not good
Poor	0.23	0.23	0.23	0.23	
Bad	> 0.23	< 0.23	< 0.23	< 0.23	
	AMBI	H	S	M-AMBI*(n)	
High	1.41	3.24	27	0.83	good
Good	2.53	2.45	20	0.63	
Moderate	3.70	1.62	14	0.41	not good
Poor	4.83	0.83	7	0.21	
Bad	>4.83	<0.83	<6	<0.20	

Table 1.8 - Updated classification system used for assessment the ecological status of Offshore circalittoral mixed sediments with Modiolula phaseolina

	AMBI	**H**	**S**	**M-AMBI*(n)**	
RefCond (Baseline)	0.7	4.1	39	0.91	
	EQR AMBI	EQR H	EQR S	EQR M-AMBI*(n)	MSFD
High	0.9	0.9	0.9	0.9	good
Good	0.68	0.68	0.68	0.68	
Moderate	0.45	0.45	0.45	0.45	not good
Poor	0.23	0.23	0.23	0.23	
Bad	> 0.23	< 0.23	< 0.23	< 0.23	
	AMBI	H	S	M-AMBI*(n)	
High	1.23	3.69	35	0.82	good
Good	2.40	2.79	27	0.62	
Moderate	3.61	1.85	18	0.41	not good
Poor	4.78	0.94	9	0.21	
Bad	>4.78	<0.83	<9	<0.22	

EQR M-AMBI*(n) index was calculated for each sampling station, the results were included into ANEMONE geodatabase and represented on map as points. In order to obtain a continuous distribution of EQR M-AMBI*(n) results, the punctual values were interpolated using ArcGIS tools (Spline with barriers tool), resulting a raster with 1.5 x 1.5 km resolution. The Spline with barriers tool applies a minimum curvature method, as implemented through a one-directional multigrid technique that moves from an initial coarse grid, initialized in this case to the average of the input data, through a series of finer grids until an approximation of a minimum curvature surface is produced at the desired row and column spacing. The raster cell values were included into two classes according to the threshold established for EQR M-AMBI*(n) and symbolized using colours: green for GOOD and red for not-GOOD. The results were superimposed on the EmodNET Seabed Habitats (2019), MSFD Benthic Broad Habitat Types layer based on EUSeaMap (2019) and the percent of each targeted habitat in GOOD or not-GOOD status was calculated.
Furthermore, as an exercise Turkish Benthic Index (TUBI), an index developed to assess the impact of organic enrichment on soft-bottom benthic community structure in Turkey (Çinar et al., 2015), although not calibrated yet for the Black Sea ecological specificities was used for the assessment. The index is based on two metrics: the Shannon-Weaver's diversity index (metric 1) and the relative abundance of ecological groups (metric 2). Scores of TUBI vary between 0 and 5, and the benthic quality status increases with increasing TUBI scores. EQRTUBI is used to define the class boundary values by dividing the continuum of metric values into five equal width classes.

1.2.4 Results

In the studied area, after samples' processing, 133 taxa were found, most of them identified by species level, while others were considered at supra-specific level. The aim of the present study is to identify the broad habitat types and specific habitats (subtypes), assess their ecological status using the common index agreed between Bulgaria and Romania (M-AMBI*(n) and determine the percentage of affected habitats in the assessment area hypothetically considered on Romanian, Bulgarian and Turkish shelf, according to the requirements of the MSFD Decision 848/2017.

Benthic broad habitats and specific habitats (sub-types) in the assessment area

On the shelf area, mainly sedimentary broad habitat in circalittoral and offshore circalittoral types were targeted by ANEMONE joint cruise. The samples were collected at depths ranging between 67 and 106m on the Romanian shelf, 48-103m on the Bulgarian shelf and 70-90m on Turkish shelf, except for one station located at 35m depth. The sediments were generally muds mixed with sands of different grain size and broken mollusk shells. Bray-Curtis similarity group average based on fourth rout transformed abundance data in all stations showed five similarity groups in the study area. SIMPER analysis on depth ranges of the stations intended to further define the five similarity groups previously identified by Bray-Curtis, but the results turned to be inconclusive, due to the differences in sediment structure determining differences biological communities' structure, too. Therefore, a new SIMPER analysis was made on two similarity groups based on dominating species: one was *Modiolula phaseolina* clearly dominating the offshore circalittoral mixed sediments on the Romanian shelf at depths ranging between 67 and 106m, permanently associated with fauna rich in polychaetes, especially *Terebellides stroemii* showing small variation in abundance ratios of the dominant species on mixed sediments situated deeper than 100m (Figure 1.40).
A similarity group made up of Bulgarian and Turkish stations situated at depths of 86-96m (BG) and 75-90m (TR) was included in this habitat, too. This group was defined as Offshore circalittoral mixed sediments with *Modiolula phaseolina*.
The other two smaller similarity groups, more diverse, were linked together by the domination of polychaetes, especially *Aricidea claudiae*, *Terebellides stroemii* and *Nephtys hombergii* and differentiated by the dominating bivalves: *Pitar rudis* for Bulgarian shelf at depths of 48-60m and *Abra alba* for Turkish shelf at 70-90m (TR2, TR3, TR6).

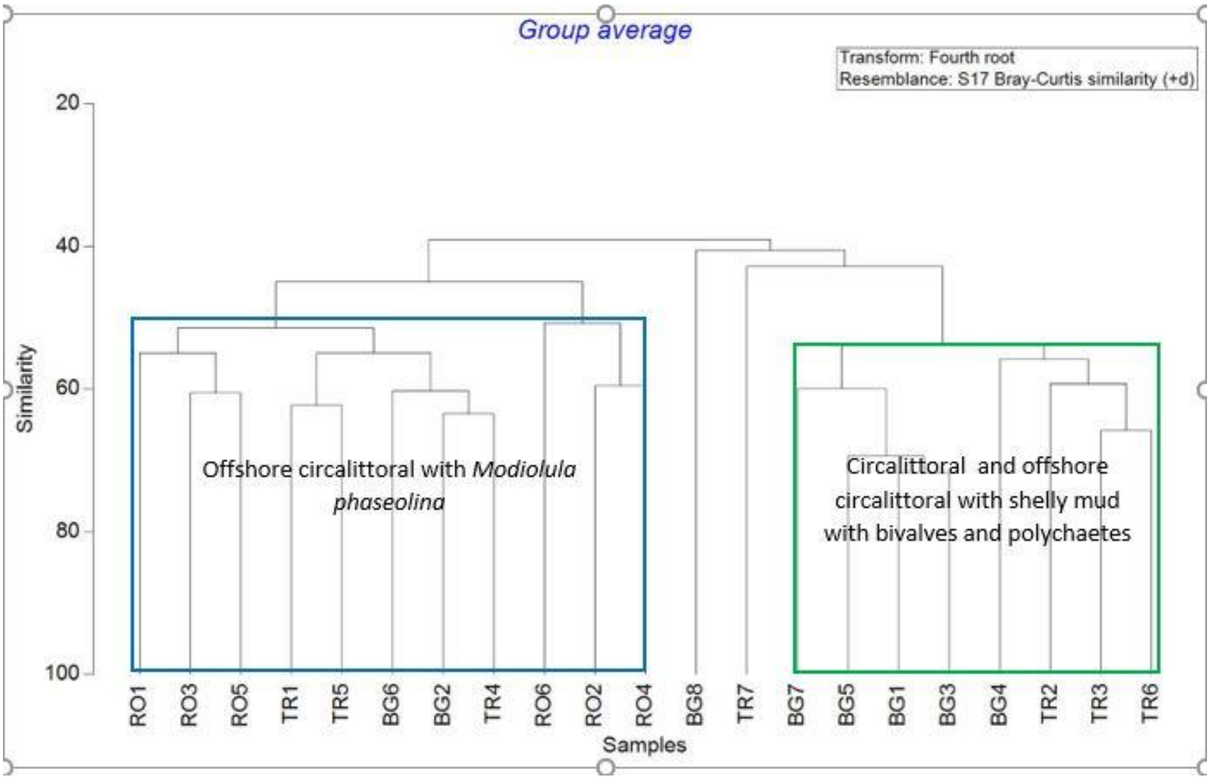


Figure 1.40 - Bray-Curtis similarity on fourth root transformed abundance data in the assessment area

Assessment of the ecological status of broad habitat types on the Black Sea western shelf based on M-AMBI*(n) index

Among the 21 sampled stations, 9 were considered in the Circalittoral and Offshore circalittoral shelly mud with pelophitic bivalves and polychaetes habitat; five of them situated in Bulgarian waters and four in Turkish waters. Results of the calculations of M-AMBI*(n) expressed in EQR showed all the stations in good ecological status (Figure 1.41), EQR values ranging between 0.77 and 1.12 (Table 1.9). The exception was represented by the station TR7 (35m), its EQR value being very close to the established threshold (0.68), proving to be very different from the others in terms of physio-geographic conditions (Figure 1.41). In this station and in the area around it the possible pressure sources that can contribute to the natural conditions' deterioration are not known, as data on pressures are not available for analysis. Most probably, being close to the shore is impacted by different anthropogenic activities occurring in the area. Nevertheless, considering the heterogeneity of this habitat type, it's necessary the collect more data and develop classification systems for specific emerging habitat subtypes.

Table 1.9 - Results of the assessment of ecological status of Circalittoral and Offshore circalittoral shelly mud with pelophitic bivalves and polychaetes based on M-AMBI(n) index and EQR*

Stations	Depth	AMBI	H	S	M-AMBI(n)	EQRAMBI	EQRH	EQRS	EQRM-AMBI
BG1	49m	1.7443	2.8524	44	1.03	1.94	0.79	1.47	1.12
BG3	60m	2.0066	3.4791	40	1.03	2.23	0.97	1.33	1.12
BG4	90m	3.5709	3.0243	24	0.71	3.97	0.84	0.80	0.77
BG5	49m	2.0919	2.5681	39	0.93	2.32	0.71	1.30	1.01
BG7	48m	3.1404	2.8156	29	0.77	3.49	0.78	0.97	0.84
TR2	90m	1.8515	2.7049	22	0.77	2.06	0.75	0.73	0.83
TR3	70m	1.9807	2.992	41	1.00	2.20	0.83	1.37	1.08
TR6	87m	2.7165	3.5195	35	0.93	3.02	0.98	1.17	1.01
TR7	35m	3.0997	2.138	22	0.63	3.44	0.59	0.73	0.69

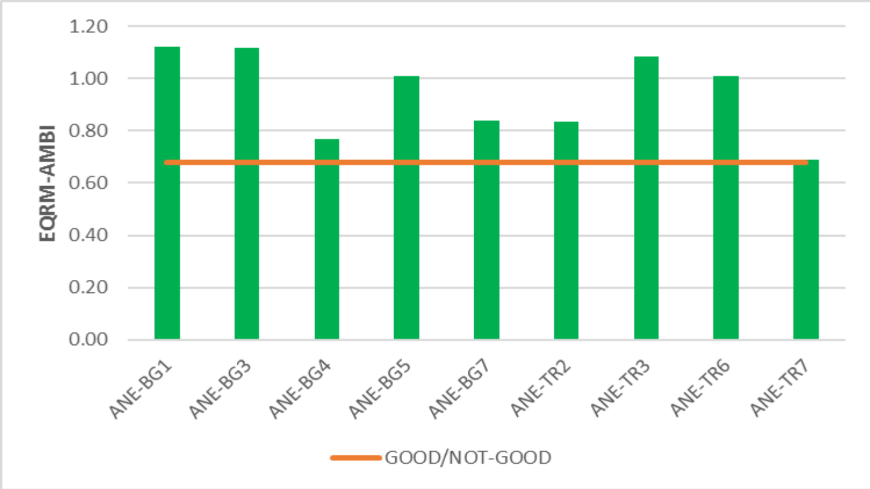


Figure 1.41 - Assessment results of Circalittoral and Offshore circalittoral shelly mud with pelophitic bivalves and polychaetes based on EQRM-AMBI

Assessment of the second habitat situated at depths ranging between 67 and 106m, comprising all six Romanian stations, three Bulgarian and three Turkish stations showed almost all of them in good status, except for one (BG8-103m) (Table 1.10). Generally, the EQR values for M-AMBI ranged between 0.88 (RO4) and 1.22 (RO5). If we compare the station in not-good status with those situated on the Romanian shelf at the same depth, it appears that the habitat is seriously disturbed (Figure 1.42). It might be that, but we cannot be sure due to the lack of data on eutrophication or other types of pressure present in the area or the habitat may be naturally different from those it was assessed with. Usually, at these depths, any anthropogenic pressures occur with lower intensity, and therefore we assume that the deterioration of habitat is rather natural. So, in order to a more thoroughly assessment, other classification system must be elaborated and used.

Table 1.10 - Results of the assessment of ecological status of Offshore circalittoral mixed sediments with Modiolula phaseolina based on M-AMBI*(n) index and EQR

Stations	Depth	AMBI	H	S	M-AMBI(n)	EQRAMBI	EQRH	EQRS	EQRM-AMBI
RO1	77m	1.992	3.8749	30	0.82	2.85	0.95	0.77	0.90
RO2	106m	1.2654	4.0521	22	0.82	1.81	0.99	0.56	0.90
RO3	76m	1.0277	3.1988	31	0.84	1.47	0.78	0.79	0.92
RO4	106m	1.2917	3.9736	21	0.80	1.85	0.97	0.54	0.88
RO5	67m	2.0457	4.7842	55	1.11	2.92	1.17	1.41	1.22
RO6	101m	1.0931	3.2371	38	0.90	1.56	0.79	0.97	0.99
BG2	86m	1.5121	4.1119	43	0.98	2.16	1.00	1.10	1.08
BG6	96m	1.769	4.591	40	0.98	2.53	1.12	1.03	1.08
BG8	103m	3.3062	3.0479	14	0.54	4.72	0.74	0.36	0.59
TR1	75m	2.3796	3.1352	31	0.75	3.40	0.76	0.79	0.82
TR4	89m	1.9558	4.191	30	0.85	2.79	1.02	0.77	0.94
TR5	77m	2.6527	4.2552	44	0.93	3.79	1.04	1.13	1.02

Based on these results, we tried to estimate the extent of the assessment area physically disturbed and the extent of the habitat adversely affected in the assessment area and to compare it with the proposed threshold at regional level (15%). This value was proposed as an intermediate value of the natural habitat extent adversely affected, including habitat loss, to be considered as an acceptable degree of change for the broad habitat type under criterion D6C5. Given the uncertainty of the assessment's outcome and considering that the pressures that might be present in the assessment area were not analyzed, and the eutrophication status is unknown too, the assessment of the natural habitat extent adversely affected is also uncertain. We only suppose that they are low. Scientific literature on the impact of anthropogenic pressures on benthic habitats' integrity is very scarce in the Black Sea region. Teaca et al. (2019) analyzed the impact of sand extraction and bottom trawling activities on the circalittoral benthic habitats on the Romanian shelf and found that this is significantly high. The results of this study show changes in benthic communities in terms of diversity, quantitative parameters (abundance and biomass) and dominance of functional groups, suggesting that sandy habitats recover slowly than the muddy ones, benthic fauna being dominated by opportunistic and disturbance-tolerant species.

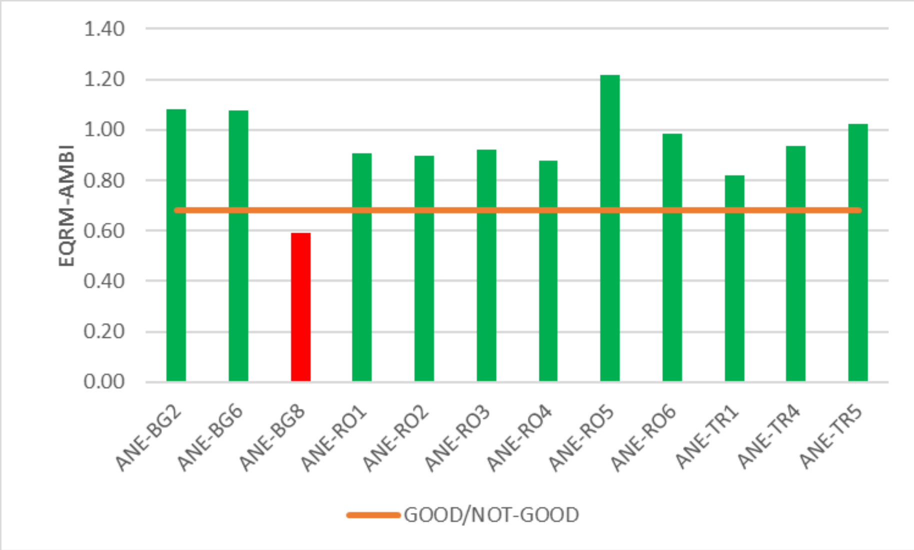


Figure 1.42 - Assessment results of Offshore circalittoral mixed sediments with Modiolula phaseolina based on EQRM-AMBI

Boundaries of the assessment area were built based on bathymetry and distribution of sampling points, covering the circalittoral zone, as follows: from 60 to 110m depth for Romanian shelf, and 40 m to 110 m for Bulgaria and Turkey. Distribution of the three circalittoral broad habitat types (circalittoral mud, offshore circalittoral mud and offshore circalittoral mixed sediments) was obtained through spatial analysis considering the data from sampling stations. First, EQRM-AMBI*(n) for all stations was intersected with the polygons of the broad habitat types from EUSeaMap. Subsequently, areas and proportion of habitats in good and not good status was calculated (Table 1.11; Figure 1.43).

Table 1.11 - Proportion of adversely affected broad habitat types in the marine reporting units based on the results of EQRM-AMBI*(n)

Assessment area/Broad habitat type	Area (km^2)	% Coverage	GOOD (% habitat)	not GOOD (% habitat)
ANEMONE overall survey area				
Circalittoral mud	7080	17	100	0
Offshore circalittoral mixed sediment	14960	37	100	0
Offshore circalittoral mud	14968	37	93	7
Other type	307	1	not assessed	
TOTAL	37315		97.5	2.5
Romania				
Circalittoral mud	95	0.45	100	0
Offshore circalittoral mixed sediment	13561	64.86	100	0
Offshore circalittoral mud	7015	33.55	100	0
Other type	238	1.14	not assessed	
TOTAL	20909		100	
Bulgaria				
Circalittoral mud	2954	31.29	100	0
Offshore circalittoral mixed sediment	1399	14.82	100	0
Offshore circalittoral mud	5107	54.10	80.03	19.97
Other type	69	0.73	not assessed	
TOTAL	9529		89.30	10.70
Turkey				
Circalittoral mud	4031	58.62	100	0
Offshore circalittoral mixed sediment	0	0.00	100	0
Offshore circalittoral mud	2846	41.38	100	0
Other type	0	0.00	not assessed	
TOTAL	6877		100	0

Summarized results of the habitats area and extent adversely affected is presented in Table 1.11. Within the overall ANEMONE survey area, the broad habitat types Circalittoral mud and Offshore circalittoral mixed sediment are in good status over 100% of their extent. Offshore circalittoral mud is adversely affected in 7% of its extent.

In the Bulgarian southern shelf area, the predominant habitat type Offshore circalittoral mud is adversely affected over nearly 20% of its extent, which equals to 10.7 % of the MRU seabed area. The extent threshold of maximum 15 % adversely affected is exceeded, therefore the habitat is assessed in not good status. The rest of the habitat types are in good status over 100 % of their extent. Overall, southern shelf is assessed in good status as regards the benthic habitats, since the overall threshold of < 15 % adversely affected over the entire MRU seabed is not exceeded.

It is important to point out that the extent of Offshore circalittoral mud adversely affected is estimated based on single sampling point (BG8), which is below EQRM-AMBI*(n) threshold, the result being extrapolated over 1.5 x 1.5 km cell using GIS tool. In the first place, it is uncertain if the point is representative of the whole cell of this size. Thus, increased sampling resolution would increase the assessment precision of the extent adversely affected. Secondly, the sampling depth at 103m suggests that the environmental conditions are possibly deteriorated due to natural conditions – hypoxia in the Black Sea below 100 m. Specific thresholds may be required to be developed for the peripheral shelf according to the characteristic natural conditions.

In the Romanian and Turkish shelf areas all present habitat types are in good status over 100 % of their extent in the respective assessment area. Therefore, both the Romanian and Turkish MRUs are assessed in good status as regards the Benthic habitats.

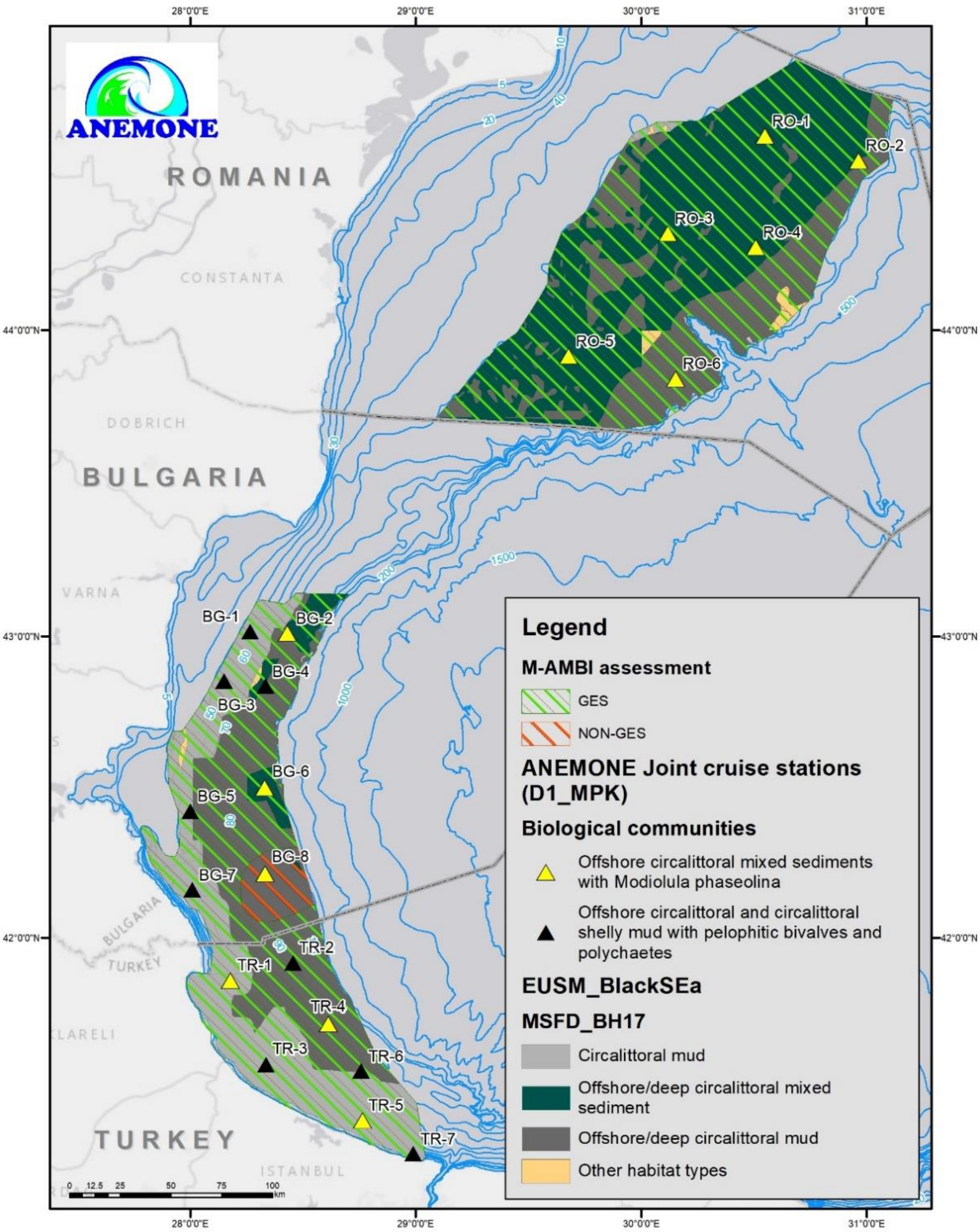


Figure 1.43 - Ecological status in the assessment area based on EQRM-AMBI(n)*

Assessment of ecological status of broad habitat types on the Black Sea western shelf based on TUBI index

Turkish Benthic Index (TUBI) values among the stations ranged between 1.43 (TR7) and 4.21 (RO5). The mean values of TUBI in stations and the ecological status of stations are indicated in Figure 1.44. In the assessment area, station TR7 had the lowest TUBI score, thus classifying the benthic quality status of the water body as poor, 14 stations possessed TUBI scores that indicated moderate ecological status and two stations on the Bulgarian coast (BG2, BG6), one station on the Romanian coast (RO2) and two stations in Turkish coast (TR4, TR5) showed good quality status. Only ANE RO5 station had high mean TUBI value (4.1±0.1) indicating high ecological status (Figure 1.44). As only good and high ecological status can be assimilated to good status in MSFD, while moderate and poor ecological status are not-good, according to the results of this index, the most part of the assessment area can be categorized into a not-good environmental status.

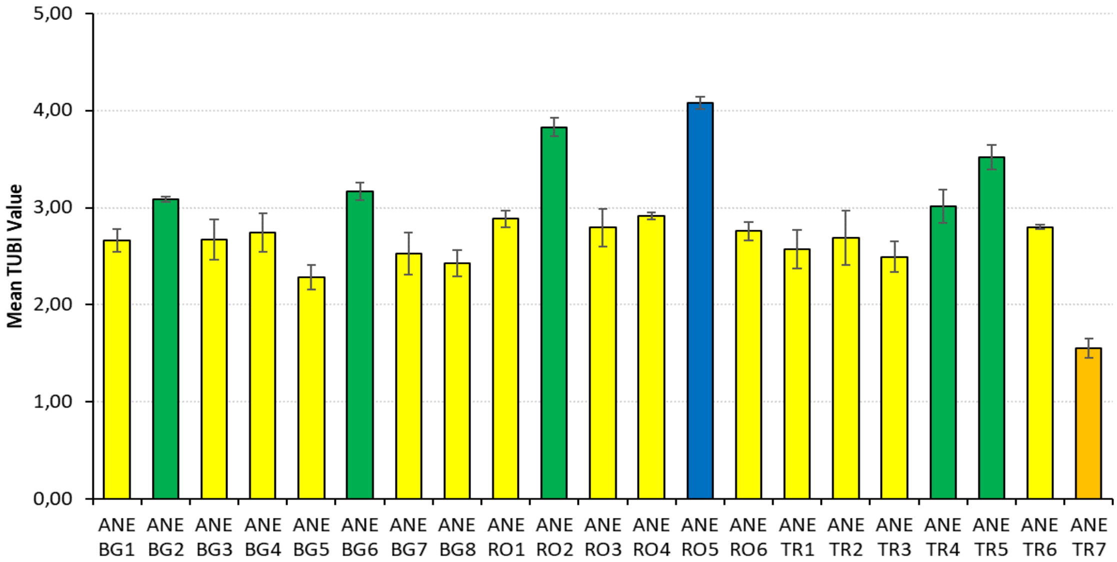


Figure 1.44 - Mean TUBI values in the assessment area

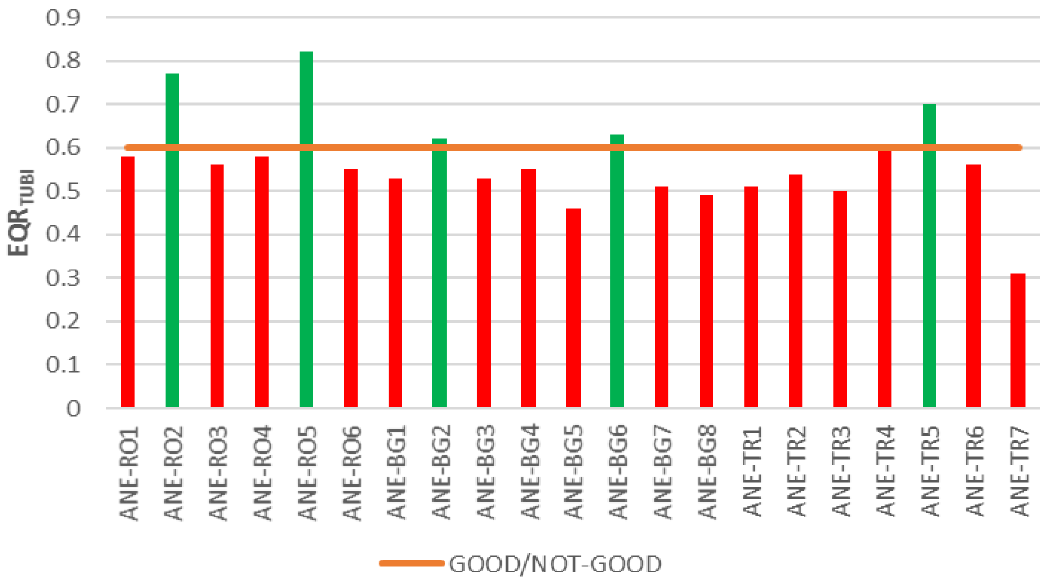


Figure 1.45 - Assessment of ecological status of benthic habitats in the assessment area based on EQRTUBI

According to TUBI results, most part of the assessment area is in not-good status, except for 5 out of 21 stations, distributed as follows: two in Romania, two in Bulgaria and one in Turkey (Figure 1.45). The percentages of the ecological groups of the species determined in the analyzed stations are given in the Figure 1.46. In the calculation of TUBI, three major ecological groups were considered; Group 1 includes sensitive (GI) and indifferent species and (GII), Group 2 includes tolerant species (GIII), and Group 3 includes second order opportunistic species (GIV) and first-order opportunistic species (GV). The GIII ecological group at stations on the Bulgarian coast has high rates (> 50%) (except for BG2). The ecological groups GI and GII were represented at high rates at the stations on the Romanian coast. The percentage of ecological groups of the stations on the coast of Turkey was various. In the station ANE TR7, the rate of GV ecological group (first-order opportunistic species) was found ~ 50%. The GIII ecological group, which includes tolerant species, was represented in more than 50% of seven stations in the research area (Figure 1.46).

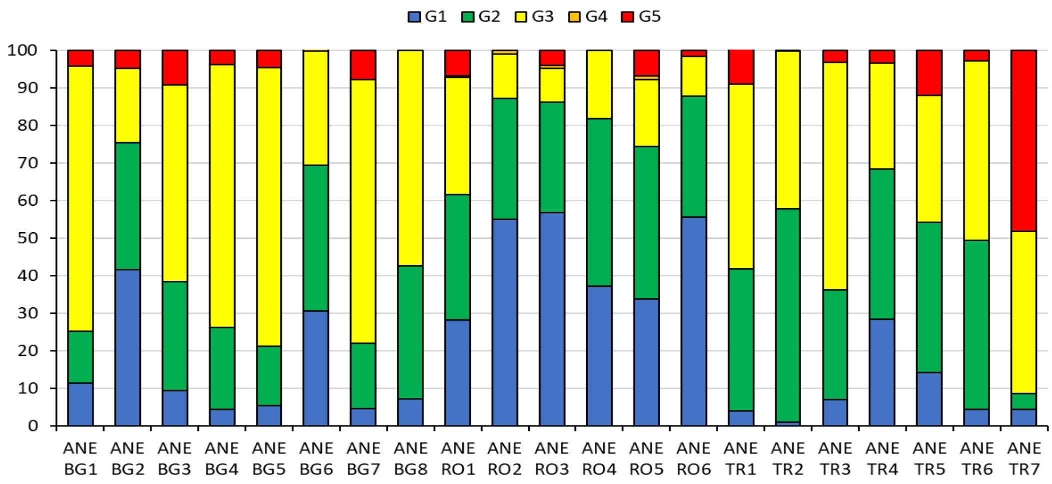


Figure 1.46 - Percentage of ecological groups detected in the sampling stations

Given the significant difference between the two indices used to assess the ecological status of benthic habitats in the assessment area, it is necessary to further calibrate TUBI index with pressures occurring in the Black Sea, and its physical, chemical, and biological features.

Conclusions

Analysis of macrozoobenthos data collected during the ANEMONE joint cruise resulted in identification of 133 taxa, most of them determined at the species level. Similarity analysis on their abundances evinced two communities distributed over three broad habitat types: Circalittoral and Offshore circalittoral shelly mud with pelophitic bivalves and polychaetes and Offshore circalittoral mixed sediments with *Modiolula phaseolina*. These communities and habitats were distributed as follows: on the Romanian shelf occurred only mixed sediments with Modiolula, while on Bulgarian and Turkish shelf both community sub-types occurred.
Application of the classification systems of M-AMBI*(n) index developed for the Romanian circalittoral and offshore circalittoral habitats have been used to assess the ecological status of the two main communities identified in the assessment area consisting of all stations sampled during the ANEMONE joint cruise. Then the assessment was transposed to the broad habitat types identified by EUSea Map. Assessment results revealed that only 3% of the Offshore circalittoral mixed sediments with *Modiolula phaseolina* was adversely affected, while the circalittoral mud and offshore circalittoral mud were in good status in 100 % of their area. Analysis of pressures on the seafloor integrity resulted from anthropogenic activities could not be performed and integrated with M-AMBI*(n) results due to lack of data in the assessment area.
Obviously, the assessment results are inconclusive, leaving room for interpretation due to:

- differences in physio-geographical areas, leading to necessity of properly identification of habitat sub-types and elaboration of respective classification systems with baseline and threshold values for GES;
- lack of data on pressures.

1.2.5 Recommendations

- Further development of M-AMBI*(n) classification systems for the habitat sub-types identified by the Black Sea countries as ecological relevant for GES assessment and validation of the index for the physical disturbance pressure.
- Identification of pressures derived from anthropogenic activities acting in the Black Sea region.
- Development of pressure indicators.
- Development of specific indicators for the impact from physical disturbance.
- Calibration of TUBI Index for the Black Sea specificity.

1.3 Marine mammals (dolphins and porpoises)

The cetacean fauna of the Black Sea includes three species/subspecies – the Black Sea harbour porpoise (*Phocoena phocoena relicta*), the Black Sea common dolphin (*Delphinus delphis ponticus*) and the Black Sea bottlenose dolphin (*Tursiops truncatus ponticus*). All three species, drastically affected by commercial killing in the 20th century, are exposed to ongoing anthropogenic threats which may cause increased mortality and morbidity, disturbance, habitat deterioration and depletion of food resources.

Coastal states have assumed international obligations to protect Black Sea cetaceans as the contracting parties to several international instruments (listed in chronological order): the International Convention for the Regulation of Whaling (1946); the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 1973); the Convention on the Conservation of Migratory Species of Wild Animals (CMS, 1979); the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention, 1979); the United Nations Convention on the Law of the Sea (UNCLOS, 1982); the United Nations Convention on Biological Diversity (CBD, 1992); the Convention on the Protection of the Black Sea Against Pollution (Bucharest Convention, 1992); and the Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and Contiguous Atlantic Area (ACCOBAMS, 1996). In addition, all three species are included in the European Marine Strategy Framework Directive (MSFD) and also are covered by Annex IV of the European Habitats Directive and therefore require strict protection by EU member states.

In 2002, all three Black Sea cetacean species were listed as "Endangered" (EN) in the Provisional List of Species of the Black Sea Importance, an annex to the Black Sea Biodiversity and Landscape Conservation Protocol of the Bucharest Convention. In 2007, the ACCOBAMS contracting parties adopted the conservation status of Black Sea populations of the harbour porpoise, common dolphin and bottlenose dolphin as "Endangered" (EN) as well. In 2008, Black Sea cetaceans were included in the IUCN Red List of Threatened Animals:

- Black Sea harbour porpoises – "Endangered" (EN),
- Black Sea common dolphins – "Vulnerable" (VU), and
- Black Sea bottlenose dolphins – "Endangered" (EN).

The European Marine Strategy Framework Directive (MSFD) aims at implementing a precautionary and holistic ecosystem-based approach for managing European marine waters. Marine mammals are included as a functional group for the assessment and reporting under Descriptor 1 - Biodiversity. Conservation of mobile marine megafauna such as cetaceans requires transboundary cooperation, which the MSFD promotes through regional instruments, such as the Regional Sea Conventions and other regional cooperation structures such as Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and Contiguous Atlantic Area (ACCOBAMS) and Black Sea Commission (BSC).

Taken together the above instruments represent a formidable framework for the conservation of Black Sea cetaceans, nevertheless major gaps in knowledge burdens the process of assessing the Good Environmental Status for the three species.

In the last years, small steps were done for synergetic approaches in data collection for abundance estimates through dedicated surveys. That is why starting with the 2013 survey in the NW Black Sea, in the waters of Ukraine, Romania and Bulgaria (Birkun et al., 2014), continuing with the ACCOBAMS funded projects in Romania, Bulgaria, Ukraine and Turkey, and not last through the ACCOBAMS Survey Initiative (ASI) and CeNoBS project - *Support MSFD implementation in the Black Sea through establishing a regional monitoring system of cetaceans (D1) and noise monitoring (D11) for achieving GES*, distance sampling by line transect method became the proposed common protocol for assessing the abundance and distribution in the ACCOBAMS area (both in the Black and Mediterranean Seas).

Still more steps are to be done, meanwhile, the information on stock distribution and animal abundance, migrations, critical habitats, anthropogenic and natural threats as well as some basic aspects of life history and pathology is critical in determining the likely impact of anthropogenic mortality and in planning and implementing relevant conservation programs and not last establishing the Good Environmental Status of the Black Sea cetacean species in accordance to the MSFD.

Implementing the basin-wide multi-sectoral monitoring programme including regular basin-wide aerial surveys as a key element, added by regional and national ship-based surveys, photo identification of local stocks (first of all, marginal coastal small groupings), passive acoustic monitoring and record of stranding and bycatch is necessary for obtaining timely updated reliable information on status of cetacean populations. Also, demographic studies based on photo identification / mark-recapture approach and analysis of age structure of stranded and bycaught individuals are necessary. Nevertheless, region-wide cooperation, sharing knowledge and data and creating data resources of common use as elements of holistic approach are crucial for monitoring highly mobile species of marine mammals.

1.3.1 Introduction

In the past the Black Sea and the Caspian Sea shared the same waters, being connected and forming a lake. In the interglacial period (100 000 – 150 000 years ago) the Black Sea became connected to the Mediterranean Sea after the opening of the Dardanelles. Was then again isolated and only about 6 000 years ago reconnected to the Sea of Marmara and Mediterranean Sea (Zaitsev and Mamaev, 1997). The Turkish Straits System - Marmara Sea, Çanakkale and Istanbul Straits - forms a transitional zone between the Mediterranean and the Black Sea. The special characteristics of this zone make it a barrier, a corridor or an acclimatization zone for different organisms (Öztürk and Öztürk 1996). To the north, the Kerch Strait, a shallow channel about 45 km long, connects the Black Sea to the Sea of Azov.

The main biotopes are sandy-bottom shallow-water areas, especially in the north-western part of the Black Sea and the Sea of Azov. The coasts of the southern Crimea, the Caucasus, Anatolia, some capes in the south-western part of the Black Sea (Kaliakra, Emine, Maslen Nos, Galata) and Zmeiny Island are mostly rocky. The seabeds are mostly mud in the zone between 10 to 20 m and 150 to 200 m depth. The total area of Black Sea coastal wetlands is about 10 000 km^2. There are sites of reproduction and feeding and wintering grounds of many rare and commercially valuable fish species, including the sturgeon family, and are therefore biotopes of special importance. Anoxic conditions occurring below 70 to 200 m delimit the vertical distribution of planktonic and nektonic organisms as well as of deep sea-bottom organisms. The structure of marine ecosystems differs from that of the neighboring Mediterranean Sea in that species variety is lower and the dominant groups are different. However, the abundance, total biomass and productivity of the Black Sea are much higher than in the Mediterranean Sea. (Alexandrov & Zaitsev, 1998; Zaitsev & Alexandrov, 2000).

The number of species in the Black Sea is around one third of that in the Mediterranean. Despite recent changes in absolute numbers, the ratio remains close to three: 10 000 species in the Mediterranean versus 3 700 species in the Black Sea (Zaitsev & Alexandrov, 2002).

Four species of mammal occur: the Mediterranean monk seal (*Monachus monachus*), which is considered to be extinct from the Black Sea, and three species of cetaceans, the bottlenose dolphin (*Tursiops truncatus* ssp. *ponticus*, Barabasch – Nikiforov, 1940), the common dolphin (*Delphinus delphis* ssp. *ponticus*, Barabasch – Nikiforov, 1935) and the harbour porpoise (*Phocaena phocaena* ssp. *relicta*, Abel, 1905). In the beginning of 1950s, the Black Sea was home to about 1 million dolphins. Although hunting for dolphins has been banned since 1966 in Romania, Bulgaria and USSR, their population decreased by the end of 1980s (1983 – Turkey committed to ban the capture of cetaceans also). Being at the top of the food chain, Black Sea cetaceans play a major role in ecological equilibrium of the marine ecosystem. Due to this position, they are very sensitive to ecological conditions and in direct competition with some human activities (Radu et al., 2013).

The historical status of cetacean populations (including *P. p. relicta*, *D. d. ponticus* and *T. t. ponticus*) is not clear. Various surveys have been conducted, primarily within Ukrainian/Russian Federation waters and Turkish waters, but the compatibility of survey results is not always clear, and the limited geographical coverage restricts the extrapolation of results to basin-wide level. This lack of information extends to the understanding of population abundance, distribution, migrations, critical habitats, anthropogenic and natural threats as well as some basic aspects of lift history and pathology.

In the 20th century, the number of Black Sea harbour porpoises was dramatically reduced by direct hunting, which only ceased in 1983. Following the prohibition to capture cetaceans, incidental catches in fishing gear may also played a role in the current population trend, but without accepted estimates of abundance and of by-catch rates, it is not possible to determine the current conservation status of any of the Black Sea cetacean species (Birkun et al., 2008).

Although historical population estimates are not available, it is beyond reasonable doubt to conclude that hunting had a significant adverse impact on the populations of harbour porpoises in the Black Sea.

The quality of the Black Sea ecosystem is dependent, in particular, on the survival and welfare of these top predator populations. It is difficult to foresee all negative consequences for the regional biodiversity, if cetaceans disappear as it had happened with the monk seal (Öztürk, 1992, 1996; Kıraç & Savaş, 1996; Güçlüsoy et al., 2004).

The three cetacean species are recognized as subpopulation and exhibit genetic differences to the Mediterranean populations of harbour porpoises, common dolphins and bottlenose dolphins. The habitats of all three species overlap, but the principal habitats differ, for example harbour porpoises and bottlenose dolphins are principally associated with the circumlittoral area over the continental shelf, whereas the common dolphin is principally associated with the open sea and is present in the circumlittoral areas as a secondary habitat.
The range of all three species includes the entire Black Sea. Harbour porpoises are also associated with the Marmara Sea, Kerch Strait and Azov Sea. Common dolphins are also associated with the Marmara Sea but are not known in the Azov Sea and are infrequently observed in the Kerch Strait. Bottlenose dolphins are also associated with the Marmara Sea, Kerch Strait and the waters of the Azov Sea near the Kerch Strait.
Information relating to critical habitats is incomplete and although migrations or seasonal movements are known to occur in all three species, it is unclear to what extent particular areas constitute critical habitat. There is basic information available on 'hotspots', but these are at best incomplete. The identification of important parts of their habitat may be difficult and costly to identify or misguided. The application of strict protection systems for the species throughout their range (rather than of critical habitats or of the species within critical habitats) is particularly relevant given the transboundary and migratory nature of Black Sea cetaceans.
Further will be presented the preliminary results of the cetacean survey performed during the ANEMONE Joint Cruise in October 2019, on board the research vessel "Mare Nigrum" (Romanian flag). The surveyed area covered the shelf waters of Romania, Bulgaria and western Turkey, an area of 9754.58 Km^2. The observations were performed following line transect sampling method, single platform (2 observers, on the left and right of the vessel bridge) (Figure 1.47) over 380.44 Km transect length. A total of 54 sightings were recorded.
Additional, data on floating marine litter and birds (Class: Aves) were collected, following strip transect method.

Figure 1.47 - Single platform positioning used during the ANEMONE JC survey for marine mammal data collection

Cetacean abundance overview in the NW Black Sea

Group of marine mammals in the Black Sea

Before presenting the result of the Joint Cruise, with which we like to bring additional information on the state of cetaceans in the Black Sea, is necessary to remind the readers, whom we are sure that have at least basic knowledge of the species, the three species inhabiting the Black Sea ecosystem. Forward we introduce the common dolphin (Figure 1.48), the bottlenose dolphin (Figure 1.49) and the harbour porpoise (Figure 1.50).

The common dolphin (*Delphinus delphis* ssp. *ponticus*, Barabasch – Nikiforov, 1935) (Figure 1.48).

Class: Mammalia
Order: Cetacea
Suborder: Odontoceti
Family: Delphinidae
Genus: Delphinus
Species: *Delphinus delphis* ssp. *ponticus*

Figure 1.48 **- Delphinus *delphis ssp.* ponticus *Barabasch-Nikiforov, 1935 (@Mare Nostrum NGO)***

The bottlenose dolphin (*Tursiops truncatus* ssp. *ponticus*, Barabasch – Nikiforov, 1940) (Figure 1.49)

Class: Mammalia
Order: Cetacea
Suborder: Odontoceti
Family: Delphinidae
Genus: Tursiops
Species: *Tursiops truncatus* ssp. *ponticus*

Figure 1.49 **- Tursiops *truncatus ssp.* ponticus *Barabasch-Nikiforov, 1940 (@Mare Nostrum NGO)***

The harbour porpoise (*Phocena phocoena* ssp. *relicta* - Abel, 1905) (Figure 1.50).

Class: Mammalia
Order: Cetacea
Suborder: Odontoceti
Family: Phocoenidae
Genus: Phocoena
Species: *Phocoena phocoena* ssp. *relicta*

Figure 1.50 - Phocena* phocoena *ssp*. relicta *Abel, 1905 (@Tonay/TUDAV)

Short summary of cetacean monitoring efforts
Within the last 10 years the effort of monitoring cetaceans in the Black Sea increased, mostly supported by the non-governmental (NGO) sectors and through NGO-research institutes partnerships. At present there are available data on abundance, density and distribution (Dede & Tonay, 2010; Gladilina et al., 2017a., Gol'din et al., 2017, Gladilina et al., 2018; Gladilina & Gol'din, 2016; Krivokhizhin et al., 2012; Paiu et al., 2019; Panayotova & Bekova, 2015; Uludüz et al., 2019), strandings (Anton et al., 2012, Candea et al., 2011; Paiu et al., 2016; Tonay et al., 2017; Öztürk et al., 2017; Bilgin et al., 2018a; Özsandıkçı et al., 2019), bycatch (Bilgin et al., 2018b; Radu & Anton, 2014; Tonay, 2016) demographic characteristics (Vishnyakova, 2017), genetic studies (Fontaine et al., 2007, 2010, 2012; Natoli et al., 2005, 2008; Tonay et al., 2016; Viaud-Martinez et al., 2007, 2008; Chehida et al., 2020) etc., in last ten years mostly for the 12 NM area (coastal area), within the surveyed area during the ANEMONE JC (N-W Black Sea). On regional scale 2 surveys were performed, one in summer 2013 in the NW Black Sea (Ukraine, Romania and Bulgaria) and one in summer 2019 covering territorial waters and EEZ`s of Ukraine, Romania, Bulgaria, Turkey and Georgia. The 2013 survey was performed in the frame of "*Studies for carrying out the common fisheries policy: adverse fisheries impact on cetacean populations in the Black Sea" project* funded by the European Commission. Itwas the first dedicated line-transect cetacean survey in the inshore and offshore waters of the western Black Sea, combined shipboard and aerial line transect survey. It was conducted to document the distribution and abundance of cetaceans in the western Black Sea (Birkun et al., 2014).
Below are presented the results related to the absolute abundance of all three Black Sea cetacean species/subspecies, including the Harbour porpoise (*Phocoena phocoena relicta*), the Common dolphin (*Delphinus delphis ponticus*) and the Bottlenose dolphin (*Tursiops truncatus ponticus*) for the NW Black Sea area in Table 1.12, which rounded to thousands reveal estimates of approx. 115000 cetaceans ± 22000 individuals.

Table 1.12 - Integral values estimated for the three species of cetaceans in the W Black Sea area[1]

Parameters	Harbour porpoise	Common dolphin	Bottlenose dolphin
Area (km^2)			119796.0
Observation effort (total length of transect lines), km			60036.5
No of observations	402	408	275
Estimate of expected values of group size	1.335	1.986	1.836
Mean group size	1.410	2.304	1.866
Estimate of density of groups per 1 km^2	0.184	0.254	0.120
Estimate of density of animals (individuals/km^2)	0.246	0.504	0.221
Estimate of the number of animals in the surveyed area	29465	60400	26462

[1] The extended table can be consulted in the report "Studies for carrying out the common fisheries policy: adverse fisheries impact on cetacean populations in the Black Sea"

The second regional survey was performed in the frame of the CeNoBS project — Support MSFD implementation in the Black Sea through establishing a regional monitoring system of cetaceans (D1) and noise monitoring (D11) for achieving GES funded by the DG Environment of the European Commission within the call — DG ENV/MSFD Second Cycle and of which the results will be available at the end of 2020.

Cetacean observation during ANEMONE Joint Cruise

The cetacean fauna of the Black Sea includes three species/subspecies – the Black Sea harbour porpoise (*Phocoena phocoena relicta*), the Black Sea common dolphin (*Delphinus delphis ponticus*) and the Black Sea bottlenose dolphin (*Tursiops truncatus ponticus*). All three species, drastically affected by commercial killing in the 20th century, are exposed to ongoing anthropogenic threats which may cause increased mortality and morbidity, disturbance, habitat deterioration and depletion of food resources.

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The European Marine Strategy Framework Directive (MSFD) aims at implementing a precautionary and holistic ecosystem-based approach for managing European marine waters. Marine mammals are included as a functional group for the assessment and reporting under Descriptor 1 - Biodiversity. Conservation of mobile marine megafauna such as cetaceans requires transboundary cooperation, which the MSFD promotes through regional instruments, such as the Regional Sea Conventions and other regional cooperation structures such as Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and Contiguous Atlantic Area (ACCOBAMS) and Black Sea Commission (BSC).

Taken together the above instruments represent a formidable framework for the conservation of Black Sea cetaceans, nevertheless major gaps in knowledge burdens the process of assessing the Good Environmental Status for the three species.

In the last years, small steps were done for synergetic approaches in data collection for abundance estimates through dedicated surveys. That is why starting with the 2013 survey in the NW Black Sea, in the waters of Ukraine, Romania and Bulgaria (Birkun et al., 2014), continuing with the ACCOBAMS funded projects in Romania, Bulgaria, Ukraine and Turkey, and not last through the ACCOBAMS Survey Initiative (ASI) and CeNoBS project - *Support MSFD implementation in the Black Sea through establishing a regional monitoring system of cetaceans (D1) and noise monitoring (D11) for achieving GES,* distance sampling by line transect method became the proposed common protocol for assessing the abundance and distribution in the ACCOBAMS area (both in the Black and Mediterranean Seas).

Still more steps are to be done, meanwhile, the information on stock distribution and animal abundance, migrations, critical habitats, anthropogenic and natural threats as well as some basic aspects of life history and pathology is critical in determining the likely impact of anthropogenic mortality and in planning and implementing relevant conservation programs and not last establishing the Good Environmental Status of the Black Sea cetacean species in accordance to the MSFD.

Implementing the basin-wide multi-sectoral monitoring programme including regular basin-wide aerial surveys as a key element, added by regional and national ship-based surveys, photo identification of local stocks (first of all, marginal coastal small groupings), passive acoustic monitoring and record of strandings and bycatch is necessary for obtaining timely updated reliable information on status of cetacean populations. Also, demographic studies based on photo identification / mark-recapture approach and analysis of age structure of stranded and bycaught individuals are necessary. Nevertheless, region-wide cooperation, sharing knowledge and data and creating data resources of common use as elements of holistic approach are crucial for monitoring highly mobile species of marine mammals.

1.3.2Material and methods

Between September 30 and October 7, 2019, a vessel cruise was performed in the frame of the ANEMONE project within the waters of Romania, Bulgaria and Turkey.
The methodology included the linear transect surveys (LTS) method, designed according to standard principles of distance sampling (Buckland et al., 2001; Buckland, 2004). The observation was made from a platform with a height of 9 m, using both eye and binoculars (7x50 WPC-CF Fujinon) for species sighting, identification and distance, angle boards for angle and. Tracks and coordinates were recorded, using the GPS navigator Garmin eTrex 30.
The two observers acted both as observers and data recorder, using voice recorders and individual sheets in order to reduce the gaps in observation period. Because of the large distance between the observers a signal was designated to alert a sighting and information, when needed, were transmitted with the help of radio-stations. The observers were on effort during the transit between the fix designated stations of the mission and went off-effort when the ships arrived in the next station. The brakes in the station usually were for at least 2 hours which provided plenty of time for the observers to rest, during the work of the other scientist in the station.
Survey speed was between 8-10 kts (14.81 – 18.52 km/h). Data collection was based on the protocol used for the vessel survey component of the Adverse Fisheries Impacts on Cetacean Populations in the Black Sea project and Distance 7.0 software of Birkun et al. (2014). Collecting environmental conditions: sea state, glare, cloud cover, turbidity and a subjective assessment of overall conditions at the beginning of each transect and whenever a change occurred. Having in mind the short period of time for data collection and unfavourable hydrometeorological forecast the observers remained active also poor conditions, sea state of 5 on the Beaufort scale.
Observers searched a 110° arc from abeam to ahead. When a sighting was made, the following data were recorded: angle to the sighting when it was observed, distance, species, group size, initial cue, estimated swim direction, behaviour, and observer making the sighting. For quality assessment, digital pictures of the whole group and individuals were taken; animals were counted, and school size were estimated. Action was performed only “on effort” mode.
Density and abundance, cluster (group) density were estimated by analytical tools based on detection probability functions for distance sampling (Buckland et al., 2001), using Distance 7.3 software (Thomas et al., 2010). The minimum value of the Akaike Information Criterion or AIC (Buckland et al., 2001), was used to choose between models and select which covariates to include in the detection function.
Encounter rate was defined as a number of group observations per km. The population density was estimated as the number of individuals per square kilometer. The type of spatial distribution (random, uniform or patchy) was estimated from the coefficient of variation for group density (Caughley, 1977). Only encounters on transect lines were used for density and abundance estimations: all the other records on the way to transect lines were only used as referring to the cetacean presence in the area (heat maps).
The total surface of the surveyed area was estimated by multiplying the transect distance by the furthest observation width. The marine mammal’s density (individuals/km^2) was calculated by dividing the recorded individuals with the surveyed area surface.
The species density maps were derived using Kernel Density interpolator in QGIS 3.12-3, which calculates the density of elements with a spatial distribution, considering their neighbourhood, using Silverman formula (Silverman, 1986). Once calculated, the density is then multiplied with the sum of the point’s values (QGIS, 2020).

1.3.3 Results

During the seven days of the cetacean survey were recorded 54 cetacean sightings (114 individuals) of all three species inhabiting the Black Sea (Table 1.13). The observations were collected over a 380.44 Km line transect. The transects covered 19.4% of the designed study area.

Table 1.13 - General characteristics of the data collected during the ANEMONE joint cruise survey

Distribution in time of the species sighted	No. of observation	No. of the observed individuals	No. of observed calves
Delphinus delphis	17	37	1
01.10.2019	8	17	
02.10.2019	3	4	
03.10.2019	1	2	
05.10.2019	2	5	
06.10.2019	2	6	1
07.10.2019	1	3	
Phocoena phocoena	20	42	7
01.10.2019	6	23	5
04.10.2019	4	5	
06.10.2019	10	14	2
Tursiops truncatus	17	35	6
01.10.2019	1	1	
03.10.2019	4	12	2
05.10.2019	7	12	1
06.10.2019	5	10	3
Grand Total	54	114	14*

***The number of calves is included in the total number of individuals and not additional. It should be read as 114 individuals, from which 14 were calves.**

Due to different conditions and areas, an integrated analysis of all 19 transects could not be performed, and separation based on the proximity and effort was done. Three areas (Figure 1.51) were defined, following the effort imposed by the cruise plan, weather and the data collection protocol. First area, entirely in the offshore waters of Romania, includes 6 transects with a total length of 137.85 Km. Will be named further as RO Sector. Second transect (BG-TK Sector) it covered the shelf waters of Bulgaria and Turkey over 12 transects, 6 in Bulgaria and 6 in Turkey, with a total length of 281.19 Km. The third BG-RO Sector consists of a single transect, during the trip back to Constanta harbour, over 61.4 km. In this case, only one sighting was recorded, 3 common dolphins. The environmental conditions posed a great setback to acquiring more data on this specific transect due to sea state more than 5 on Beaufort scale.

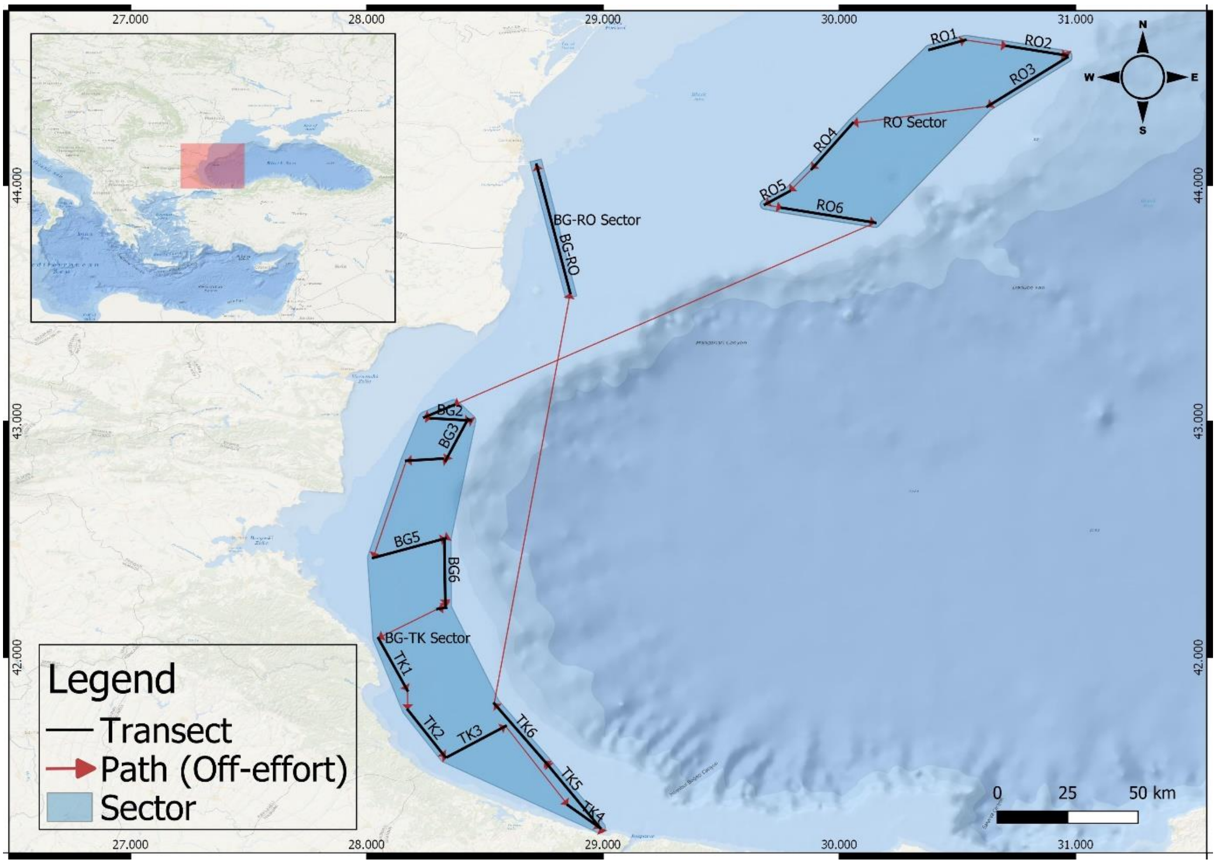


Figure 1.51 - Line transect survey plan in the western Black Sea area

As stated, during the third sector (BG-RO Sector) only one sighting, common dolphin, was sighted and recorded. Within the other two sectors (RO and BG-TR) all the three species were registered with a similar density of individuals/km^2, 0.012 for RO sector, respective 0.013 for BG-TR sector.
Below are presented the results related to the absolute abundance of all three Black Sea cetacean species/subspecies, including the harbour porpoise (*Phocoena phocoena relicta*), the common dolphin (*Delphinus delphis ponticus*) and the bottlenose dolphin (*Tursiops truncatus ponticus*) for the W Black Sea area in the Table 1.14 for RO sector and Table 1.15 for BG-TR Sector.

Table 1.14 - Integral values estimated for the three species of cetaceans in RO Sector

Parameters	**Harbour porpoise**	**Common dolphin**	**Bottlenose dolphin**
Area (km^2)	3850.25		
Observation effort (total length of transect lines), km	137.85		
No. of observations	6	11	1
Mean group size	3.83	1.90	-
Estimate of density of animals (individuals/km^2)	0.032	0.059	0.005
Estimate of number of animals in surveyed area	126	231	21

Distance sampling analysis performed within the DISTANCE 7.3 software (Thomas et al., 2010) provides a cetacean abundance 95%CI ranging between 99 and 1835 individuals with a high CV of 0.65 for the RO Sector.
The animals sighted in the 18 sightings within the area of RO sector are presented below (Figure 1.52).

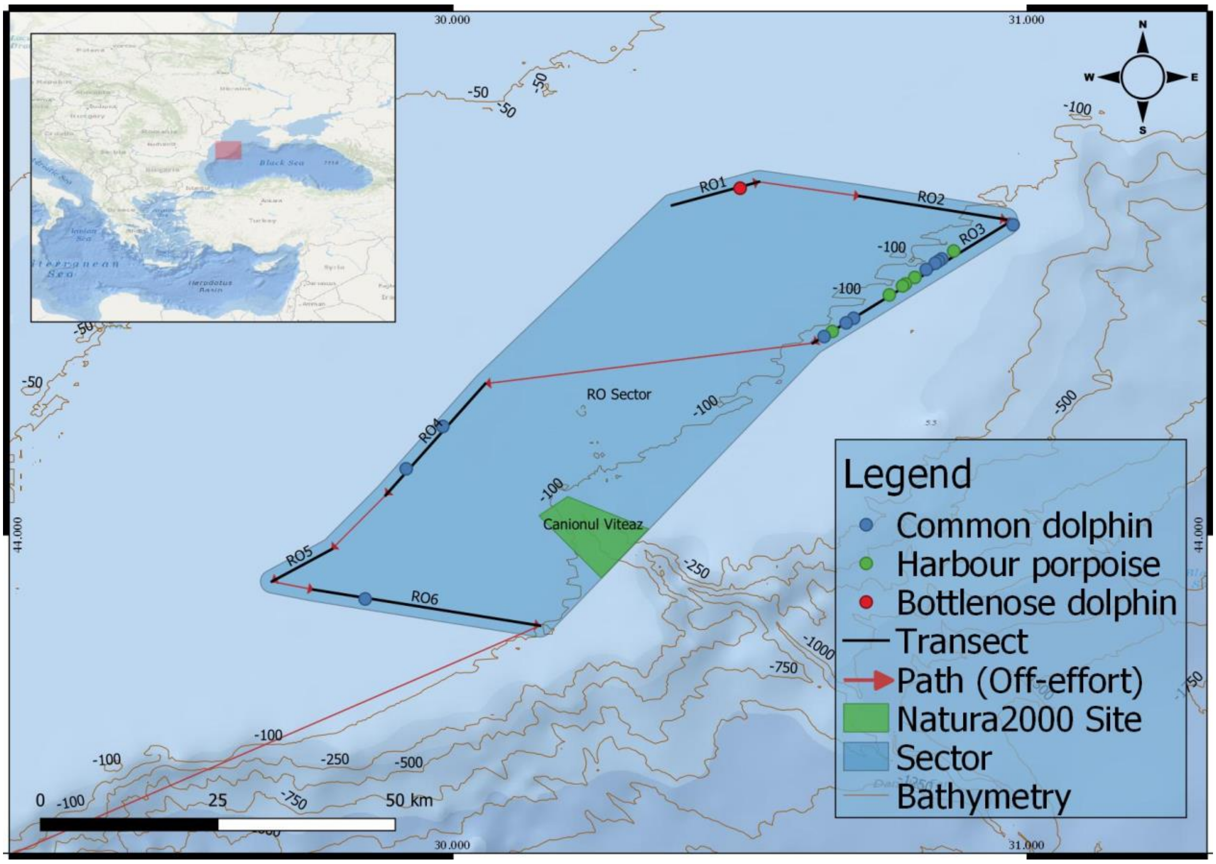


Figure 1.52 - Cetacean sightings distribution by specie recorded in the RO sector

Table 1.15 - Integral values estimated for the three species of cetaceans in BG-TR Sector

Parameters	Harbour porpoise	Common dolphin	Bottlenose dolphin
Area (km^2)	3850.25		
Observation effort (total length of transect lines), km	137.85		
No. of observations	14	5	16
Mean group size	1.35	2.6	2.12
Estimate of density of animals (individuals/km^2)	0.050	0.034	0.090
Estimate of number of animals in surveyed area	287	196	513

Distance sampling analysis performed within the DISTANCE 7.3 software (Thomas et al., 2010) provides a cetacean abundance 95%CI ranging between 545 and 4103 individuals and a CV of 0.48 for the BG-TR Sector.

The animals sighted in the 35 sightings within the area of BG-TR sector are presented below (Figure 1.53).

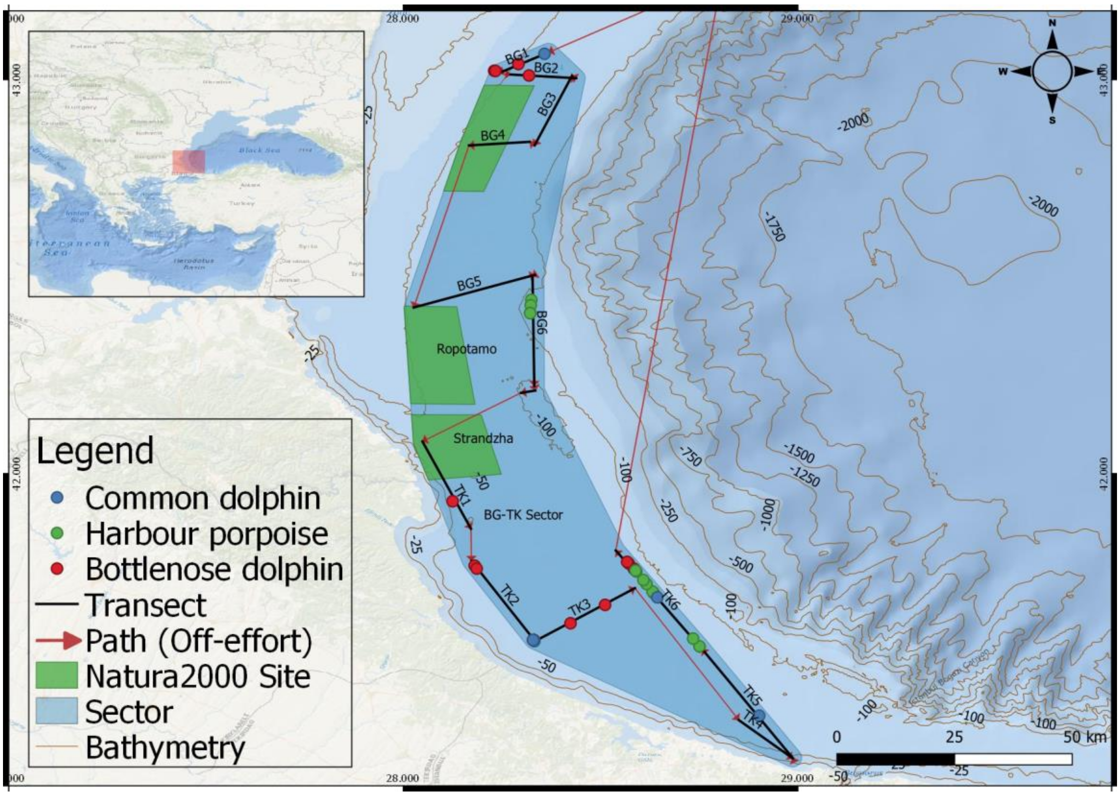


Figure 1.53 - Cetacean sightings distribution by specie recorded in the BG-TR sector

Density maps present two major cetacean hotspots, one in each sector (Figure 1.54). In RO Sector in the NE part and for the BG-TR Sector in the S part, both of them are marked with a red circle on the map below.

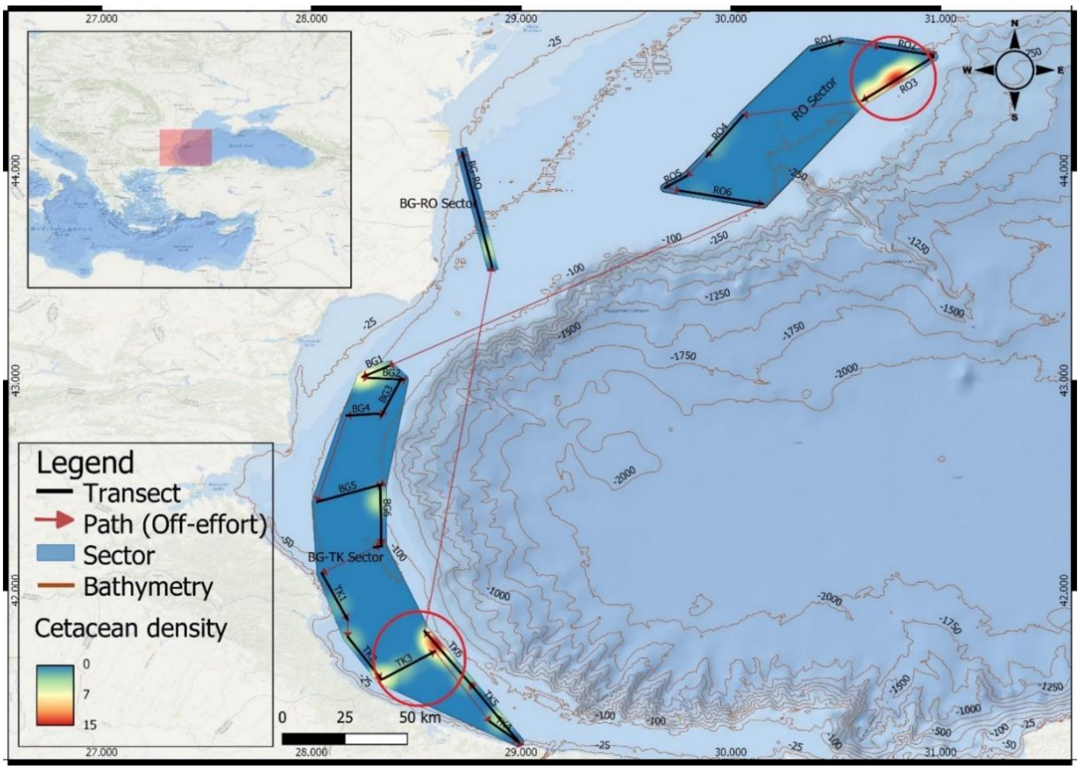


Figure 1.54 - Cetacean density heatmap for the ANEMONE JC. Red circles highlight the major hotspots

1.3.4 Conclusions

Due to the precarious conditions in which the survey was performed it is highly advisable to use with caution the presented results. The survey was performed with the restraints imposed by the ANEMONE Joint Cruise plan, which were not complying totally with the needs for a proper cetacean survey.
Once again, it proves that dedicated surveys are needed for marine mammals monitoring, since the methods used are highly dependent of good meteorological conditions. So far, to some extents, common expeditions are possible for marine mammals and birds and should be taken in consideration.
Information relating to critical habitats is incomplete and although migrations or seasonal movements are known to occur in all three species, it is unclear to what extent particular areas constitute critical habitat. There is basic information available on 'hotspots', but these are at best incomplete. The identification of important parts of their habitat may be difficult and costly to identify or misguided.
The application of strict protection systems for the species throughout their range (rather than of critical habitats or of the species within critical habitats) is particularly relevant given the transboundary and migratory nature of Black Sea cetaceans.
The collected information is useful for qualitative studies on presence and distribution of the species for the surveyed area, and poor for quantitative studies.

2 Eutrophication

"Human induced eutrophication is minimised, especially the adverse effects thereof, such as losses in biodiversity, ecosystem degradation, harmful algal blooms, and oxygen deficiency in bottom waters."

2.1 Introduction

The Black Sea has special natural features and a drainage basin six times higher than its surface. Therefore, it has been vulnerable to anthropogenic pressures and pollution sources (BSC, 2008). Until the '60s, the Black Sea was known as one of the most productive seas, with luxuriant pelagic fauna, being an example of natural eutrophic ecosystem due to the permanent Danube's nutrients input (Gomoiu, 1981). Further, with anthropogenic activities enhancement, increased use of fertilizers, wastewater discharges, detergents, etc., the nutrients regime has undergone significant changes. These changes were found into the river nutrients input that increased significant (Mee & Topping, 1999, Cociașu et al., 2008) and led to alterations in the northwestern Black Sea ecosystem. At the beginning of '80s, phytoplankton has developed excessive and intense blooms became annually and extended in time and frequency. During 1983-1988, more than 20 blooms were observed, produced by 8 species, of which 5 (*Prorocentrum minimum, Skeletonema costatum, S. subsalsum, Eutreptia lanowii, Emiliania huxleyi*) with highest densities known until then at the Romanian coastal area. The average phytoplankton biomass was 4777 mg/m^3, 10 times higher than in 1959-1963. The algae species with more than 100000 cells/L abundance, increased from 34, in 1960-1970, to 54, in 1983-1988 (Bodeanu et al., 2004, Gomoiu, 1992).

Therefore, the Black Sea eutrophication has been a major topic since at least the 80s. when its effects appeared: the transparency decreasing, organic matter decomposition and oxygen depletion (Gomoiu, 1992) and bottom became seasonally hypoxic or even anoxic (ICPDR – ICBS, 1999) transforming the northwestern part of the Black Sea into a highly eutrophic one (Zaitsev in Mee, 1999). In the early '90s, there have found decreasing nutrients input resulted in the first recovery signs (decreasing of phytoplankton blooms, improvement of bottom oxygen regime, increasing of benthic macro fauna (Gomoiu, 1992). Unfortunately, due to absence of measures, eutrophication effects continued from year to year and considerable changes in the pelagic ecosystem at a basin-wide scale became noticeable in the second half of the 1980s and the beginning of the 1990s (Yunev, Moncheva & Carstensen, 2005). Thus, during the 1980s and early 1990s, the Black Sea ecosystem was in a catastrophic condition (Kideys, 2002). Laying downwards the Danube's discharge mouths, the Romanian coast was particularly affected by eutrophication due to the Danube's increased nutrients input from point and diffuse sources of pollution. After 1992-1993, the nutrient limitation abruptly shifted from nitrogen to phosphorus, which then severely reduced plankton production and the system maintained low biomass of bacterioplankton, zooplankton, and total marine living resources, but moderate *Noctiluca scintillans* and gelatinous biomass (Oguz & Velikova, 2010). During the decade following the regime shift of the 1990's, fish stocks gradually improved because of good recruitment and a possibly favourable climate, shrinking fishing effort and diminishing *Mnemiopsis leidyi* biomass, and the outcome was a partial recovery to pre-shift conditions (Daskalov et al., 2017) considered as an alternative pristine state dominated by jellies and opportunistic species than the fish-dominated healthy pristine state (Oguz & Velikova, 2010).

Recently, the emphasized spatial and seasonal variability and the extreme phenomena from the NW Black Sea coast makes the current eutrophication state definable as a moderate - good, equivalent to an eutrophic - mesotrophic state which, under the action of climatic factors and human impact more pronounced in the coastal zone, can easily pass to extreme states like unsatisfactory (hypertrophic) or very good (oligotrophic), conditions occasionally encountered in the waters of the NW Black Sea, often seasonally (Lazar et al., 2013, Daskalov et al., 2016).

The eutrophication reduction (EcoQ3) is the subject of both Black Sea Strategic Action Plan (2009) implemented by the Black Sea Commission and the Marine Strategy Framework Directive through the Descriptor 5. The latter consider that the Good Environmental Status (GES) has been achieved when human-induced eutrophication is minimized, the biological community remains well-balanced and retains all necessary functions in the absence of undesirable disturbance associated with eutrophication (e.g. excessive algal blooms, low dissolved oxygen, declines in sea grasses, kills of benthic organisms and/or fish) and/or where there are no nutrient-related impacts on sustainable

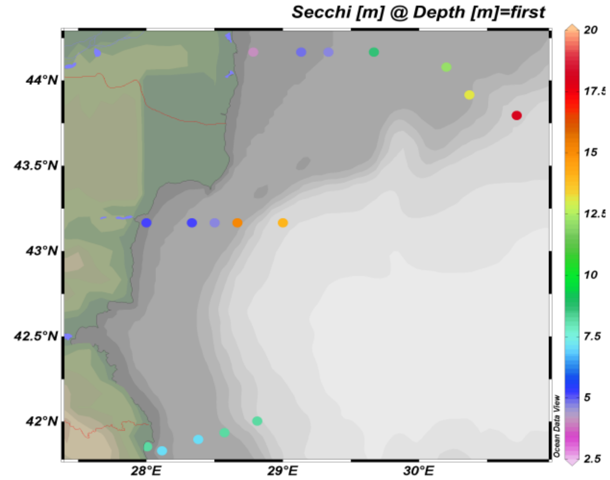
use of ecosystem goods and services (Borja, 2013).
Subsequently, in 2010, Decision EU/2010/477 considered for Descriptor 5 that the assessment of eutrophication in marine waters needs to consider the assessment for coastal and transitional waters under Directive 2000/60/EC in a way which ensures comparability, taking also into consideration the information and knowledge gathered and approaches developed in the framework of regional sea convention (European Commission, 2010). But to ensure that the second cycle of implementation of the marine strategies of the Member States further contributes to the achievement of the objectives of MSFD and yields more consistent determinations of good environmental status, Decision EU/2010/477 was reviewed to achieve a clearer, simpler, more concise, more coherent and comparable set of GES criteria and methodological standards and develop specific guidance to ensure a more coherent and consistent approach for assessments in the next implementation cycle (European Commission, 2017). Consequently, in 2017 it came into force Decision EU/2017/848 introducing primary and secondary criteria instead of direct and indirect effects of nutrient's enrichment (Lazăr et al., 2019).
In this context, besides of the riparian EU countries obligations (Romania and Bulgaria) to meet the requirements of the EU Directives (particularly WFD and MSFD) there is a strong need to have powerful tools to assess the Black Sea eutrophication at regional level. Therefore, this assessment aims to a harmonized approach of the eutrophication assessment at the (sub) regional level – the North, northwestern, western and southwestern Black Sea.

2.2 Material and methods

A core set indicator was chosen for the eutrophication assessment in respect with the Descriptor's 5 criteria grouped as follows:

- causes of eutrophication – nutrients levels – concentrations of phosphate, silicate, nitrogen oxidised forms, ammonium in water column (Grasshoff et al., 1999)
- direct effects of eutrophication – chlorophyll *a* concentration in the water column (Jeffrey & Humphrey, 1975) and *Noctiluca scintillans*
- indirect effects of the eutrophication – water dissolved oxygen content (Winkler method, onboard – Grasshoff et al., 1999) for stations with bottom depth up to 50 m, transparency (in-situ measurements, Secchi disk) and benthic habitats status (M-AMBI (n)).

Based on the data achieved in the cruise (average data from the upper homogenous layer), on the reference values and acceptable deviations of these parameters (specific for each country) it was used the integrative tool for the eutrophication assessment, BEAST (Black Sea Eutrophication ASsessment Tool).
All maps were made with Ocean Data View (ODV) software version 5.2.1. ODV is a computer program for the interactive exploration that displays data in two basic ways: (1) either by showing the original data at the data locations as colored dots of user-defined size or by projecting the original data onto equidistant or variable resolution rectangular grids and then displaying the gridded fields. Method 1 produces the most elementary and honest views of the data, instantly revealing occasional bad data values and regions of poor sampling. In contrast, method 2 produces nicer plots and avoids the overlapping of the colored dots that occurs with method 1 (Figure 2.1), especially for large dot-sizes. Users should note, however, that the gridded fields of method 2 (Figure 2.1) are actually data products and that small scale or extreme features in the data may be modified or lost as a consequence of the gridding procedure (weighted-average gridding) (Schlitzer, 2014). All ODV representations done within the scope of this assessment have used method 2 with sampling stations marked as black dots (Figure 2.1).



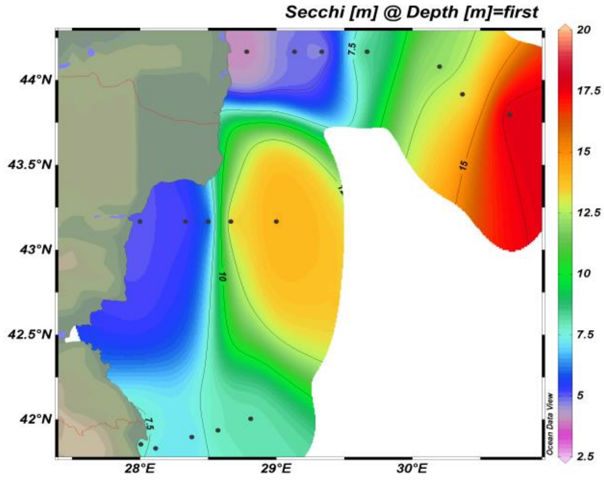


Figure 2.1 – Examples of ODV maps done by method 1 - original data (left) and method 2 – data products by weighted average gridding (right)

Table 2.1 - D5 - Eutrophication - Criteria, Indicators, Targets and Threshold values

Criteria	Name	Type	Indicators	GES/ Targets	Threshold values Beyond coastal waters
D5C1	Water column nutrients	Primary	Dissolved Inorganic Phosphorus (DIP) Total Phosphorus (TP) Dissolved Inorganic Nitrogen (DIN) Total Nitrogen (TN)	GES – Nutrients concentrations are at levels which do not indicate eutrophication effects. Targets RO - percentile 75 is less than the threshold values	UA DIP – 9.8 mM RO DIP – 0.23 mM DIN – 10.50 mM BG DIP – 0.15 mM TR DIP – 0.03 mM
D5C2	Water column chlorophyll *a*	Primary	Chlorophyll *a* concentration	GES – Chlorophyll *a* concentrations are at levels which do not indicate nutrients enrichment. Targets RO – percentile 75 in warm season (May - September) is less than the threshold values	UA 1.8 (µg/L for ShW_UA1) 1.2 µg/L (for ShW_UA3) 0.75 µg/L (for ShW_UA5) 0.67 µg/L (for ShW_UA7) RO 4.11 µg/L – north area 2.79 µg/L – south area BG 1.2 µg/L TR 0.69 µg/L
D5C3	Harmful Algal Blooms	Secondary	*Noctiluca scintillans* biomass (mg/m^3)	GES - Harmful bloom intensity is at levels which do not indicate nutrients enrichment. Targets RO – percentile 50 of *Noctiluca scintillans* biomass is less than the threshold values (seasonal)	UA 40% (for ShW_UA1) 37% (for ShW_UA3) 33% (for ShW_UA5) 52% (for ShW_UA7) RO 30% BG

Criteria	Name	Type	Indicators	GES/ Targets	Threshold values Beyond coastal waters
					30% TR 30%
D5C4	Photic limit (transparency) of the water column	Secondary	Transparency (m)	GES – seawater transparency is not reduced because of algae in suspension and is at levels which do not indicate nutrients enrichment. Targets RO - percentile 10 of the warm season transparency is higher than the threshold values	UA RO 6.8m BG 9m (in spring); 11-13m (in summer) TR
D5C5	Bottom dissolved oxygen	Primary Could be substituted with D5C8	Bottom dissolved oxygen concentrations	GES – dissolved oxygen concentration is not reduced because of the nutrients enrichment and is not at levels which indicate negative effects on benthic habitats. Targets RO - percentile 10 is higher than the threshold values	UA RO – stations with bottom depth up to 50m 5mg/L not less than 60% BG not less than 100 – 115% TR
D5C8*	Macrozoobenthic communities	Secondary	M-AMBI (n)	GES - Species composition and relative abundance are at levels which do not indicate negative effects from nutrients and organic matter enrichment Targets RO - *Mytilus galloprovincialis* – percentile 75 of M-AMBI (n) is higher than the threshold value. *Modiolula phaseolina* percentile 75 of M-AMBI (n) is higher than the threshold value.	M-AMBI*(n) ≥ 0.68 în biogenic reefs with *Mytilus galloprovincialis* from circalittoral M-AMBI*(n) ≥ 0.61 in circalittoral mud with *Modiolula phaseolina*

***C6 and C7 are not applicable to the shelf waters where macroalgae community is not specific**

2.3 Water column nutrients

Due to the natural variability, the influence of the nutritional factor in the temperate zone is generally based on the following facts: maximum nutrients concentrations are found at the end of the winter and early spring, shortly before phytoplankton blooms; the sharp decrease of the nutrient concentrations after spring blooms which often persisted until autumn; changes into nutrients ratio are similar with those from phytoplankton populations. Thus, the biogenic elements reservoir controls the phytoplankton development directly, and the Liebig Law (of the minimum) permits us to state that that nutrient with minimum concentrations directly controls this development. Normal nutrition requires a stable ratio (Redfield ratio) within the main elements, C:N:P=106:16:1. Additionally, diatoms need, among other nutrients, silicic acid to create biogenic silica for their frustules. As a result, the Redfield-Brzezinski nutrient ratio was proposed for diatoms and stated to be C:Si:N:P = 106:15:16:1 (Brzezinski, 1985). If this ratio is profoundly impaired (mainly due to the anthropogenic influence), the photosynthetic activity is altered. Usually, the nutrients concentrations of the phytoplankton are higher than those of the seawater (Riley, 1971); thus, it outlines the role of the biological regeneration, nutrients input from the water masses circulation, resuspension from sediments.

The nutrient level assessment considered phosphate, silicate, and inorganic nitrogen forms at the surface (horizontal distribution) and within the water column (vertical distribution).

The coupling between N and P in the ocean might be weakened by variation in the N:P stoichiometry of phytoplankton. In recent years, researchers have begun to investigate apparent particulate and dissolved N:P deviations from the modern oceanic mean of 16:1 (Arrigo et al. 1999, Weber & Deutsch, 2010). Of particular importance is the question of whether the N:P of organic matter exported out of the surface ocean could vary on a global basis over time. N fixation requires two metals — iron and molybdenum — that could limit this process's rate and thus interfere with the N fixation feedback. It has been argued that the scarcity of iron in the modern ocean (see below) contributes to the widespread tendency toward N deficit in the global ocean by suppressing N fixation rates (Falkowski 1997). More dramatically, it has been hypothesized that the long spell of slow evolution in life from 2.0 to 0.6 billion years ago was due to molybdenum limitation of N fixers, which slowed ocean productivity, organic carbon burial, and the build-up of oxygen in the atmosphere (Anbar & Knoll 2002, Sigman & Hain, 2012).

Phosphate and silicate horizontal distribution had similar profiles, with both peaks in BG-2 station (Figure 2.2).

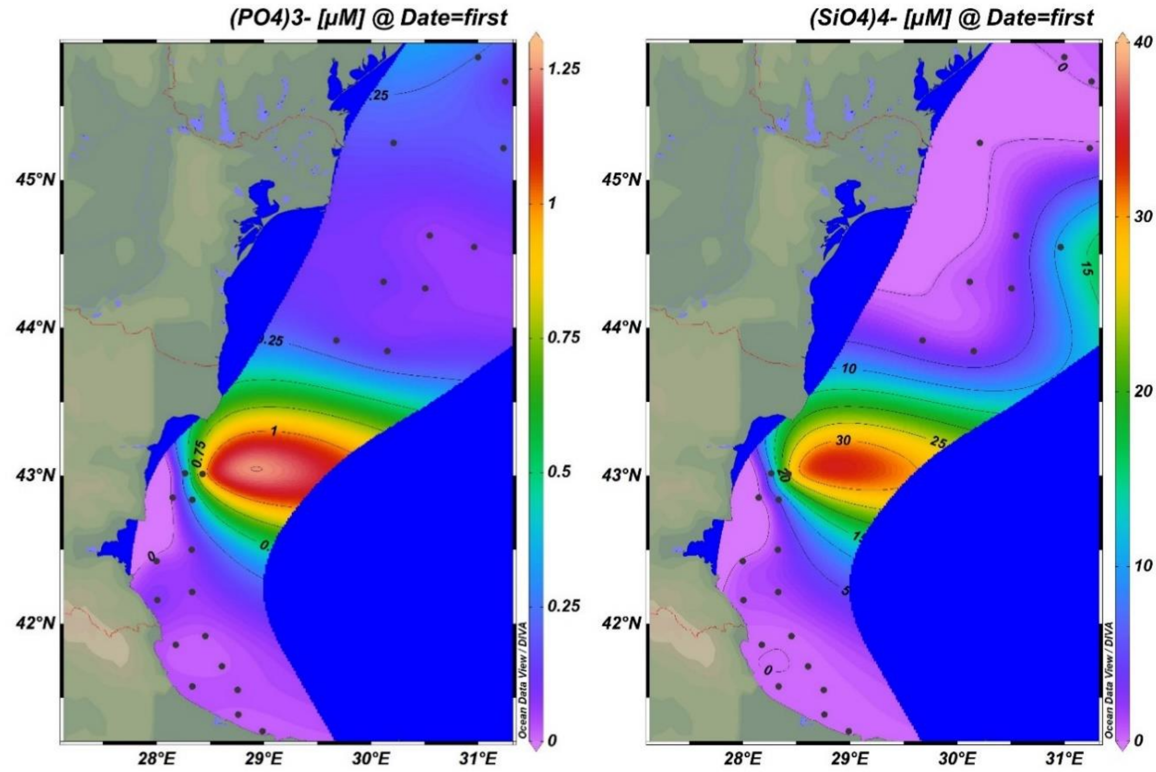


Figure 2.2 – Phosphate and Silicate concentrations - Horizontal distribution

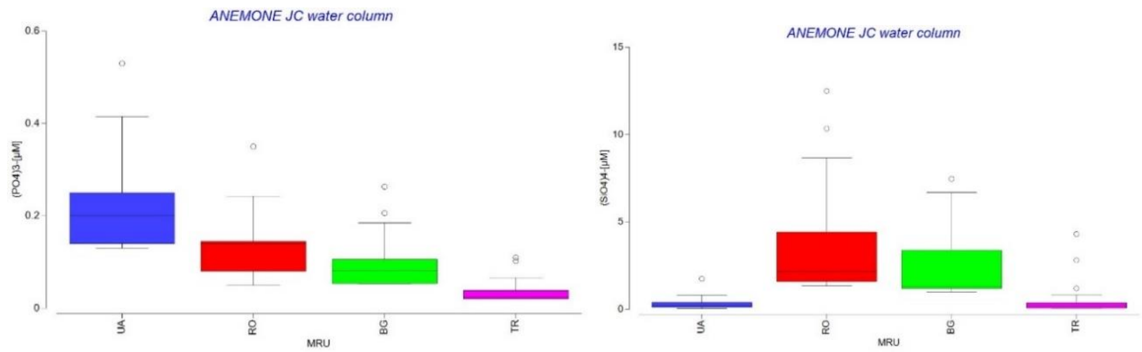


Figure 2.3 – Phosphate and Silicate concentrations - Horizontal distribution

Apart from the high values from the surface (BG-2), phosphate (SHL) decreasing gradient is shaped from north to south while silicate reached its peaks in RO and BG (SHL) (Figure 2.3).
The inorganic forms of nitrogen (oxidized forms, TNOx, as the sum of nitrite and nitrate and ammonium, NH_4^+) had different distributions, linked with their potential sources. Thus, for TNOx, the riverine load from the northwestern shelf is highlighted by concentrations above 2 µM in UA-16, UA-17, and RO-3 (Figure 2.4). Nevertheless, the levels are relatively low.

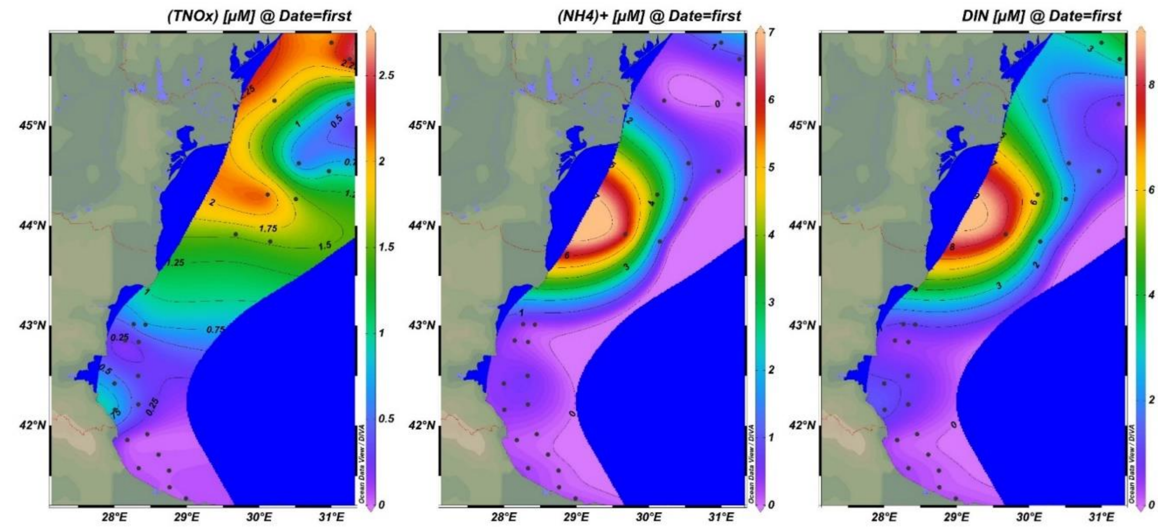


Figure 2.4 – Total oxidized inorganic nitrogen, ammonium and dissolved inorganic nitrogen - Horizontal distribution

In the SHL the highest TNOx concentrations are in the Romanian waters whilst for ammonium in the Bulgarian ones (Figure 2.5). None of them are exceeding the reference values for GES.

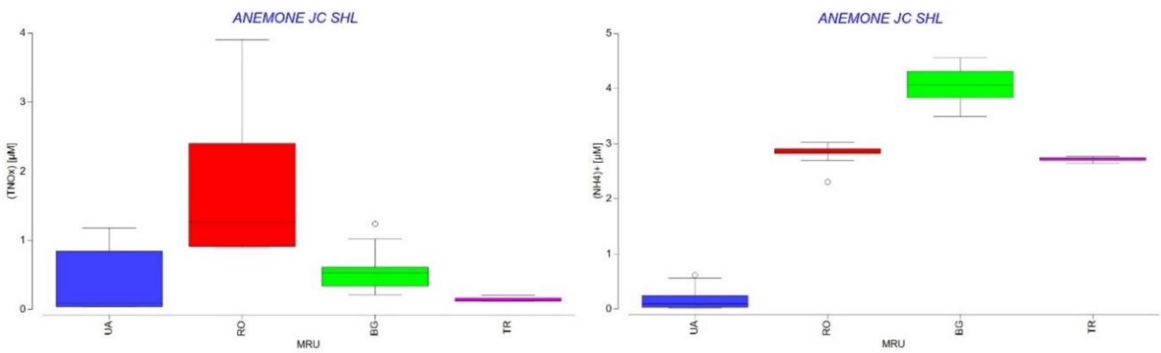


Figure 2.5 - Total oxidised inorganic nitrogen and ammonium (SHL)

Water column distributions

The water column assessment was done for the section with bottom depts within 86-106 m (10 stations – RO-2, 4, 6, BG-2, 4, 6, 8, TR-2, 4, 6).

The seasonal variations of the nutrients concentrations appear in the surface layer as biological activity result and are influenced by thermocline formation, generally starting in April-May. In spring, the intensity and the length of the light cause the phytoplankton's blooms, which results in a sharp decrease of the nutrients from the photic zone. Zooplankton and fish consume a large part of phytoplankton, and the nutrients are returned to the sea as metabolic products. Simultaneously, in spring, the vertical mixing contributes to the replenishment by bringing the nutrients rich water from the bottom in the euphotic zone. However, in early summer, the sun heating causes the thermocline's development (at 20-40m) that inhibits the vertical mixing. Subsequently, the upper thermocline layer is nutrients depleted, and the thermocline is acting as a barrier.

Overall, at the beginning of autumn, the thermocline is delineated at ~20-70 m, although the NW is narrower (Figure 2.6). CIL is present in station RO-2, 4, 6 and BG-2 in the layer 69-76 m, which is characteristic of the Black Sea (Yakushev et al., 2006). It was noticed in the southernmost stations a 10m thickness upper mixed layer with constant temperature and salinity. The near-bottom layer had a constant temperature of 8.38 – 8.56 °C (average 8.47 °C, std. dev. 0.06 °C).

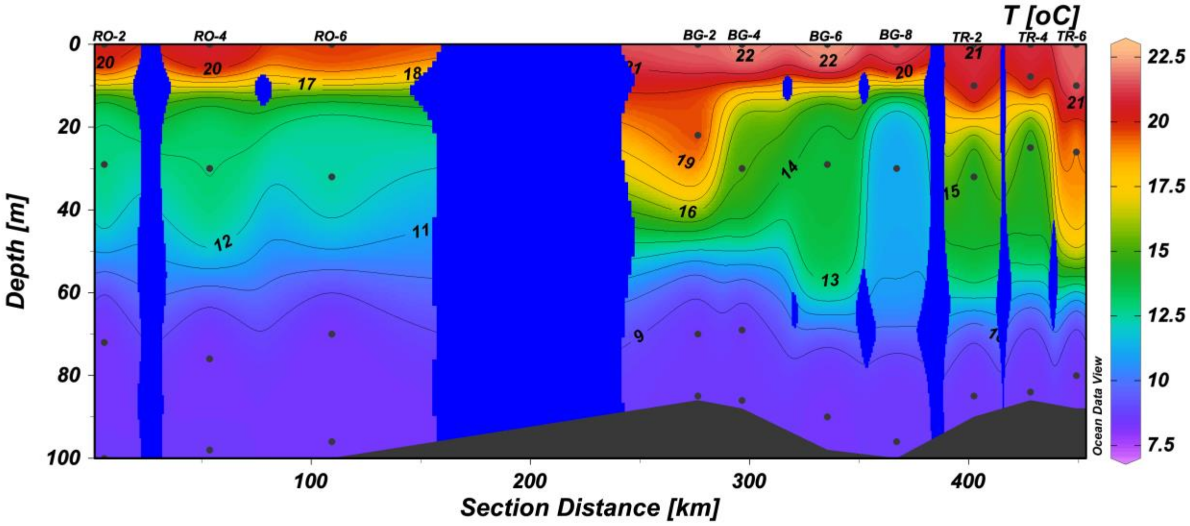


Figure 2.6 – Water column (0-100m) temperature

Salinity has increased by depth, reaching its maximum in the near-bottom layer, 18.93 -20.12 PSU (average 19.47 PSU, std.dev. 0.42 PSU). The stronger increase (~1 PSU) was below thermocline, outlining thus the stratification of the water masses, which plays a vital role in sea circulation (Figure 2.7).

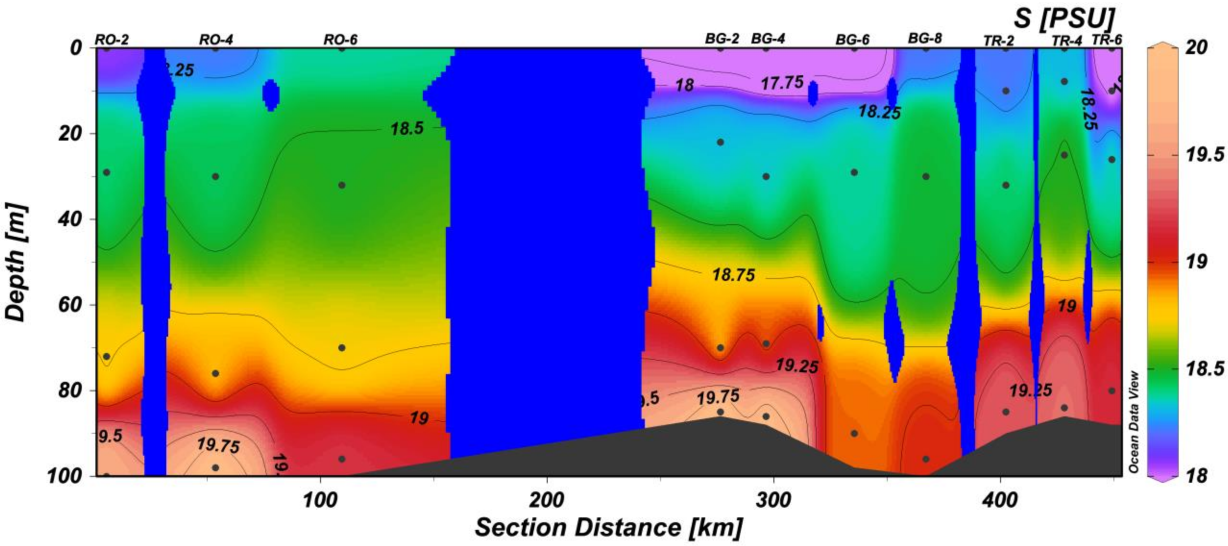


Figure 2.7 – Water column (0-100m) salinity

Phosphates (DIP)

The phosphate concentrations from the surface layer depend on water masses exchange from deeper layers and become higher in the upwelling areas, in the open waters. There is a remarkable correlation between phosphate concentrations in the surface layers and zooplankton content (Dafner, 2007). The latter represents an indirect measure between phytoplankton availability as food and productivity as well. The phosphates concentrations sharply increase near thermocline due to the detritus oxidation and zooplankton activity (Dafner, 2007). The maximum concentrations usually occur nearby oxygen minimum or carbon dioxide maximum. The vertical diffusion makes the distribution more or less uniform in the deeper layers (below maximum phosphate depth).
In the water column, the highest values arose, as expected in the near-bottom layer. Thus, at RO-2

and RO-4, phosphate reached, at the near-bottom layer (100 m), 1.15 µM, 1.32 µM, respectively (Figure 2.8). Generally, there are two DIP peaks - one below the DCM, in the upper and below thermocline layer (mean 0.30 µM) and the other at the bottom (mean 0.56 µM) (Figure 2.9).

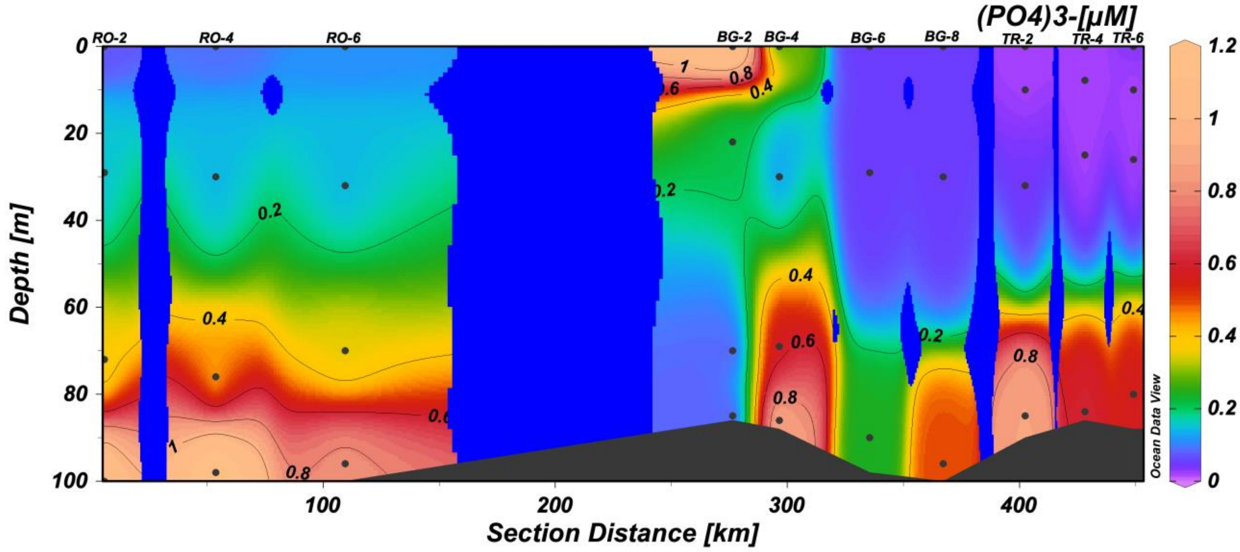


Figure 2.8 - Water column (0-100m) phosphate

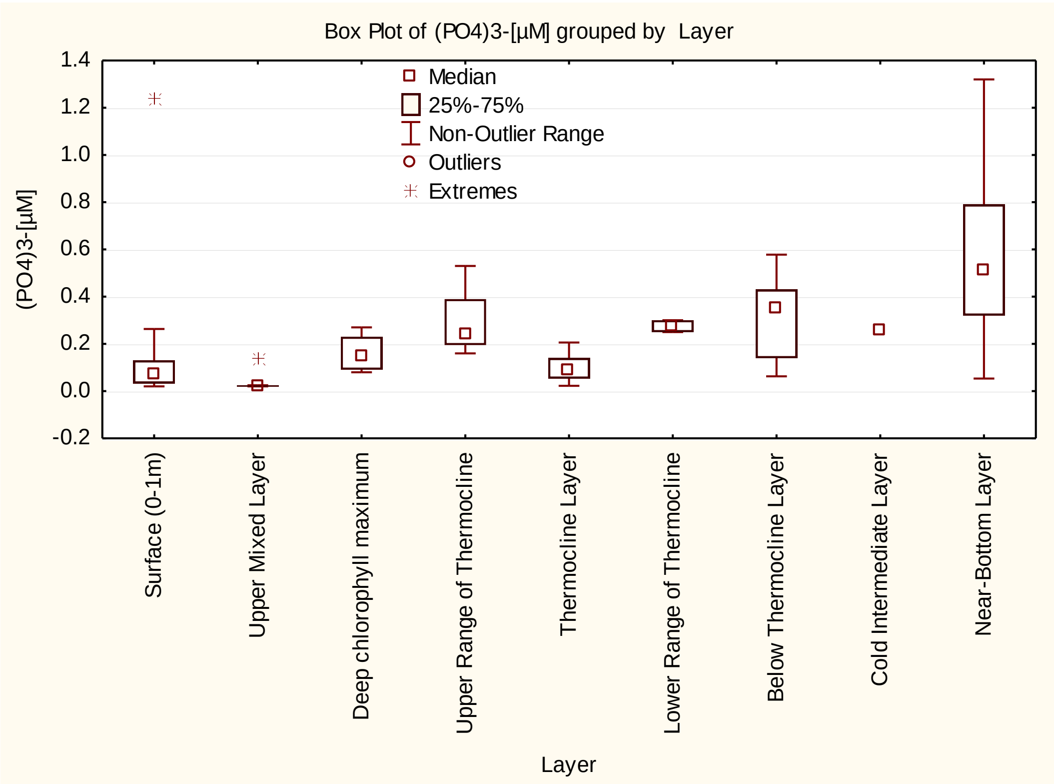


Figure 2.9 - Box plots of DIP grouped by layers

Silicates

Due to the phytoplankton blooms, particularly diatoms, the silicate seasonal variations are similar with the phosphates: sharp decreases in spring and start of the regeneration in summer up to highest values from winter, sometimes interrupted by the autumnal bloom (Riley, 1971). In the open waters, the concentrations are usually low, excepting the upwelling events. Generally, they go up with the depth (Figure 2.10). The pattern is similar to DIP and specific to stratified waters (Figure 2.11).

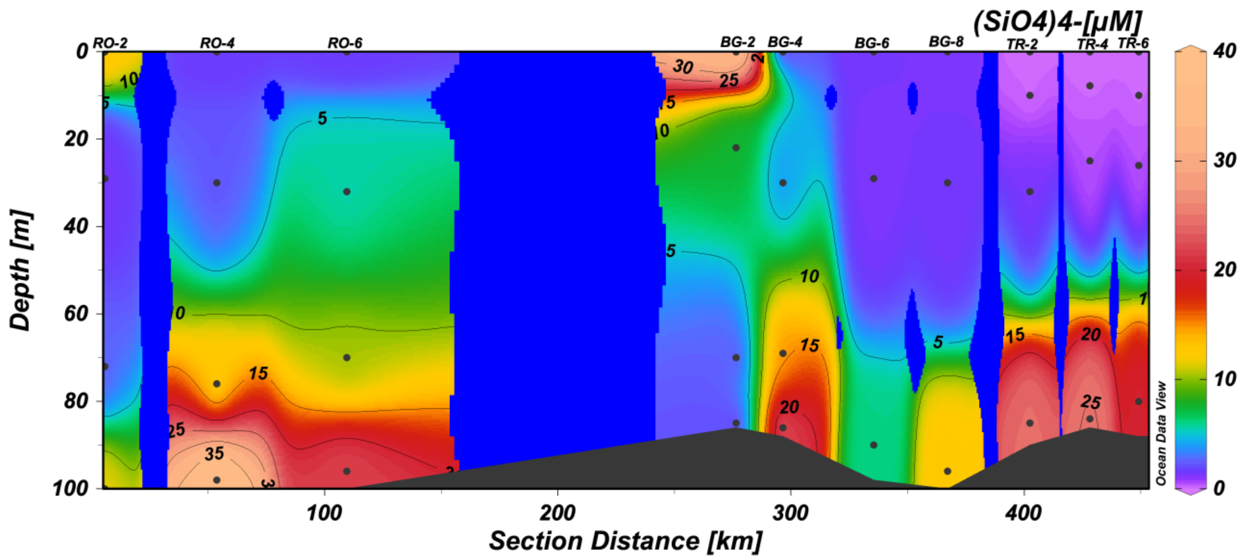


Figure 2.10 - Water column (0-100m) silicate

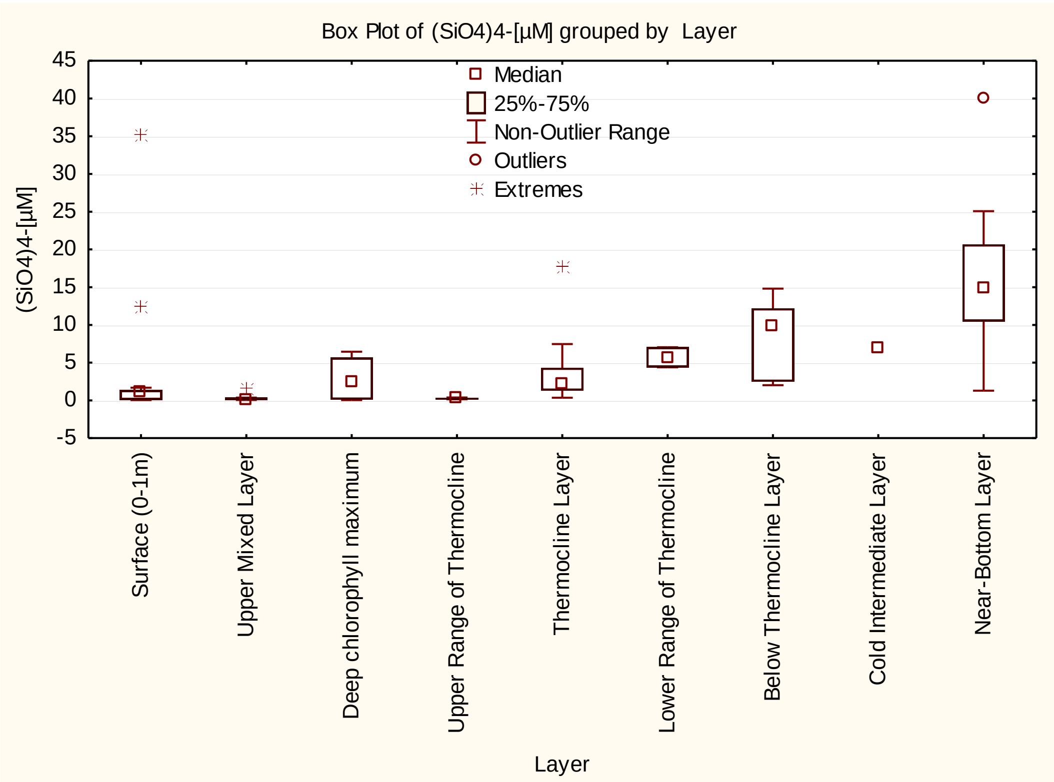


Figure 2.11 - Box plots of SiO4 grouped by layers

Nitrogen forms

The nitrate, nitrite and ammonium's seasonal variations occur in the surface layer due to biological activity. In spring, the phytoplankton proliferation into the coastal waters results in the sharp decrease of the inorganic nitrogen concentrations from the euphotic zone. Zooplankton and fish consume the phytoplankton, and the nitrogen reappeared as excretion products (e.g. ammonium and urea). The vertical mixing contributes as well to the replenishment of the inorganic nitrogen stock. In the summer, due to the thermocline's presence, the vertical mixing is inhibited, and the upper mixed layer is depleted of inorganic nitrogen. Usually, at this time, the main form is ammonium from excretions and rapidly assimilated by phytoplankton, again driving to the constant regeneration of

nitrate. Nitrification is complete until the thermocline breaking allows the homogenous nitrate distribution into the water column (Peres, 1961; Riley, 1971; Horne, 1969; Jensen, 2009).
The oxidized nitrogen forms (sum of nitrite and nitrate), TNOx, generally showed the lowest levels in the upper mixed layer (Figure 2.12 and Figure 2.14). The maximum, 20.39 µM, occurred in RO-6, in the near-bottom layer, where nitrification processes prevail, and nitrate is regenerated (Figure 2.12 and Figure 2.14).

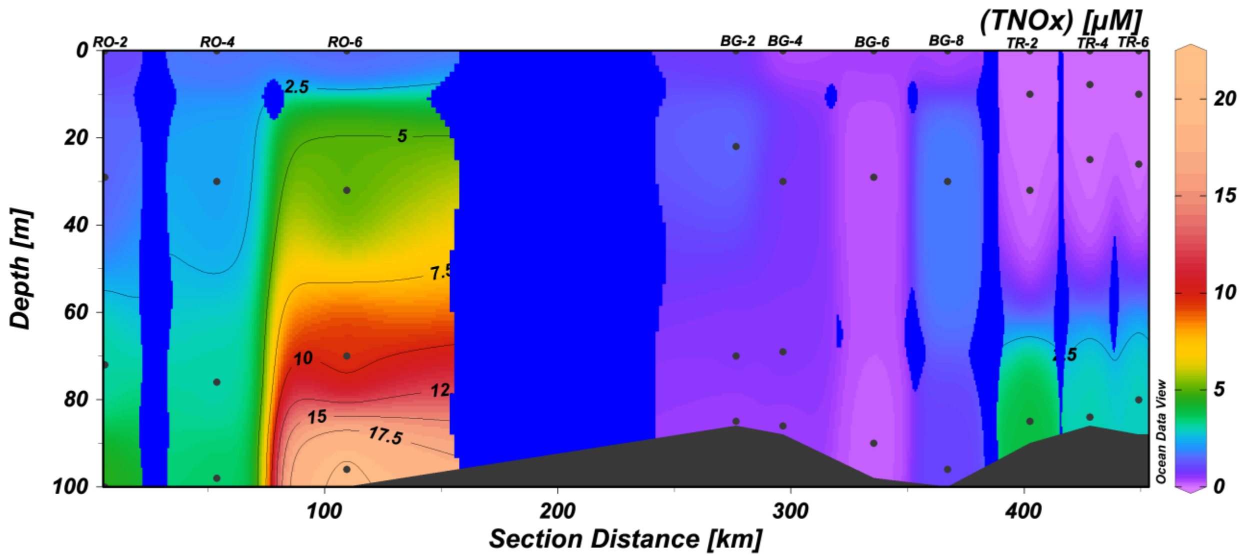


Figure 2.12 - Water column (0-100m) TNOx (nitrite+nitrate)

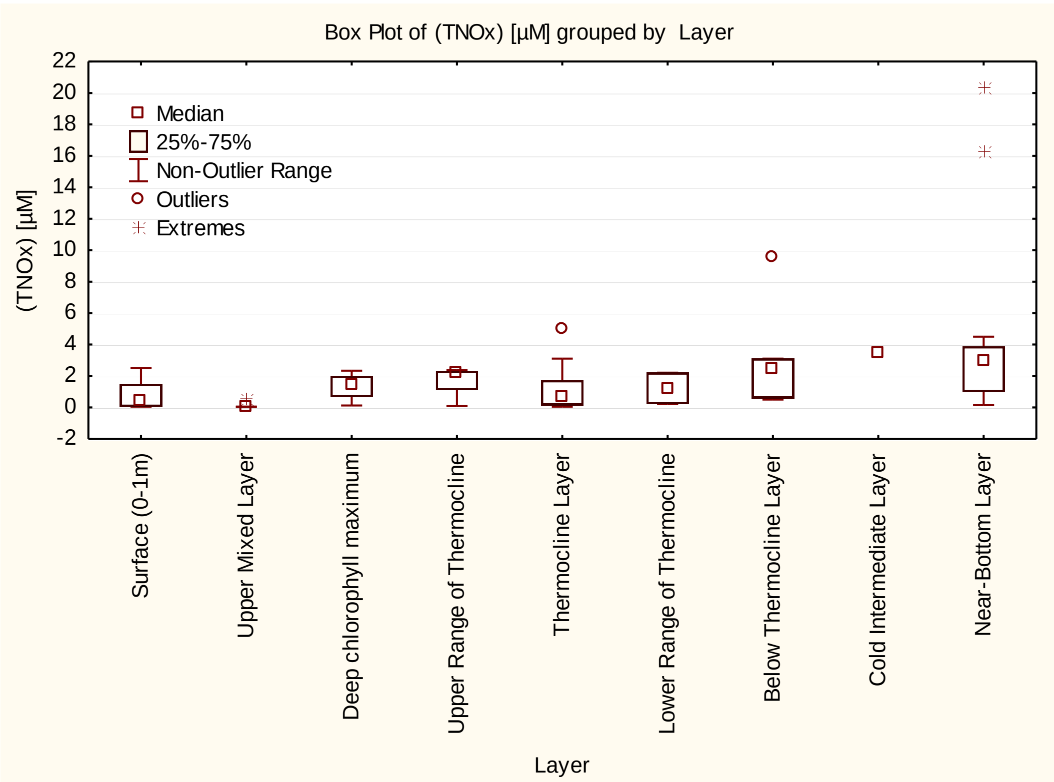


Figure 2.13 - - Box plots of TNOx grouped by layers

Ammonium generally showed homogenous and low concentrations in the oxic layer. The peak occurred in the oxicline (Figure 2.14Figure 2.15

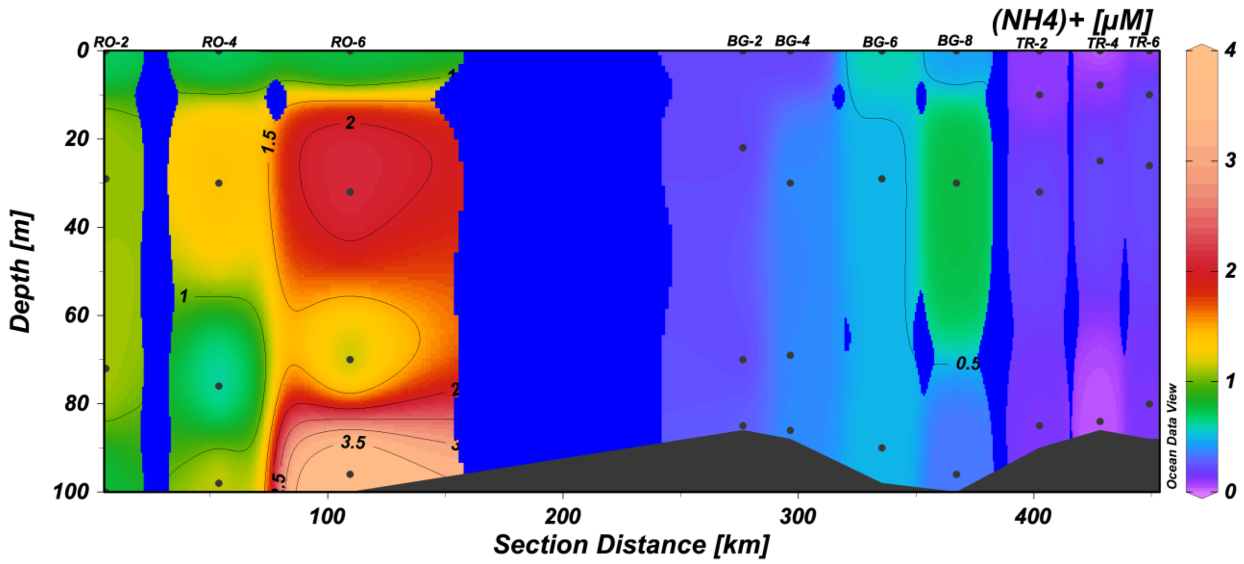


Figure 2.14 - Water column (0-100m) ammonium

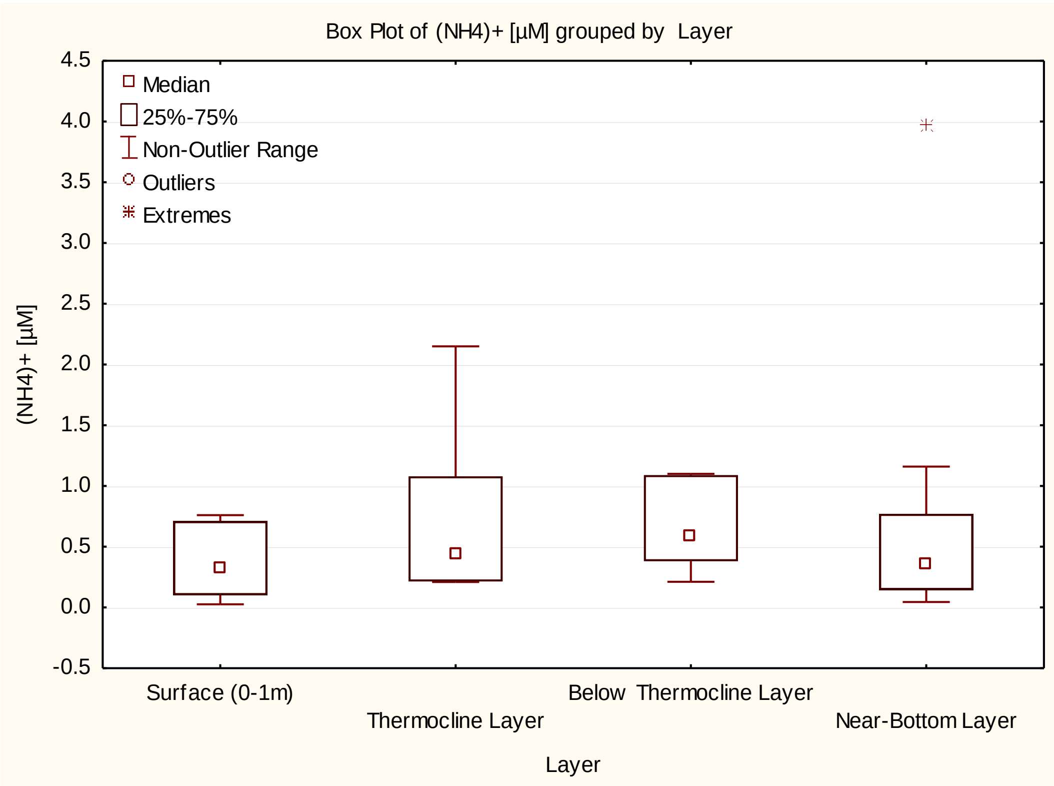


Figure 2.15 - Box plots of NH4 grouped by layers

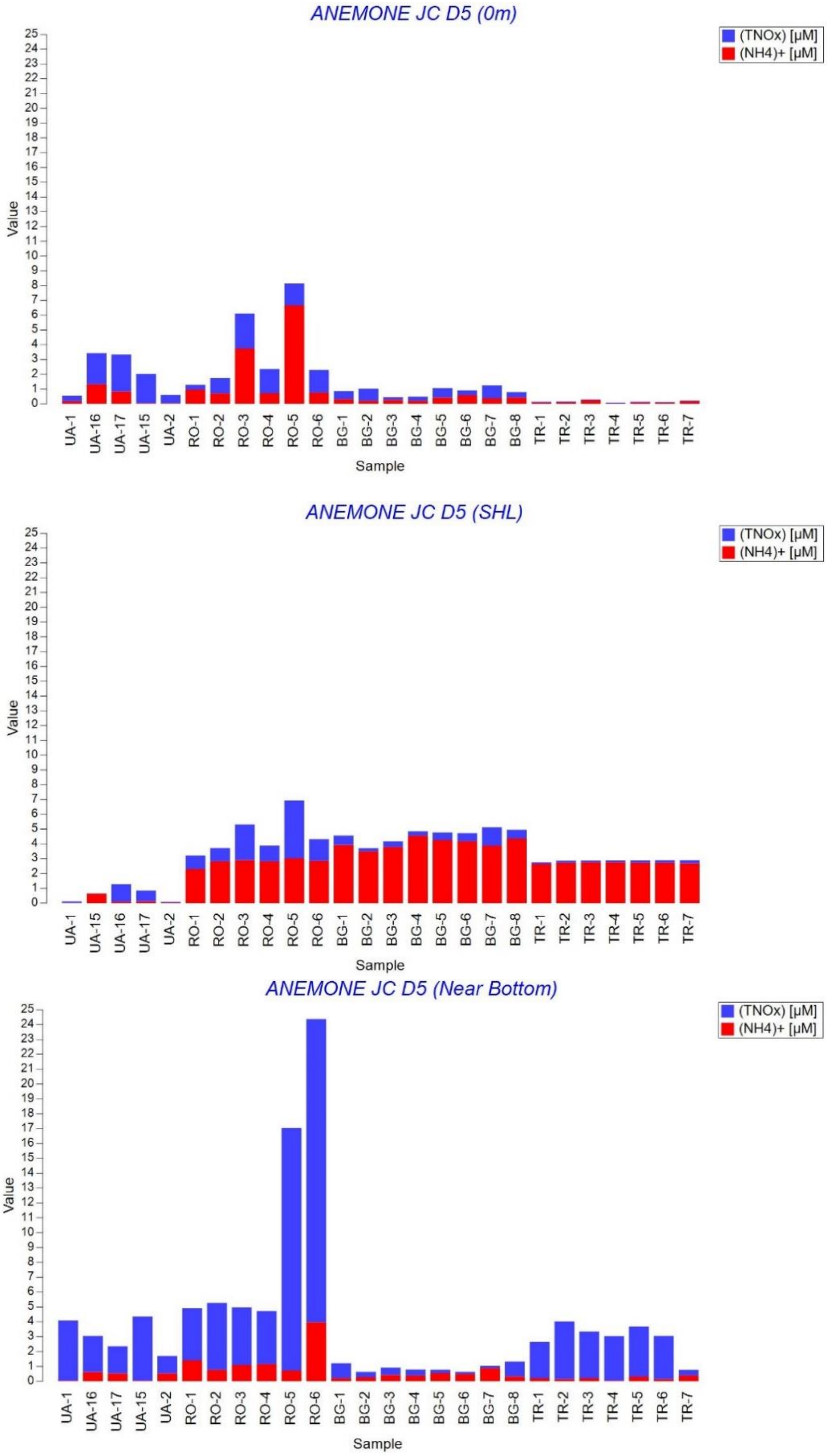


Figure 2.16 – Total oxidised inorganic nitrogen and ammonium dominance at surface (up), in SHL (middle), and near bottom (down)

Nutrient Ratios

The optimal N/P ratio for phytoplankton growth is 16:1 (based on molar concentrations) and is called the Redfield ratio. Significant deviations from 16 at low N/P-ratios (below 10) might indicate nitrogen limitation and at high N/P ratio (above 17) potential phosphorus limitation of phytoplankton growth. Deviations in the range 10-17 indicate that either of the nutrients may be limiting (Ekhlom, 2008). This might affect the biological state of the ecosystem, particularly the phytoplankton biomass, species composition and eventually food web dynamics. Anthropogenic eutrophication in the coastal environment results from increased delivery of land-based nutrients considerably enriched in nitrogen (N) and phosphorus (P) compared to silicon (Si). These nutrient inputs strongly modify the nutrient balance N:P:Si in the coastal waters for phytoplankton stoichiometry, i.e. N:P=16 for marine phytoplankton (Redfield et al., 1963) and N:Si=1 for coastal diatoms (Bzrezinski, 1985). This, in turn, modifies the composition of the phytoplankton community characterized by a dominance of opportunistic non-siliceous species (Officer & Ryther, 1980; Billen et al.,1991).

In contrast to N and P riverine fluxes (which have been strongly modified in the past 50 years), silica fluxes (which originate essentially from the weathering of rocks) has remained relatively constant or even decreased due to eutrophication and/or trapping in reservoirs. Therefore, silica has become a limiting factor for river diatoms in the large rivers' main branch, resulting in lower Si/N and Si/P ratios in estuaries and coastal regions. Whereas increased N, P deliveries to the coastal zone are recognized as a significant threat to the ecological functioning of nearshore coastal ecosystems, less attention has been paid to their imbalance concerning silica (Officer & Ryther, 1980; Conley et al., 1993; Turner & Rabalais, 1994; Justic et al.,1995a; Justic et al., 1995b; Billen & Garnier, 1997; Turner et al., 1998; Conley, 1999; Humborg et al., 2000; Cugier et al., 2005; Billen & Garnier, 2007; Humborg et al., 2008). However, Si/P and Si/N ratios determine the phytoplankton community structure, especially the shift from diatoms to non-diatoms and these changes may have significant impacts on water quality in the proximal, i.e. nearshore part of the coastal zone (Turner et al., 2003; Cugier et al., 2005; Howarth & Marino, 2006).

Ratios were calculated based on inorganic nitrogen concentrations (sum of oxidized forms and ammonium) as N, phosphate concentrations as P and silicate concentrations as Si. The most impaired ratio was Si/P which almost doubled due to phosphorus limitation, particularly at the surface. N/P ratio reached its maximum in the thermocline (Figure 2.14).

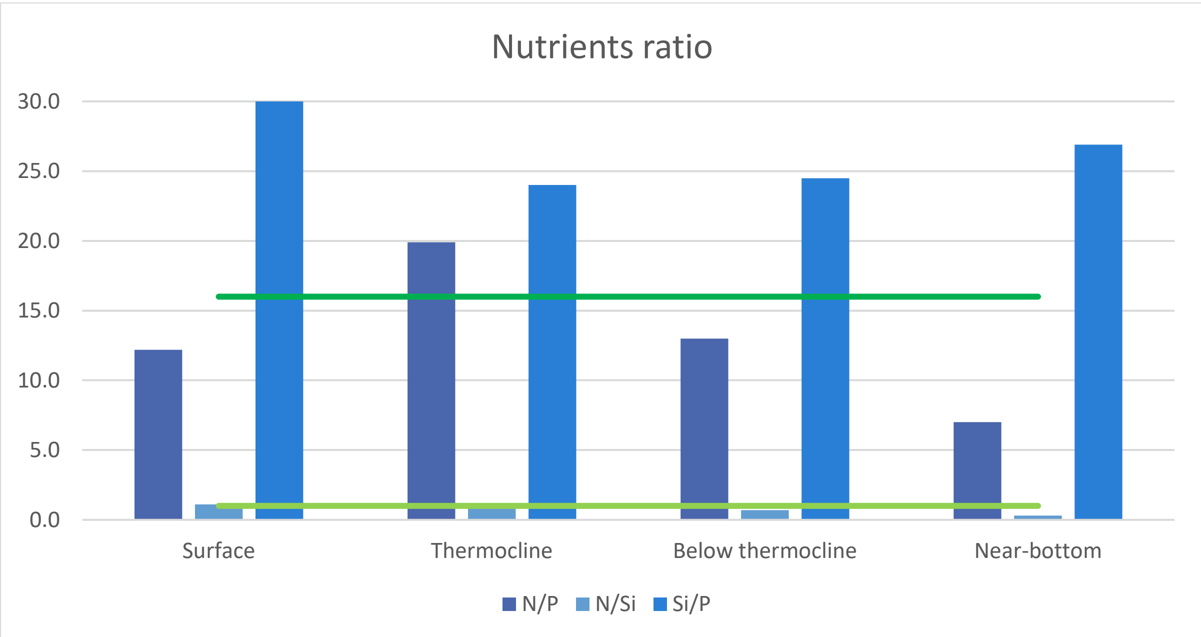


Figure 2.17 - Nutrients ratio in the water column

2.4 Water column chlorophyll *a*

The use of chlorophyll *a* as a status indicator has many advantages - both spectrophotometric and fluorometric analytical methods are time and cost-effective, and reproducible, while the results are easily comparable among datasets (Domingues et al., 2008); the sensitivity of chlorophyll *a* to nutrient concentrations in the water column is well documented (Håkanson & Eklund, 2010; Harding et al., 2013; Mozetič et al., 2012). Therefore, it is not surprising that chlorophyll *a* datasets are among the most widely used in the assessment systems (Giovanardi et al., 2018; Höglander *et al.*, 2013). However, there are also some disadvantages of the use of chlorophyll *a*, e.g., the non-linear relationship between chlorophyll *a* concentration and species biomass expressed in carbon content due to environmental factors and interspecific differences (Kruskopf & Flynn, 2006) or shifts in the baselines due to other pressures related to global change (Carstensen et al., 2011).
In the Black Sea region, chlorophyll *a* is the only phytoplankton parameter considered together with nutrient concentrations, water transparency, and bottom oxygen in the Eutrophication Assessment Tool (BEAST) used as Descriptor 5 integrated assessment tool. Threshold values for chlorophyll *a* for broad pelagic habitats and marine reporting units (MRU) by countries are presented in Table 2.2.

Table 2.2 - Threshold values for Chlorophyll a (mg/m^3) for broad pelagic habitat shelf by countries (summer)

Country	**Broad pelagic habitat shelf**	**Chl a threshold value**
Ukraine	ShW_UA_3	1.8
	ShW_UA_5	1.2
	ShW_UA_1	0.75
	ShW_UA_7	0.67
Romania	BLK_RO_RG_MT01 (30 - 200m)	2.79
Bulgaria	BLK-BG-AA-Shelf-South (30-200m)	1.3
Turkey	TR-KARD1 (10-100m) west BS1 shelf	0.69

Spatial distribution

In line with the recent trends, the average surface chlorophyll *a* concentration for the entire shelf area was low, 0.61±0.36 µg/L, and varied within the absolute values 0.26 -1.76 µg/L, the highest value was in the UA shelf (UA-17) and the lowest in the RO shelf (RO-4). The lowest average surface chlorophyll *a* was measured in the RO shelf (0.33±0.05 µg/L) followed by BG shelf (0.49±0.12 µg/L) (Figure 2.18). In the TR shelf the average surface chlorophyll *a* concentration (0.61±0.13 µg/L) was close to that in the BG shelf and about 2 times higher than in the RO shelf but at very low range of variation (Figure 2.19). The highest surface average (1.27±0.5 µg/L) was observed in the UA shelf (exceeding that in the RO, BG and TR shelf respectively about 3.8, 2.6 and 2.1 times, but also low for the area) to a certain extent related to the shallow depth range of the stations (< 50m), proximity to the shore and riverine influence and the different period (conditions) of sampling (for example the salinity at station UA-1 was 14.4 PSU, where relatively high surface chlorophyll *a* concentrations were measured, 1.57 µg/L) (Figure 2.18 and Figure 2.19).
There was no specific pattern of surface distribution neither from north to south, nor with stations depth (innershelf stations <50m and the outershelf stations > 50m) (statistical analysis not shown).

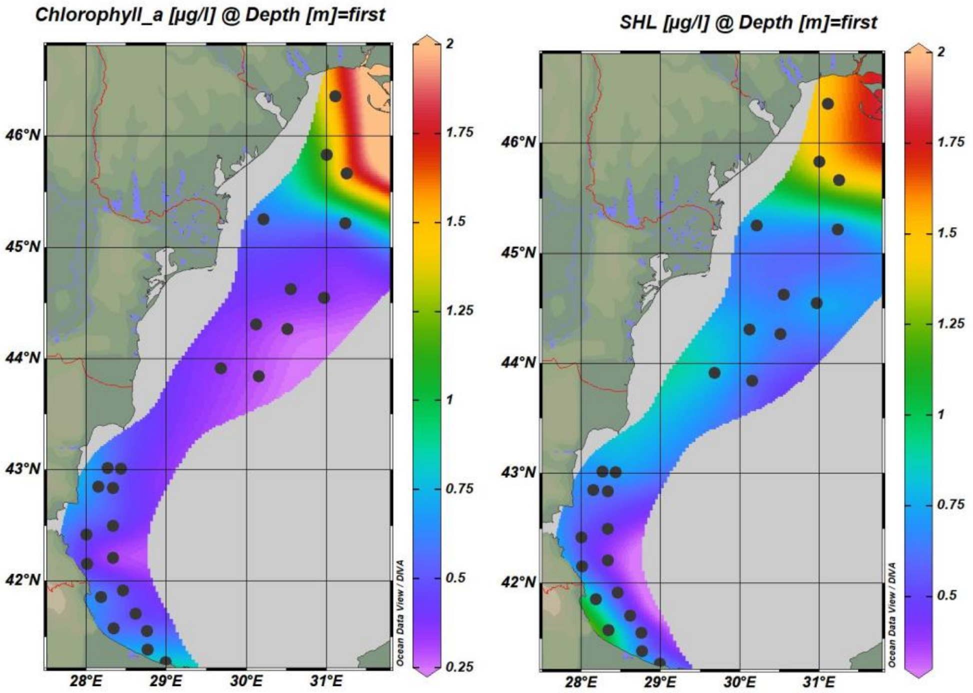


Figure 2.18 - Chlorophyll a (µg/L) pattern of distribution: surface (L) and Surface Homogenous Layer (SHL)(R)

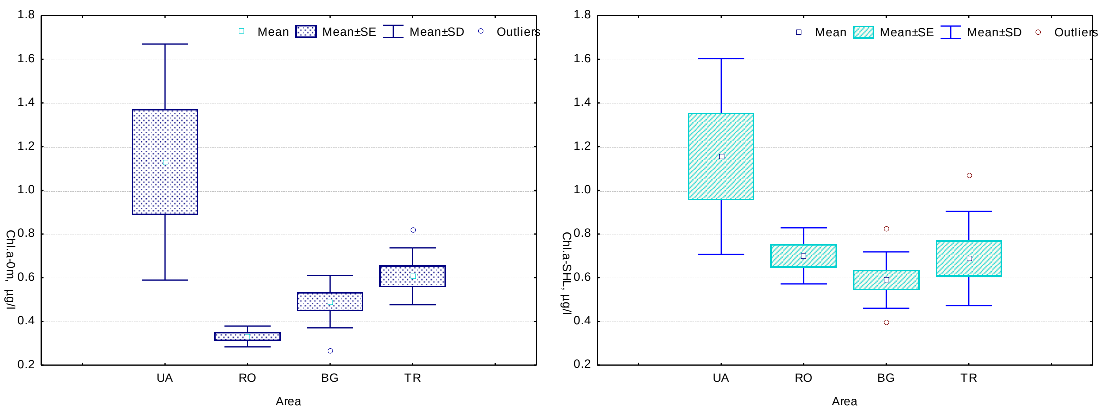


Figure 2.19 - Box-Whisker plots of chlorophyll a (µg/L) surface (L) and SHL (R)

The Kolmogorov-Smirnov test was applied to test the data's normal distribution, Levene's test for homogeneity of variance and t-test for two independent means to test statistically significant differences between the shelf regions. Box-Whisker (StatSoft Inc., 2004) and cluster analysis was applied to the data set to identify possible patterns of chlorophyll *a* distribution (Primer, 5.0).

In addition, satellite data were used to assess the general model of chlorophyll *a* distribution in the entire northwestern Black Sea area during the cruise sampling period. A k-means clustering analysis was applied to Sentinel 3/Ocean and Land Color Instrument (OLCI) pigment concentrations retrieved from regional empirical algorithm (Slabakova et al., 2020). The coefficient of the algorithm was derived from the cubic polynomial regression of log transformed in situ chlorophyll *a* and ratio of remote sensing reflectance at 490 nm and 560 nm with R2=0.88 (N=186). The chlorophyll *a* product was derived from Sentinel 3 (S3) single swath OLCI Level 2 Full Resolution water-leaving reflectance by applying the equation with corresponding coefficients. Using the Sentinel Application Platform (SNAP) Level 3 Binning Operator (Campbell et al, 1995) the S3/OLCI Level 2 regional chlorophyll *a* products were spatially averaged into Level 3 daily gridded files with 1km resolution. Pixels were filtered according to a Water Quality and Science Flags (WQSF) expression that removes clouds, land, inland water, etc. and pixels with out-of-range OLCI data, allowing only the highest quality pixels.

Then the daily gridded files were used as input for generation of a six days composite map, corresponding to the period of Anemone Joint cruise.
The Kolmogorov-Smirnov Test of Normality statistic (D = 0.20183, and p= 0.3154) shows that data does not differ significantly from that which is normally distributed. The Levene's Test showed that homogeneity is met for the two main clusters with average chlorophyll *a* 0.58 µg/L and 0.33 µg/L identified by cluster analysis (Figure 2.20) respectively (f-ratio=3.82156 and p=0.065 at significance $p < 0.05$).
The cluster analysis applied to the composite satellite data constructed for the week of sampling also shows homogenous distribution of the surface chlorophyll *a* concentration (Figure 2.20), with the lower values of mean chlorophyll *a* concentrations (0.31 - 0.86 µg/L) contributing about 88% of the total pixels. The increased mean chlorophyll values (>1.64 µg/L) were mostly distributed in the northwestern Black Sea coastal and inner shelf waters and some coastal regions in the western (Varna and Burgas Bay) and southwestern Black Sea (near Bosporus region, which were out of the sampling area) but give a general picture of the chlorophyll *a* pattern of distribution (Figure 2.21).

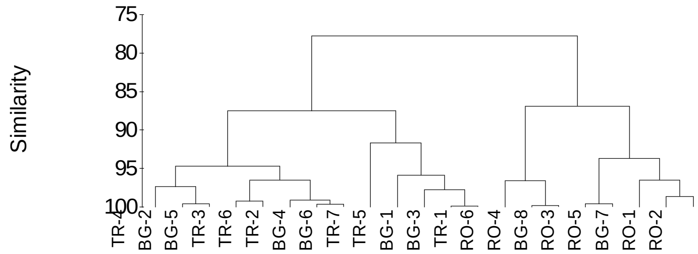


Figure 2.20 – Cluster analysis of the sampling stations based on surface chlorophyll* a *(µg/L)

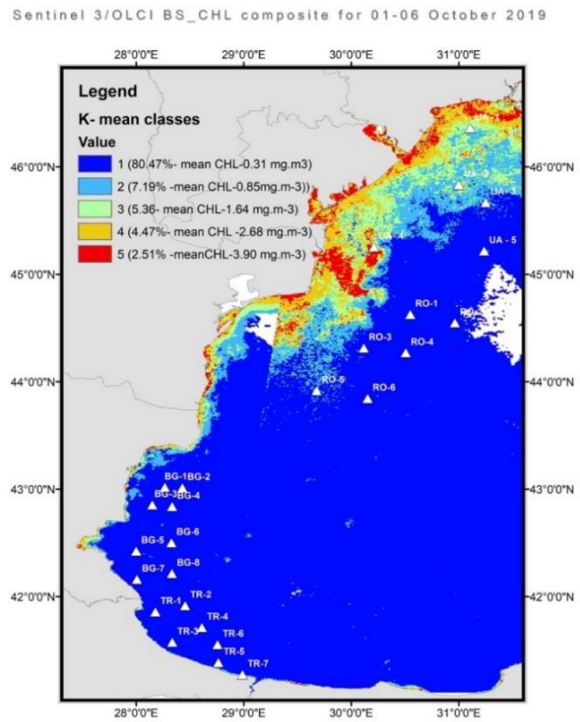


Figure 2.21 - Sentinel 3/OLCI pigment (chlorophyll* a *concentration), 01-06 October 2019

Vertical distribution

At all stations the integrated SHL chlorophyll *a* was higher than that at the surface with close average per shelf pelagic habitat respectively for RO, BG and TR (0.70±0.13 µg/L; 0.59±0.13 µg/L and 0.69±0.22 µg/L), excluding UA where there was no difference between the surface and the SHL concentrations (SHL, 1.29±0.38 µg/L) (Figure 2.18, Figure 2.19). The pairwise Levene's test showed that homogeneity is met for the three pairs accordingly for RO-BG (f-ratio=0.419 and p=0.529), for BG-TR, (f-ratio =1.994 and p=0.181) and for RO-TR (f-ratio=0.996 and p=0.340) at significance p <0.05 While similar to the surface the mode of distribution did not show any consistent pattern from north to south and with stations depth, the data manifest a type of clustering suggesting also homogenous distribution but with few stations with higher values distributed randomly within the pelagic habitats of the area (Cluster 1), most likely related to the pattern of currents (Figure 2.22 and Figure 2.23).

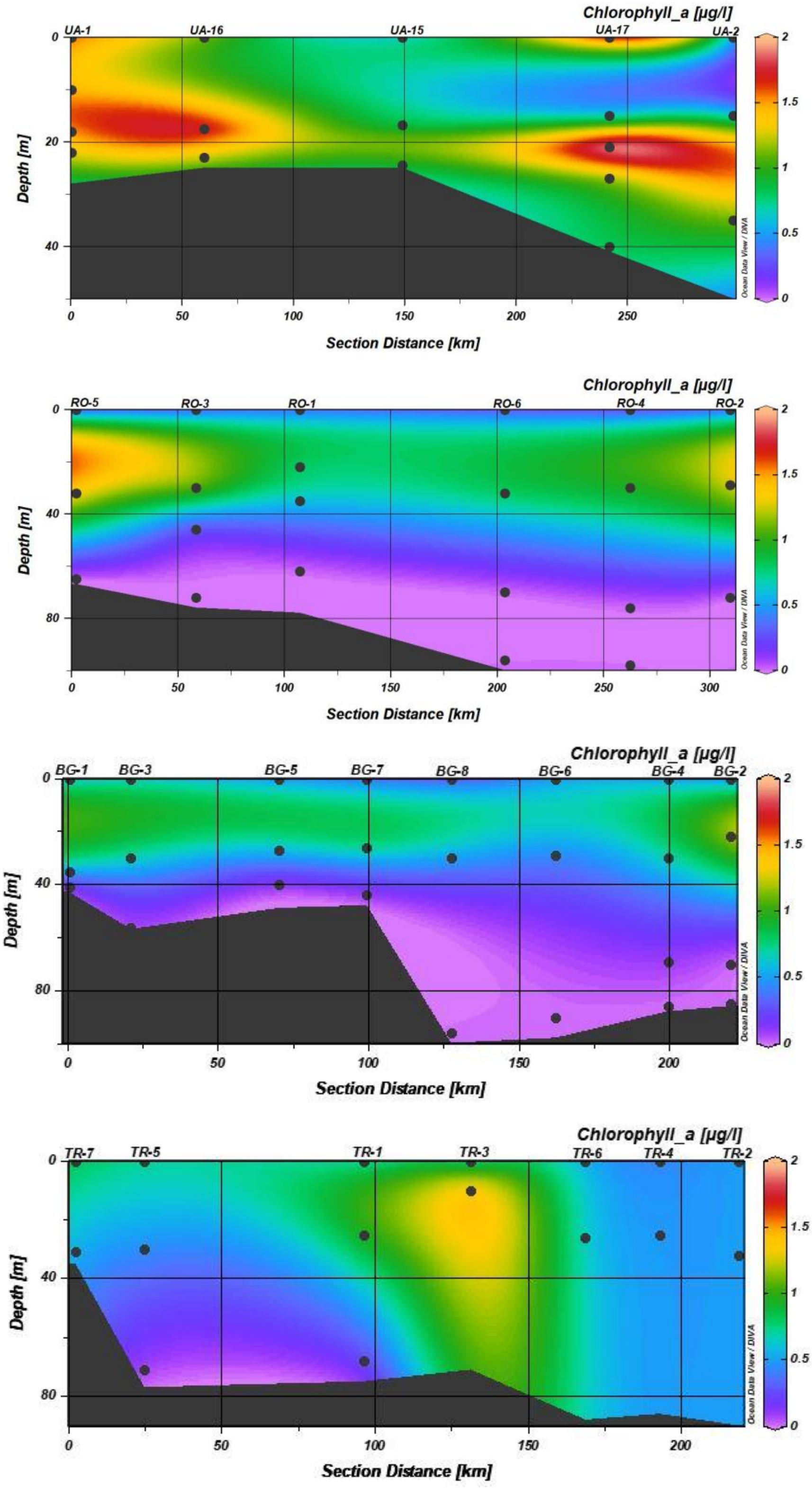


Figure 2.22 - Vertical distribution of chlorophyll a concentration (µg/L) by stations and pelagic habitats

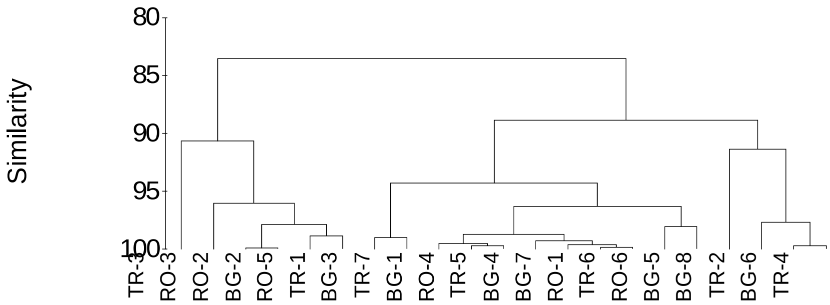


Figure 2.23 - Cluster of the sampling stations based on SHL chlorophyll a (µg/L)

Chlorophyll *a*, below the thermocline was "undetected" excluding the stations <50m where it was less than 0.1 µg/L, typical for the summer distribution in the Black Sea under the conditions of strong vertical stratification.

2.5 Photic limit (transparency) of the water column

Transparency is a measure of the water's clarity showing its attenuation of light penetration into the water column. It is influenced by the properties of absorption and water diffusion, in turn, dependent on the existing amount of particulate matter and dissolved substances. Generally, in the seawater is found alive or dead organic particles (e.g. phytoplankton), small particles and inorganic coloured solutes (e.g. humic acids). Thus, transparency integrates many of the concrete effects of eutrophication, such as the disappearance of perennials and flowering or algal blooms intensification. Transparencies (N=19) ranged from 8.0 m to 12.0 m (mean 10.0 m, std. dev 1.2 m) with no significant correlations with chlorophyll *a* concentration and reflecting the good ecological status (Figure 2.24).

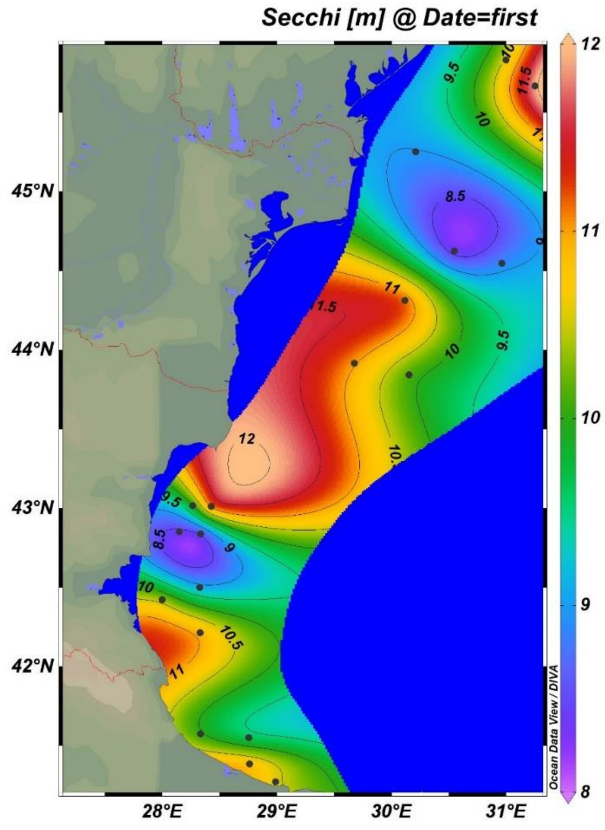


Figure 2.24 – Seawater transparency

2.6 Bottom dissolved oxygen

The dissolved oxygen regime variability depends on several antagonistic factors. Thus, the seawater oxygen enrichment's contributing factors are currents and winds regime, atmospheric contact, and photosynthetic processes. While, there are more numerous and varied factors contributing to the depletion of seawater oxygen content: the supersaturated water masses contact with the atmosphere, which may sometimes benefit from the contribution of oxygen to maintain the balance at the air-water interface, respiration, biological and chemical oxidation processes (of reducing agents (e.g. H_2S, FeS), dissolved and particulate organic matter, sediments, enzymatic processes, bacterial oxidation, water masses stratification (Riley, 1971; Horne, 1969; Peres, 1961; Best, 2007).
The Black Sea represents a stratified system. The vertical distribution of dissolved oxygen in the Black Sea reflects its specific features as the density stratified basin with a permanent H_2S zone under the pycnocline.
The upper layer biogeochemistry, below the permanent anoxic waters, involves four distinct layers (BSC, 2008; Sorokin, 2002; Konovalov, 2000). In ANEMONE JC we identified only the first two (Figure 2.25 and Figure 2.26).

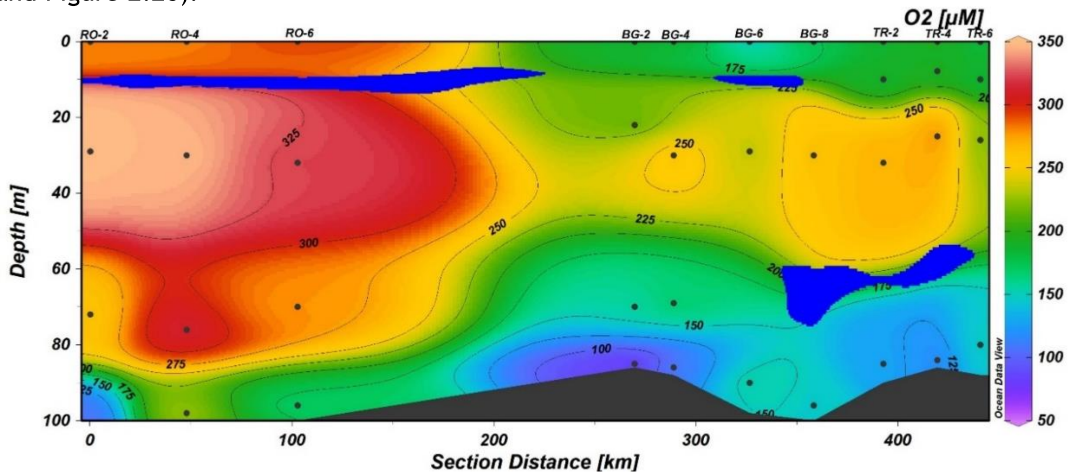


Figure 2.25 – Dissolved oxygen water column content

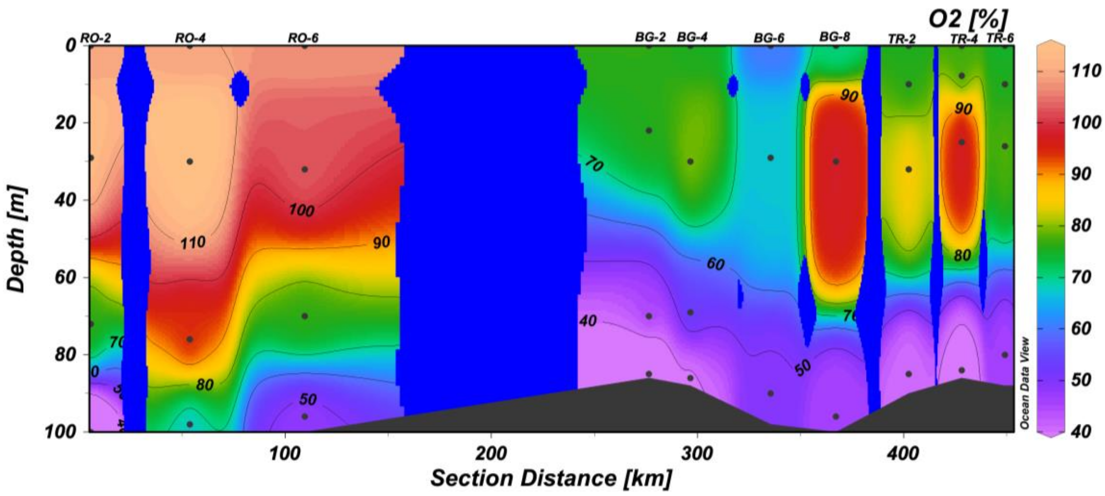


Figure 2.26 – Dissolved oxygen saturation water column content

Oxic layer – situated approx. in the range 0-65m, is characterized by biological processes, with high dissolved oxygen levels and quite low nutrients concentrations. In this layer, below the thermocline, the nutrients concentrations started to increase due to the regeneration. Thus, the highest values of the oxygen concentrations were found there due to the phytoplankton enhanced activity sustained.
Oxicline – (part of the oxic layer) the oxygen concentrations started to decrease was situated approx. at 80 -100m. In the oxicline layer, the dissolved oxygen content reduces to ~50 Mm.

2.7 Assessment of trophic status and water quality with E - TRIX

A universal method for assessing the level of eutrophication (trophicity) of marine waters and generally accepted manuals for practical assessment do not exist to date. For each study on this problem, a subjective author's approach prevails, that usually determines the choice of indicators and their number when calculating various environmental indices. Usually, the proposed assessment methods are limited with the number of measured hydrochemical and biological parameters and indicators of the marine environment. The most frequently recommended for scientific research and use in monitoring programs for the state of the natural marine environment is the calculated E-TRIX index, which has been widely used in recent years.
E-TRIX is an integral indicator related to the characteristics of the primary production of phytoplankton and nutritional factors. In the calculation formula of the index E-TRIX is composed of the following indicators of ecosystem: the concentration of chlorophyll-a - analog, which replaces the index of phytoplankton autotrophic biomass; the deviation of oxygen saturation from 100% - an indicator of the primary production intensity of the system, which covers the phase of active photosynthesis and the phase of respiration predominance; the concentration of total phosphorus and mineral nitrogen-indicators of the nutrients presence (Vollenveider, 1998) .
E-TRIX is calculated by the formula:

$$TRIX = [log(Ch \cdot D\%O \cdot N \quad \cdot P) + 1.5]/1.2$$

where *Ch* – chlorophyll concentration, µg/L;
D%O - deviation in absolute values of dissolved oxygen from 100% saturation;
N - concentration of the sum of mineral nitrogen dissolved forms, µg/L;
P - concentration of total phosphorus, µg/L.
The E-TRIX index changes according to the conditions of water trophic status in the range from 0 to 10, and the assessment of the category of trophic level and the state of water quality is carried out according to the index value (Table 2.3)

Table 2.3 - Characteristics of waters according to TRIX value

MSFD	Water quality	Value of E-TRIX	Trophic level	Characteristics of water
GES*	High	≥0 - ≤4	Low	High transparency of water, lack of colour anomalies of water, lack of satiety and lack of saturation of dissolved oxygen
	Good	>4 - ≤5	Moderate	Occasional cases of reducing transparency of water, lack of water colour anomalies, hypoxic bottom waters.
Not GES	Moderate	>5 - ≤6	High	Low water transparency, water colour anomalies, hypoxia of bottom waters, and occasional cases of anoxia.
	Bad	>6 - ≤10	Very high	High water turbidity, large areas of colour anomalies of water, regular hypoxia over a large area and frequent anoxia of bottom waters, death of benthic organisms

Methodological aspects in determining the E-TRIX index by the averaged data of individual measurements, and by calculation for the initial data and subsequent averaging of index values, were discussed in (Ukrainsky, 2010). In the calculation, formula uses standard and most frequently measured hydrochemical and hydrobiological characteristics of marine waters, the number of parameters does not change, which makes it possible to compare the values of E-TRIX for different areas of the sea and oceans.
For the assessment of trophic status and water quality with the E-TRIX of the Black Sea shelf, we use the data collected in the Ukrainian part of the Black Sea shelf for the period of 08/29/19 to 09/31/2019. Data for the shelf of the Romanian, Bulgarian and Turkish regions were collected in the period of 10/01/19 - 10/06/2019.
In general, based on the results of the E-TRIX assessment, the quality of the Black Sea shelf waters in the study areas was assessed as GES for both surface and near-bottom waters, which is shown in Figure 2.27 and Figure 2.28.
The exception is two stations in the Ukrainian part of the shelf, where the water quality does not fit the GES limits. In the central area of the NWBS st. UA16, surface water quality does not fit GES, E-TRIX value is 5.17, which pointed out “Moderate” water quality and “High” trophic level. At this station, the total phosphorus concentration was 34.43 µg/L, mineral nitrogen 48.0 µg/L. Among the

mineral forms of nitrogen, the maximum was noted for ammonium (18.58 μg/L). The chlorophyll concentration was 1.14 μg/L; also, low oxygen saturation was noted (73.38%). The high concentration of ammonium nitrogen suggested its recent entry into the surface waters of this area.

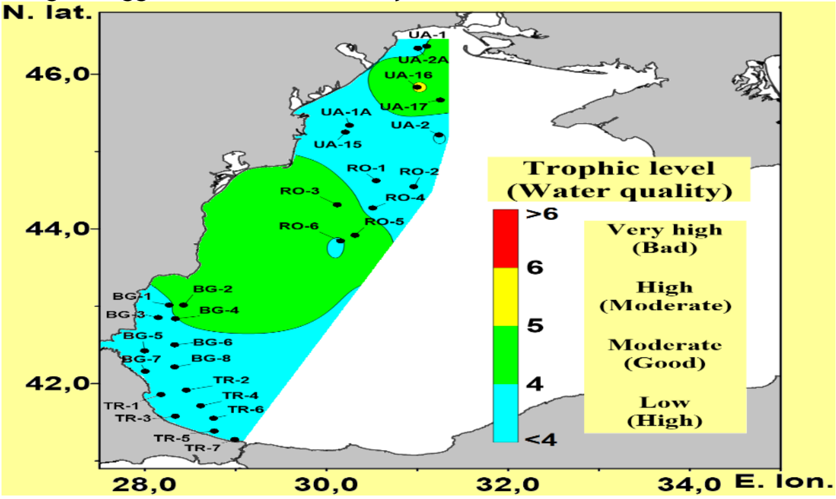


Figure 2.27 - Assessment of trophic status and water quality with the E-TRIX of the Black Sea shelf (surface)

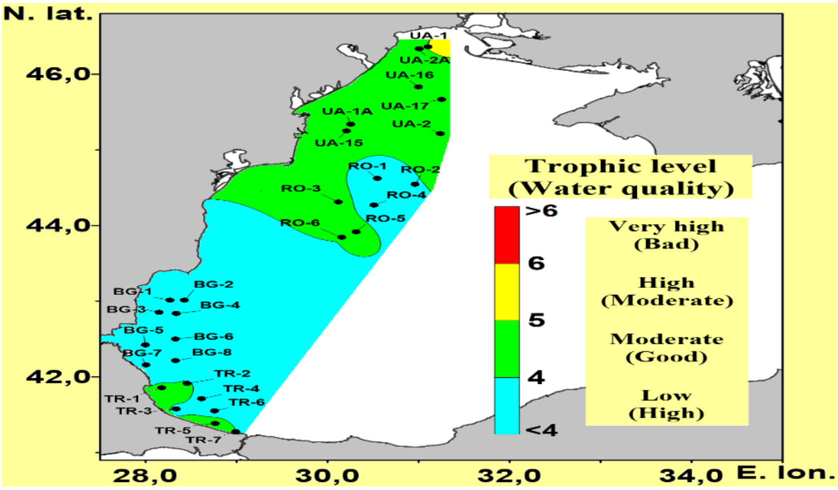


Figure 2.28 - Assessment of trophic status and water quality with the E-TRIX of the Black Sea shelf (bottom)

Also, in the near-bottom waters (depth 28 m) in the Ukrainian part of the shelf at station UA-1, the water quality did not fit GES; the E-TRIX value was 5.17, which indicated “Moderate” water quality and “High” trophic level. At this station, the concentration of total phosphorus was 27.16 μg/L, of mineral nitrogen 64.3 μg/L. Chlorophyll concentration was 1.3 μg/L, oxygen saturation was also low (79.3%). In this situation, based on the maximum of mineral nitrogen in the form of TNOx, it can be assumed that the path of nutrient intake is directed from bottom sediments to near-bottom waters. In general, according to the results of the assessment by the E-TRIX method, the quality of the Black Sea shelf waters in the study areas matched GES for both surface and near-bottom waters. The exception is two stations in the Ukrainian part of the shelf, where the water quality was lower than GES. In the central area of NPMS at st. UA-16, the surface water quality was not GES, the E-TRIX value is 5.17, which pointed out Moderate Water quality and High Trophic level. There were registered high concentrations of ammonium nitrogen (18.58 μg/L) and a low oxygen concentration (73.38%), which indicated the recent enrichment of this region's surface waters with nutrients. Also, at UA-1 station in the near-bottom level (depth 28 m) in the Ukrainian part of the shelf, the water quality was not GES; the E-TRIX was 5.17, which indicated “Moderate” water quality and “High” trophic level. The maximum of TNOx (56.75 μg/L), chlorophyll concentration of 1.3 μg/L, and low oxygen saturation (79.3%) were also noted. These factors indicated the flow of nutrients from bottom sediments into bottom waters at this time of the study.

2.8 Black Sea eutrophication integrated assessment - BEAST

BEAST (Black Sea Eutrophication Assessment Tool) was developed in the frame of Baltic2Black project based on the HELCOM Eutrophication Assessment Tool (HEAT 2.0). HEAT 2.0 it was developed based on the OSPAR “Common Procedure” and taking the requirements of the MSFD Commission Decision into consideration.
Thus, BEAST categories are divided into three criteria: C1 - causes of eutrophication, C2 - direct effects and C3 - indirect effects. Each criterion could have a set of indicators (based on availability and expert choice). The result of each indicator status is done by EUTRatio and it is included, according to its own weight (chose by expert), into a qualitative response: high, good, moderate, poor, and bad (Table 2.4 and
Table 2.5).

Table 2.4 - Category 1 - Nutrients levels - BEAST

C1: Nutrient levels	RefCon	AcDev	EUT_Target	Unit	Resp	EUT_T-score	EUT_status	EUT_S-score	EUT_Ratio	Ind_Conf	Weight	C1_EUT_sum	C1_EUT_status	C1_conf	C1_Weight
DIP	0.10	50%	0.15	μM	+	H M L	0.06	H M L	0.400		50%				
TNOx	1.00	50%	1.50	μM	+	H M L	0.44	H M L	0.293		50%				
Add new indicator …															
											100%	0.347	HIGH		

Table 2.5 - Glossary of terms used in BEAST

RefCon	Reference conditions
AcDev	Acceptable deviation (from reference conditions)
EUT_Target	Eutrophication target (or calculated from RefCon ± AcDecv
Resp.	Numerical response to nutrient enrichment (+ or ÷)
EUT_T-score	Confidence score assigned to the eutrophication target (H = high; M = moderate; L = low)
EUT_status	Eutrophication states (based on monitoring data from a given period/year)
EUT_S-score	Confidence score assigned to the eutrophication status (H = high; M = moderate; L = low)
EUT_Ratio	Eutrophication Ration (calculated from: EUT_status/EUT_Target)
Ind_conf	Indicator confidence (calculated from EUT_T-score and EUT_S-score)
C1_EUT_sum	Eutrophication Sum for Criteria 1 (the sum of individual EUT_ratio's)
C1_EUT_status	Eutrophication Status for Criteria 1 (five classes: High, Good, Moderate, Poor and Bad)
C1_conf	Confidence (weighted) for Criteria 1
C1_Weight	Weight factor assigned to Criteria 1 (100; 50 or 33%; pending the number of criteria covered)

Within the categories, BEAST is averaging the parameters or taking a weighted mean (according to the significance of the parameter or the data quality) while, between the categories, the One-Out-All-Out-principle (OOAO) is applied (the worst assessment of a quality element determines the overall assessment result). The result is another qualitative response, the “Final eutrophication status”: high, good, moderate, poor and bad.

For the Black Sea eutrophication assessment, based on one summer cruise (report citation), it was used a core set of indicators (due to their availability, reference conditions availability and relevance) as follows:

- C1 - causative factors – nutrients (DIP - ortophosphate, TNOx – sum of nitrate and nitrite) weighted as 50% each.
- C2 - direct effects – phytoplankton blooms – chlorophyll *a* (as an estimate of the Total biomass)
- C3 - indirect effects – bottom dissolved oxygen (%) (effective only for coastal and shelf waters up to 50m bottom depth due to the natural features of the Black Sea)

Each country project partner expert provided the reference conditions used. The EU MS (Romania and Bulgaria) acquired them as an MSFD obligation, while Ukraine and Turkey achieved in national project's results.
By applying BEAST, we had 26 qualitative results (for each network station) grouped in "high", "good", "moderate", "poor", and "bad" eutrophication status. The threshold between Good-Moderate status as GES boundary considered the GES status (Table 2.6). The results highlighted 50% of the stations in non-GES status or the other way because of the stations' distribution (Figure 2.29). Thus, we consider the network stations' not appropriate for the human-induced eutrophication (D5) assessment and advocate for specific monitoring networks and frequencies.

Table 2.6 - BEAST status - ANEMONE Joint Cruise

Transect	Station	Qualitative status	Assigned value	GES
Ukraine	UA-1	Poor	4	x
	UA-2	Good	2	√
	UA-16	Poor	4	x
	UA-17	Poor	4	x
	UA-15	Good	2	√
Romania	RO-1	High	1	√
	RO-2	Good	2	√
	RO-3	Good	2	√
	RO-4	Good	2	√
	RO-5	Good	2	√
	RO-6	Moderate	3	x
Bulgaria	BG-1	Moderate	3	x
	BG-2	Poor	4	x
	BG-3	High	1	√
	BG-4	Moderate	3	x
	BG-5	Good	2	√
	BG-6	Moderate	3	x
	BG-7	Moderate	3	x
	BG-8	Good	2	√
Turkey	TR-1	Bad	5	x
	TR-2	Good	2	√
	TR-3	Poor	4	x
	TR-4	Good	2	√
	TR-5	Moderate	3	x
	TR-6	Good	2	√
	TR-7	Moderate	3	x

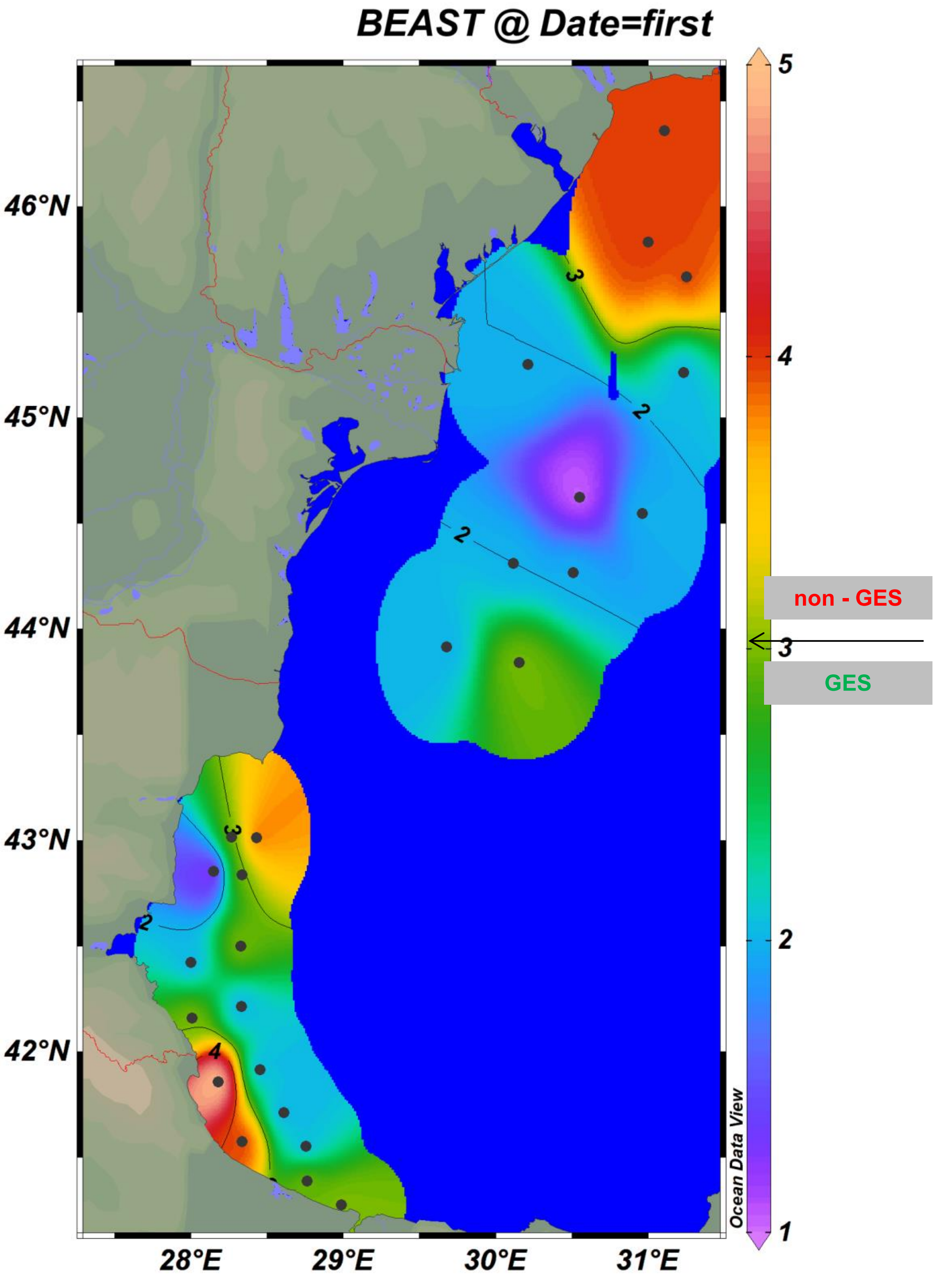


Figure 2.29– BEAST surface distribution – ANEMONE JC

2.9 Gaps and Recommendations

There is a need to establish the core set indicators to assess eutrophication at (sub) regional level based on their relevance, not availability, for having comparable results. It is imperative to have the capability to achieve the data and implement the dedicated monitoring program for eutrophication on a specific designed monitoring network considering the inland runoff.
The integrative tool (ex. BEAST) was done based on reference values established by each country and core set parameters identified due to each laboratory capability. There is no joint decision on the integration of different indicators into weights for BEAST. Considering that BEAST principle works on reference values and accepted deviations, it will be difficult to introduce in the integrated assessment biological parameters like "species shift in floristic composition" or others.
No atmospheric deposition of nutrients was quantified.
The assessment was based on one cruise (summer), which does not allow us to make an accurate assessment of the eutrophication state.
Need to understand better/define the threshold between natural variability (including seasonality and climate change) and the anthropogenic impact, which is a "must" for descriptor 5.

GAPS	RECOMMENDATIONS
No/few data on open sea waters	Systematic use of additional tools such as remote sensing of surface chlorophyll, ferry boxes, and smart buoys is recommended if data are validated with in-situ data. Develop reliable algorithms (more research needed related to the CDOM, seawater optical properties, etc.) for satellite-derived chlorophyll *a* in the shelf waters.
No/few data on atmospheric deposition of nutrients	Monitoring of atmospheric deposition of nutrients. Coupled atmosphere-river-coastal sea models need to be developed at the regional scale for the estimate of critical nutrient loads from terrestrial sources, concerning transitional/ coastal retention, and chemical and biological target indicators
No info on the threshold between natural variability (including climate change) and anthropogenic impact, which is a must in the descriptor 5.	Research on natural background nutrient enrichment (e.g. import by upwelling; import from pristine/ good status rivers) for determination of pristine state and separation of natural productive status from anthropogenic impacted eutrophic status; climate change impacts on availability and transformation of nutrients and organic matter from land to the sea.
The link to land-based inputs is not well established.	Identification of critical nutrient loading thresholds beyond which the whole system is changing into an alternative steady-state;
No indicators/parameters considered for assessing the impact of human-induced eutrophication on the vertical distribution of nutrients, DO, chlorophyll *a*, etc.	More research is needed for developing indicators/parameters that considered the effects of eutrophication on nutrients, DO, chlorophyll distribution within the water column (with particular emphasis on open waters). Data/information from literature, past and recent cruises should be considered for assessing the temporal variability of the position and magnitude of suboxic layer, nutricline, DCM, etc. (models to be developed).
Need to distinguish between natural range and increase of spatial extension of anoxic sediments due to anthropogenic organic loading.	Research on factors that govern the occurrence and extension of hypoxic/ anoxic sediment surface. Additional continuous monitoring tools (benthic observatories, etc.) to be used for hypoxic/anoxic events study (and factors governing) in the sensitive areas.
No assessment tools that account for shifts in species composition and frequency of blooms in the scoring	Development of phytoplankton assessment tools that account for shifts in species composition and frequency of blooms in the scoring Development of monitoring tools that account for rapid changes in algal communities, allowing detection of bloom peaks (continuous measurements, ships-of-opportunity, remote sensing tools, algorithm development, real-time monitoring, etc.).
Non integrative tool to assess eutrophication.	BEAST, E-TRIX, NEAT must be robust, integrated, sufficiently sensitive, comparable, and with recognized scientific merit.
Need for Quality Assurance guidelines for the descriptor - an essential requirement for successful monitoring, allowing for appropriate intercalibration and comparative assessment.	The procedures aim to ensure that monitoring results meet the required levels of precision and confidence. Those procedures can take the form of standardizing sampling and analytical methods, replicate analyses and laboratory testing schemes.

3 Contaminants

"Concentrations of contaminants are at levels not giving rise to pollution effects".

3.1 Introduction

Same as terrestrial ecosystems, marine ecosystems are submitted to increasing anthropogenic disturbances (Richir & Gobert, 2016). Based on expert judgment (Halpern et al., 2008), the impact of 17 anthropogenic drivers of ecological change (e.g., pollution, fishing, ocean acidification, species invasion etc.) on marine ecosystems was mapped. Their analysis indicated that no area remained unaffected by human activities, that a large fraction of the oceans was strongly affected by those drivers (41%), but that some large, less-impacted oceanic areas still remained (3.7%, particularly near the poles). The ecologic, economic and social importance of marine ecosystems being irrefutable, a well-planned approach of managing the marine space is essential to achieve sustainability (Salomidi *at al.*, 2012). Otherwise, entire ecosystems will stop functioning under their actual form, which is likely to lead to the complete loss of goods and services derived from these ecosystems (Worm et al., 2006).

Specific features of the Black Sea make it very vulnerable to disturbances of its environment and ecosystems. The Black Sea is a nearly enclosed basin, having a limited interaction with the Aegean Sea through the Turkish Straits System. The Black Sea receives freshwater inflows all around the basin but the important ones (Danube, Dniepr and Dniestr) discharge into the northwestern coastal waters. The River Danube being one of the largest rivers in Europe introduced significant effects on the Black Sea ecosystem.

Eutrophication, pollution, and irresponsible fishing resulted in an overall decline of biological resources, the diversity of species and landscapes, and of the aesthetic and recreational values of the Black Sea, thereby bringing its ecosystems to the edge of collapse.

Having in mind that 87% of the sea water is naturally anoxic, the Black Sea is highly sensitive to anthropogenic impacts due to the huge catchment area and almost landlocked nature. Every year, about 350 cubic kilometers of river water pours into the Black Sea. This water brings a variety of products originated from the activity of more than 170 million people, who live in some of the most populated areas of the 17 different countries along riverbanks. Observed changes to its ecosystem during the last 50 years clearly indicate its vulnerability to the anthropogenic effects. Marine resources in the Black Sea have declined due to over-fishing, unplanned development of coastal zones and intense maritime traffic.

The sea continues to suffer from a long list of problems (BSC, 2019):

- pollution by land-based and sea-based sources.
- losses of biodiversity as a consequence of pollution, invasive species and the destruction of habitats.
- overexploitation of marine living resources leading to a collapse of fisheries and having a significant impact on the ecosystem health.

Pressures suffered by the Southern European seas (Mediterranean Sea and Black Sea) make them vulnerable ecological units, in particular because there are too small dimensions to ecologically self-counterbalance (Richir and Gobert, 2016). Thus, the point of saturation of the contaminants discharged in the Mediterranean and Black Sea will be more quickly achieved than in the oceans (Turley, 1999). As regards the specific chemical contamination by trace elements, for example, the high levels currently measured in the Mediterranean indicate non-stationary geochemical cycles which result from an increase of external inputs (Saliot, 2005). In addition, the almost total absence of tide does not allow the dilution of contaminants and prevents the natural phenomena of depuration as encountered in larger water bodies (i.e., in oceans) (Richir & Gobert, 2016). The Mediterranean and Black Sea also shows a deficiency in the movement of deep-water masses and of surface currents which "turn in circles" in those almost closed basins. The consequence of these specific features is that the answer of these basins to environmental disturbances due to anthropogenic pressures is more rapid than in the larger oceans (Augier, 2010).

According to the Commission Decision (EU) 2017/848 laying down criteria and methodological standards on good environmental status of marine waters and specifications and standardized methods for monitoring and assessment, and repealing Decision 2010/477/EU, the following recommendations, with relevance for contaminants, could be underlined:

- compared to the elements set out in previous Decision 2010/477/EU, the number of criteria that need to be assessed could be reduced, applying a risk-based approach to those which are retained in order to focus on the main anthropogenic pressures affecting marine waters.
- the collective pressure of human activities needs to be kept within levels compatible with the achievement of good environmental status, ensuring that the capacity of marine ecosystems to respond to human-induced changes is not compromised.
- the extent to which good environmental status is being achieved could be express as the proportion of their marine waters over which the threshold values have been achieved or as the proportion of criteria elements (species, contaminants, etc.) that have achieved the threshold values.
- it is important to focus on the predominant pressures and their environmental impacts on the different ecosystem elements in each region or subregion in order to monitor and assess their marine waters in an efficient and effective manner and to facilitate prioritization of actions to be taken to achieve good environmental status.
- criteria, including threshold values, methodological standards, specifications and standardized methods for monitoring and assessment should be based on the best available science. However, additional scientific and technical progress is still required to support the further development of some of them and should be used as the knowledge and understanding become available.
For the purpose of the assessment of the contamination status in the framework of ANEMONE Scientific Joint Cruise, criteria and methodological standards on good environmental status of marine waters for Descriptor 8 and Descriptor 9 were selected (Commission Decision EU/2017/848).
At European level, MSFD Expert Network on Contaminants, established by the Joint Research Centre (JRC) to support the MSFD implementation, works towards comparable MSFD Descriptor 8 and 9 assessments, compiling information related to substances, matrices and threshold values/reference levels (Tornero et al., 2019), aiming at equal levels of protection across European Seas. This is part of an on-going process to help regulators to assess relevant contaminants in their jurisdictional area, thus aiming at EU national authorities but also at Regional Sea Conventions in the shared marine regions. So far, environmental quality standards are established by European legislation for a part of contaminants, only in seawater and biota, as there are no regulated thresholds values in sediments.
In cases where no threshold values are laid down, countries should establish threshold values through European, regional or sub regional cooperation, for instance by referring to existing values or developing new ones in the framework of the Regional Sea Conventions. Until such threshold values are established, EU recommendation is that Member States should be able to use national threshold values, directional trends or pressure-based threshold values as proxies.
According to Commission Decision EU/2017/848, threshold values should reflect, where appropriate, the quality level that reflects the significance of an adverse effect for a criterion and should be set in relation to a reference condition. Threshold values should be set at appropriate geographic scales to reflect the different biotic and abiotic characteristics of the regions, subregions and subdivisions. This means that even if the process to establish threshold values takes place at EU level, this may result in the setting of different threshold values, which are specific to a region, subregion or subdivision.

3.2 Material and methods

Seawater, sediments and biota samples have been collected during 30.09 – 07.10.2019 ANEMONE Joint Cruise along northwestern and western Black Sea shelf from RO, BG and TR transects on board RV Mare Nigrum. The Ukrainian team contributed with samples collected during July-August 2019 along 5 stations located in the shelf (Table 3.1).
Water samples for pollutants were collected from the surface layer from the 5 -10 l Niskin bottles of the Rosette System. About 1 liter seawater was transferred into bottles, respectively 5 litters of seawater were poured into a polypropylene tank in case of Ukraine, which were stored at refrigerator temperature until their subsequent analysis in laboratory.
Sediment samples were collected with a Van Veen bodengreifer. Containers containing sediments were kept at −20 °C until processing. Sediments were freeze-dried and then well homogenized, and the coarse fragments (> 0.5 mm) were removed by sieving. Then, they were further extracted for each class of compounds.
Biota samples (*Mytilus galloprovincialis*) were collected using a biological dredge, cleaned and stored frozen. Whole soft tissue of mollusks was dissected, freeze-dried, homogenized and further processed for heavy metals and organic pollutants. One composite sample represents tissues dissected from at least 5 - 10 individuals from each location.

Table 3.1 - Stations network (UA, RO, BG and TR) for contaminants investigation, 2019

Station	Date	Longitude	Latitude	Bot. Depth [m]	Matrix
UA-1/NMS-UA-1	29.07.2019	31.1055	46.3596	25	W, S
UA-2/NMS-UA-16	31.08.2019	31.0000	45.8325	25	W, S
UA-3/NMS-UA-17	31.08.2019	31.2506	456667	40	W, S
UA-4/NMS-UA-15	03.09.2019	30.2106	45.2533	25	W, S
UA-5/NMS-UA-2	30.07.2019	31.2350	45.2159	53	W, S
RO-1	01.10.2019	30.5490	44.6253	78	W, S, B
RO-2	01.10.2019	30.9641	44.5468	106	W, S
RO-3	01.10.2019	30.1173	44.3124	76	W, S
RO-4	01.10.2019	30.5072	44.2679	103	W, S
RO-5	02.10.2019	29.6778	43.9162	67	W, S
RO-6	02.10.2019	30.1526	43.8430	103	W, S
BG-1	03.10.2019	28.2660	43.0166	43	S
BG-3	03.10.2019	28.1496	42.8525	57	B
BG-5	04.10.2019	28.0001	42.4222	49	B
BG-7	04.10.2019	28.0072	42.1601	48	B
TR-1	05.10.2019	28.1781	41.8573	75	W, S
TR-2	06.10.2019	28.4555	41.9155	90	W, S
TR-3	05.10.2019	28.3357	41.5747	71	W, S
TR-4	05.10.2019	28.6108	41.7115	86	W, S
TR-5	06.10.2019	28.7636	41.3864	77	W, S, B
TR-6	06.10.2019	28.9395	41.5542	88	W, S, B
TR-7	06.10.2019	28.9883	41,2735	35	W, S

W – seawater; S – sediment; B – biota;

Analytical methods for organic pollutants

Different extraction methods of organic compounds from **water** were applied in each area: extraction with hexane using a high-speed mixer followed by organic phase separation in a separating funnel in the Ukraine laboratory, extraction with hexane/dichloromethane (3/1) mixture in separating funnel in the Romanian laboratory or stir bar sorptive method in the Turkey laboratory.
Sediment samples were spiked with internal standards and extracted on an accelerated solvent extraction unit under pressure (PLE) with a hexane/dichloromethane/methanol mixture (60% / 20% / 20%) in the Ukraine laboratory and with hexane: acetone (1:1 v/v) in microwave in the others two laboratory. Sulphur was removed with activated copper. Extraction was followed by purification on florisil column for organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs), respectively silica/alumina column for polyaromatic hydrocarbons (PAHs) and concentration using the Kuderna-Denish concentrator or rotary evaporator and nitrogen flow in the Romanian and Turkey laboratories and on a silica gel column and concentration in a turbo evaporator under nitrogen flow in the Ukraine laboratory.
Biota samples (whole soft tissue of mollusks) were freeze-dried and further processed for organic pollutants. The extraction of OCPs and PCBs from biota samples was done with 30 ml acetone/hexane (1:1, v:v), in microwave extraction system for 30 min at 120°C in the Romanian laboratory and Soxhlet extracted for 8 hours using 250 ml of mixture of hexane and dichloromethane (50:50) followed by concentration at rotary evaporator down to 10-15 ml in Turkey laboratory. Internal standards were added to the samples for quantifying the overall recovery of the analytical procedures. The extractable organic matter (EOM) was determinate by evaporating a measured small volume of the extract. Then the lipids removed with concentrated sulfuric acid. Further processing of the samples was done by clean-up on florisil column and concentration using the Kuderna-Denish concentrator and nitrogen flow.
Persistent organic pollutants were analyzed by gas chromatography. GC-ECD method was used for OCPs and PCBs and GC-MS method for PAHs in the Romanian and Ukraine laboratory and GC-MS method was used for OCPs, PCBs and PAHs in the Turkey laboratory.
Quality Control: quality control was carried out by analysis of reference materials.

Analytical methods for trace metals

Surface **water samples** collected for metals analysis were filtered through the membrane with pore size 0,45 µm and dissolved metals have been determined in Ukraine, whereas Romania and Turkey labs worked on unfiltered seawater samples, acidified up to pH=2 with Ultrapure HNO_3, thus analyzing dissolved and acid soluble suspended forms of metals. Nitric acid has a role not only in the preservation of samples and the solubilization of particulate metals, but also as a matrix modifier, diminishing the interferences caused by salts (Grasshoff, 1999).
Sediment samples were treated with a mixture of concentrated acids (complete digestion): HNO_3, HCl, and HF in Ukraine and Turkey, and HNO_3 (partial digestion) in Romania (UNEP, 1995), using microwave digestion procedure. After cooling, the vessel contents were diluted to 50 mL or 100 mL with deionized water. The diluted samples were preserved in polyethylene bottles prior instrumental analysis.
Biota samples were digested with concentrated acid (HNO_3), in sealed Teflon bombs on a hot plate (at 120^{0}C), in Romania or with a mixture of concentrated HNO_3 and HCl (3/1) in the microwave digestion system at 180°C in Turkey. At the end of digestion, the samples were resumed in the 50 mL or 100 mL bottles, with deionized water.
Instrumental analysis and quantification: metals were analyzed by Graphite Furnace Atomic Absorption Spectrometry (GF-AAS) in UkrSCES and NIMRD laboratories (IAEA-MEL,1999), and Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) in TUBITAK laboratory. The calibration was carried out with prepared working standards for each element, starting from stock solutions. Calibration curves were prepared at 3, 5 or 9 points, between 0.5-150 µg/L concentration range for all elements. At least 3 instrumental readings were made for each sample and an average value was reported.
Quality Control: the accuracy of trace metal determination is indicated by a good agreement between determined values and those reported for the certified reference materials.

3.3 Heavy metals

Heavy metal contamination of the marine environment may be correlated with urban or industrial sources such as factories, thermoelectric plants, port activities, sewage treatment stations. The influence of rivers on coastal areas is significant, constituting a major source of metals, especially in particulate forms, extreme hydrological events (floods) contributing to the intensification of this input (Sakson et al., 2018). Atmospheric fluxes, demonstrating both natural and anthropogenic influences, are also considered to have an important contribution for European seas, both in coastal and basin areas, depending on the variability of the meteorological and local climatic conditions. Biogeochemical processes and natural levels of metals in the marine environment depend on numerous factors, such as sedimentary rock type, oxygen content, currents, salinity, pH, etc. The spread of metals in water, sediment and atmosphere results from their presence in the earth crust. In their natural concentrations metals play an essential role in many biochemical processes in living organisms, but any concentration that exceeds the background, as a result of anthropogenic activities, can become toxic (OSPAR, 1992).
Metals fall into the category of non-degradable pollutants and, by this persistent character, can sometimes quite strongly alter the natural biogeochemical balance in contaminated environments. Processes that remove metals from seawater primarily include active biological absorption processes, but also passive deposition processes, i.e. the combined process of superficial adsorption on a wide variety of high affinity surfaces associated with the particulate material, followed by particle deposition. Much of this particulate material (along with associated metals) is recycled either in the water column or in the superficial sediments. Weakly bound metals may be released from the surface of the depositing particles, replenishing the stock of dissolved metals. Marine sediments can also act as a source of metals by releasing them back into the water column. Primary flow processes between sediments and water column are re-suspension and deposition, bioturbation, advection, upwelling/downwelling, diagenetic processes and diffusion. Due to these remobilization processes, the effects of metal pollution on the local environment can be substantial and long-lasting, even in the case of restoration efforts (Richir & Gobert, 2016*)*.
Numerous studies on biogeochemical processes and the distribution of heavy metals in the NW Black Sea have demonstrated the importance of the metals input from the Danube and other rivers, together with the influence of the redox cycles of the Mn and Fe complexes. For example, Cu and Ni were found in higher concentrations in the area of the Black Sea continental shelf than in the oxic

layer of the deep-sea basin, reflecting the significant impact of rivers and anthropogenic inputs on this semi-enclosed sea. High concentrations of dissolved lead observed in surface waters in the open sea area were attributed to atmospheric intakes combined with less efficient metal capture in these waters poorer in particle matter (Tankere S.P.C., 2001).
Once entered the marine system, trace metals are removed from the surface water body by internal fluxes like sedimentation on biogenic or terrigenic particles, by diffusive exchange of dissolved species across interfaces or by advective vertical transport. As has been demonstrated by Pohl et al. (2006) in the brackish semi-enclosed Baltic Sea, the accumulation in sediments is the only noteworthy sink of heavy metals due to long water residence time. Consequently, heavy metals that are particle reactive, like Pb, have very low residence time, vertical sedimentation (sinking associated with particles) and lateral transport, as much as atmospheric input are in the same order of magnitude, while the metals (Cd, Cu, Zn) with "nutrient-like" behaviour have a residence time of several decades primarily due to their coupling to biological processes, in their case the lateral transport being more important than vertical sedimentation. (Pohl et al. 2006). This demonstrates that the system reacts very fast for particle reactive elements like Pb, while for Cu and Cd sedimentation processes are not the preferential sink and can be neglected (Pohl et al., 2006).
Measurements of heavy metals only in marine water are insufficient for assessing the state of the ecosystem due to high variability, fluctuating inputs, and low residence time. With a combined action of adsorption, hydrolysis and co-precipitation, only a small part of the free metal ions remains dissolved in water, while a large amount of them is stored in sediments. However, when environmental conditions change, sediments can be converted from heavy metal deposits into sources for water column. Therefore, the content of heavy metals in sediments is measured to provide vital information for the assessment of environmental risks on a long term (Zhuang & Gao, 2014).

Water

Metals concentrations in surface seawater collected during ANEMONE Joint Cruise 2019 varied within the following ranges: 0.29-11.70 µg/L Cu; 0.001-1.26 µg/L Cd; 0.001-8.72 µg/L Pb; 0.58-8.26 µg/L Ni; 0.05-3.36 µg/L Cr, 0.01-0.053 µg/L Hg; 2.60-63.90 µg/L Zn; 6.00-23.00 µg/L Fe; 0.50-0.84 µg/L Co. (Table 3.2).
In comparison with MAC-EQS values for Cd, Pb, Ni and Hg from Directive 39/2013/EU, none of the seawater samples investigated surpassed the thresholds, thus evincing a good status with respect to trace metal levels in seawater.

Table 3.2 - Heavy metals concentrations in seawater samples from NW, W and SW Black Sea, 2019

	Nr. samples	Mean	Median	Minimum	Maximum	Percentile - 25th	Percentile - 75th	Std. Dev
Hg (µg/L)	5	0.026	0.010	0.010	0.053	0.010	0.047	0.022
Zn (µg/L)	5	30.00	16.00	2.600	63.900	7.400	60.100	29.634
Fe (µg/L)	5	12.60	6.000	6.000	23.000	6.000	22.000	9.044
Co (µg/L)	5	0.568	0.500	0.500	0.840	0.500	0.500	0.152
Cu (µg/L)	18	3.484	3.035	0.296	11.700	0.414	6.120	3.549
Cd (µg/L)	18	0.200	0.021	0.001	1.260	0.012	0.406	0.333
Pb (µg/L)	18	1.022	0.148	0.001	8.720	0.029	1.090	2.142
Ni (µg/L)	18	1.275	0.840	0.580	8.260	0.682	1.010	1.759
Cr (µg/L)	18	0.626	0.407	0.050	3.360	0.358	0.500	0.738

Copper, especially, and to a lesser extent cadmium and lead concentrations in surface waters presented a decreasing gradient from north to south direction, some individual values being higher in some stations from NW and W areas, in comparison with SW Black Sea. Nickel and chromium values were more homogenous distributed, only one outlier value for Ni in UA-1 station and one for Cr in UA-4 station were measured (Figure 3.1; Figure 3.2). Zn, Hg, Co and Fe concentrations were measured in seawater only in UA stations, and their distribution is depicted in Figure 3.3.
Data obtained during this cruise are comparable with metals concentrations in surface seawater collected during MISIS Joint Cruise, July 2013, from RO, BG and Tr transects, that varied within the following ranges: 0.10 - 2.99 µg/L Cu; 0.05 - 0.76 µg/L Cd; 1.16 - 3.70 µg/L Pb; 0.14 - 12.38 µg /L Ni; 1.14 - 6.06 µg/L Cr (MISIS Joint Cruise Scientific Report, 2014).
According to literature data from other marine regions, concentrations of cadmium in seawater are normally situated below 0.10 µg/L (IPCS, 1992), and nickel between 0.20 and 0.70 µg/L (Alzieu, 1999). Copper was reported from 2 µg/L in open sea waters to 15 µg/L in estuarine areas, with

variation ranges of 1-5 µg/L in coastal areas from Baltic and Mediterranean seas. Dissolved chromium in oceans ranges within 0.12 µg/L at surface, up to 0.35 µg/L in deeper waters, this element being strongly represented by suspended form, rather than dissolved. Background dissolved lead in coastal and marine waters varies between 0.10 – 0.45 µg/L, this element being also rapid adsorbed by suspended matter (Alzieu, 1999).

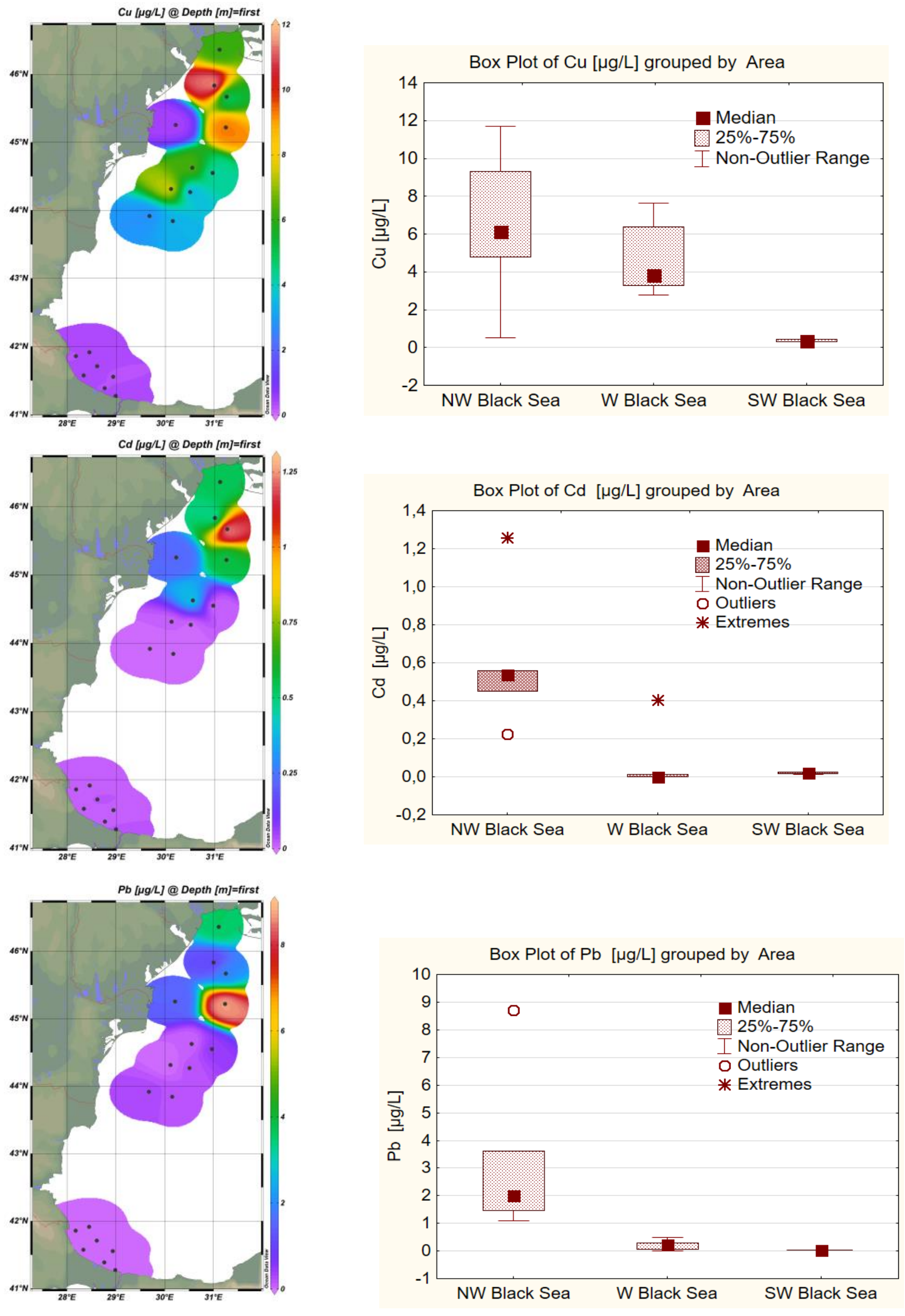


Figure 3.1 - Cu, Cd and Pb concentrations in seawater samples from NW, W and SW Black Sea, 2019

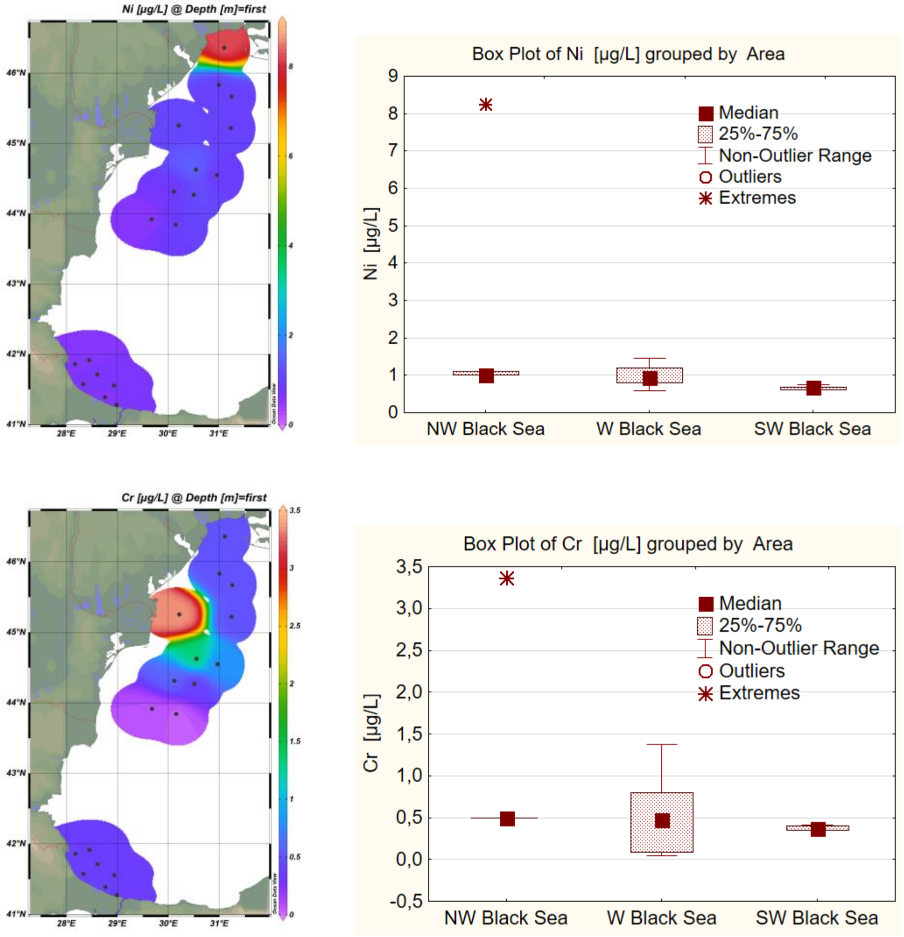


Figure 3.2 - Ni and Cr concentrations in seawater samples from NW, W and SW Black Sea, 2019

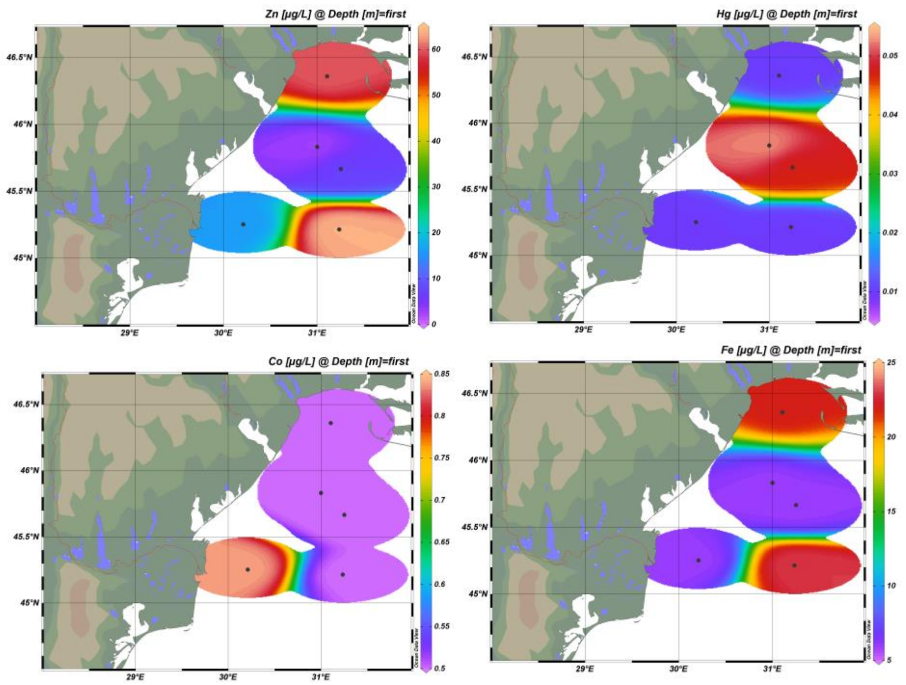


Figure 3.3 - Zn, Hg, Co and Fe concentrations in seawater samples from NW Black Sea, 2019

Sediments

Sediments are an important repository for various pollutants and also play a significant role as sensitive indicators for monitoring contaminants in aquatic systems. (Ozkan and Buyukisik, 2012). Sediments are considered to be an important carrier as well as a sink of heavy metals in the hydrological cycle and reflect the current quality of the system as well as provide information on the impact of pollution sources (Kruopiene, 2007). The distribution of heavy metals in sediments is influenced by the contribution of natural and anthropogenic sources and depends on the mineralogic and granulometric characteristics of sediments.

Sediments with a finer texture and a higher organic content tend to accumulate higher concentrations of heavy metals compared to coarse sediments and this depends on specific hydrodynamic conditions that influence the fine particle (silt and clay) distribution (Naifar et al., 2018). In marine areas characterized by a low depositional energy the accumulation of fine particles and pollutant is facilitated, whereas in coastal areas characterized by high depositional energy (wave, currents), sediments are dominated by coarse-grained particles (sand).

Concentrations of heavy metals in sediments samples from NW, W and SW Black Sea investigated in 2019 varied within the following limits: 8.81-55.07 µg/g Cu; 0.024-0.610 µg/g Cd; 5.37-57.74 µg/g Pb; 9.23-70.42 µg/g Ni; 7.67-97.88 µg/g Cr; 28.00-123.00 µg/g Zn; 257.00-1637.00 µg/g Mn; 55.131-112.50 µg/g V; 0.43-14.28 µg/g Co; 4.69-13.74 µg/g As; 0.032-0.190 µg/g Hg; 4420.00-96413.00 µg/g Al; 4520.00-36900.00 µg/g Fe (Table 3.3).

Table 3.3 - Heavy metals concentrations in sediments samples from NW, W and SW Black Sea, 2019

	No. samples	Mean	Median	Minimum	Maximum	Percentile 25th	Percentile 75th	Std. Dev
TOC (%)	8	2.07	1.65	0.360	3.81	1.42	3.15	1.20
Cu (µg/g)	18	35.23	34.94	8.810	55.07	27.89	43.63	12.81
Cd (µg/g)	19	0.24	0.19	0.024	0.61	0.06	0.32	0.17
Pb (µg/g)	19	27.67	22.24	5.370	57.74	19.20	39.22	13.68
Ni (µg/g)	19	44.60	45.90	9.230	70.42	36.71	54.21	14.67
Cr (µg/g)	19	48.86	40.30	7.670	97.88	31.80	68.45	26.70
Zn (µg/g)	15	69.09	64.67	28.000	123.00	54.99	93.96	26.69
Mn (µg/g)	15	514.26	459.00	257.000	1637.00	363.00	501.99	324.92
V (µg/g)	7	82.71	73.85	55.131	112.50	63.80	105.20	21.78
Co (µg/g)	15	8.93	8.85	0.430	14.28	6.67	12.28	3.84
As (µg/g)	15	9.16	9.68	4.690	13.74	6.35	10.81	2.54
Hg (µg/g)	15	0.10	0.10	0.032	0.19	0.07	0.12	0.04
Al (µg/g)	15	47293.58	41200.00	4420.000	96413.70	31200.00	71600.00	29151.18
Fe(µg/g)	9	16655.56	10600.00	4520.000	36900.00	8640.00	22700.00	11283.97

Grain size composition was determined only in TR sediments samples, and the results showed for most of them the predominance of clay-silt fraction (Table 3.4).

Table 3.4 - Grain size composition of TR sediment samples, 2019

Station Code	% Moisture	>2mm (%)	2mm-200 µm (%)	200µm-63 µm (%)	<63 µm (%)	% Shell	Grain Size Character
		Gravel	Coarse sand	Fine sand	Clay-silt		
TR-1	4313	3.73	0.49	29.88	65..89	4.92	Slightly gravelly sandy mud
TR-2	45.89	0.11	0.00	8.03	91.86	0.09	Slightly gravelly mud
TR-3	52.00	0.31	0.00	10.13	89.56	0.31	Slightly gravelly mud
TR-4	48.2	0.76	1.02	3.50	94.73	0.76	Slightly gravelly mud
TR-5	52.6	14.62	11.29	17.47	56.63	21.81	Gravelly sandy mud
TR-6	51.51	7.55	18.25	7.98	66.22	11.57	Gravelly sandy mud
TR-7	30.27	2.68	2.26	88.02	705	7.77	Slightly gravelly sand

Spatial distribution of heavy metal concentrations in surface sediments evinced for some elements an increasing gradient toward south-western part of the investigated area (except Pb and Ni, both presenting increased concentration in the western part) (Figure 3.5, Figure 3.6, Figure 3.7, Figure 3.8). Strong correlation of these elements (especially Cu and Cr) with Al content confirms that background (natural) heavy metals concentrations depend on the mineralogic and granulometric characteristics of sediments. (Figure 3.4) Aluminum represents aluminosilicates, the main group of minerals generally found in the fine sediment fractions. Aluminum is supposed to: a) derive with the detrital minerals from the continent to sea; b) have negligible anthropogenic input; c) behave conservatively in normal marine environments, that why this element is widely used for marine sediment normalization. Generally, metals concentrations that falls outside 95% confidence bands of Me-Al regression lines could be considered of anthropogenic origins (UNEP, 1995).

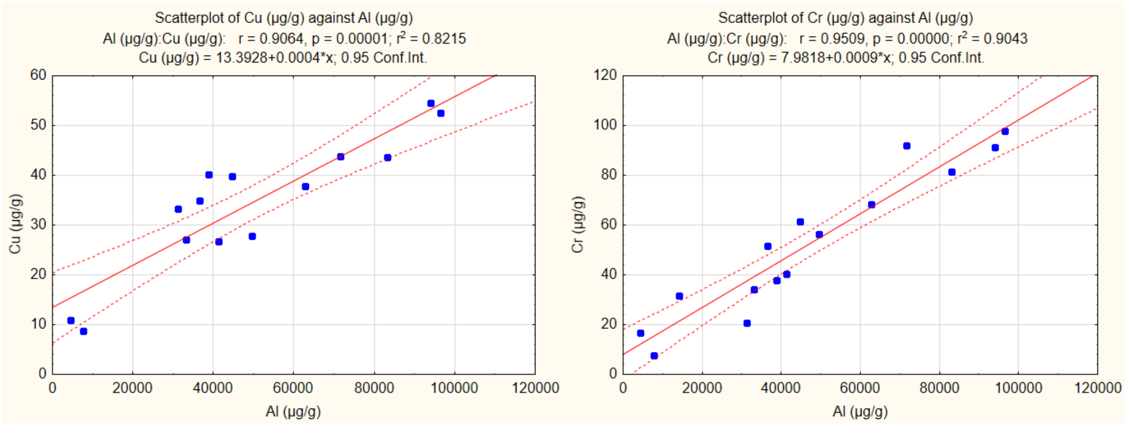


Figure 3.4 - Cu: Al and Cr:Al scatter plots for sediments from NW, W and SW Black Sea, 2019

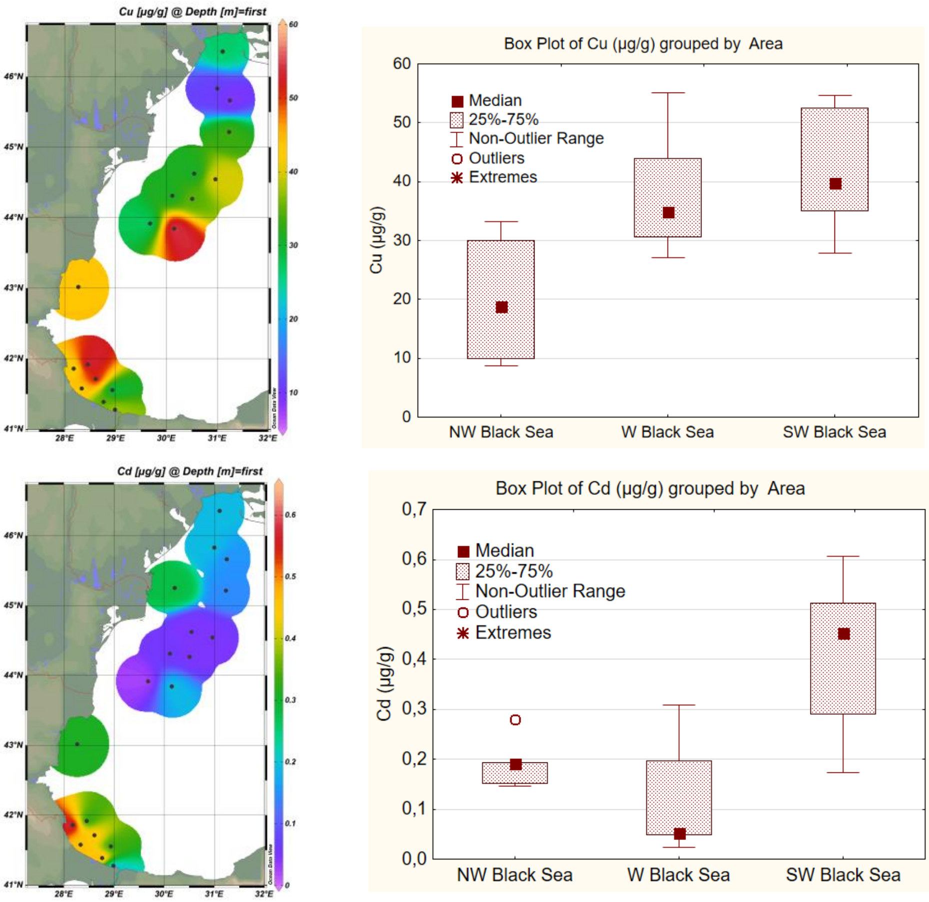


Figure 3.5 - Cu and Cd concentrations in sediments samples from NW, W and SW Black Sea, 2019

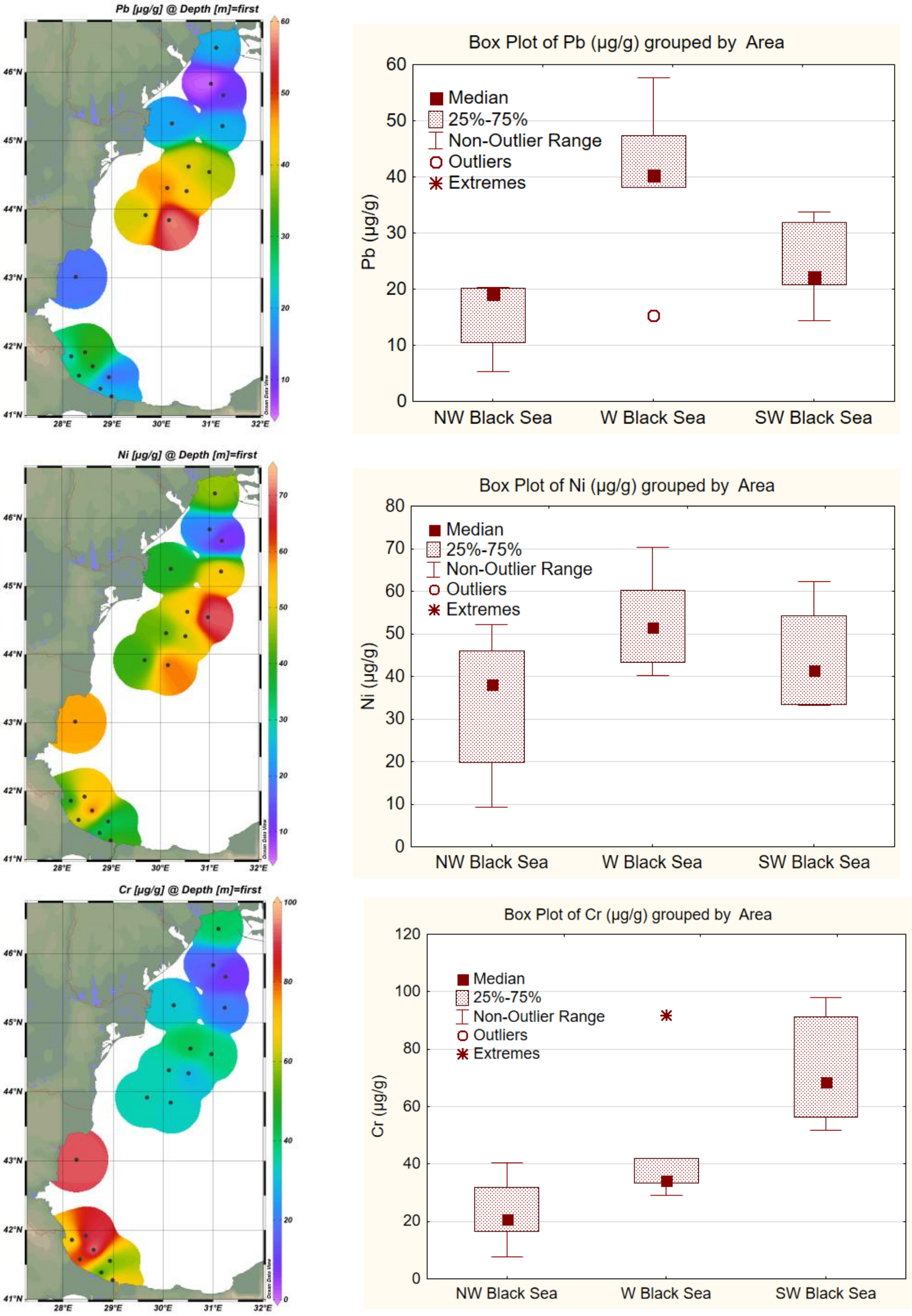


Figure 3.6 - Pb, Ni and Cr concentrations in sediments samples from NW, W and SW Black Sea, 2019

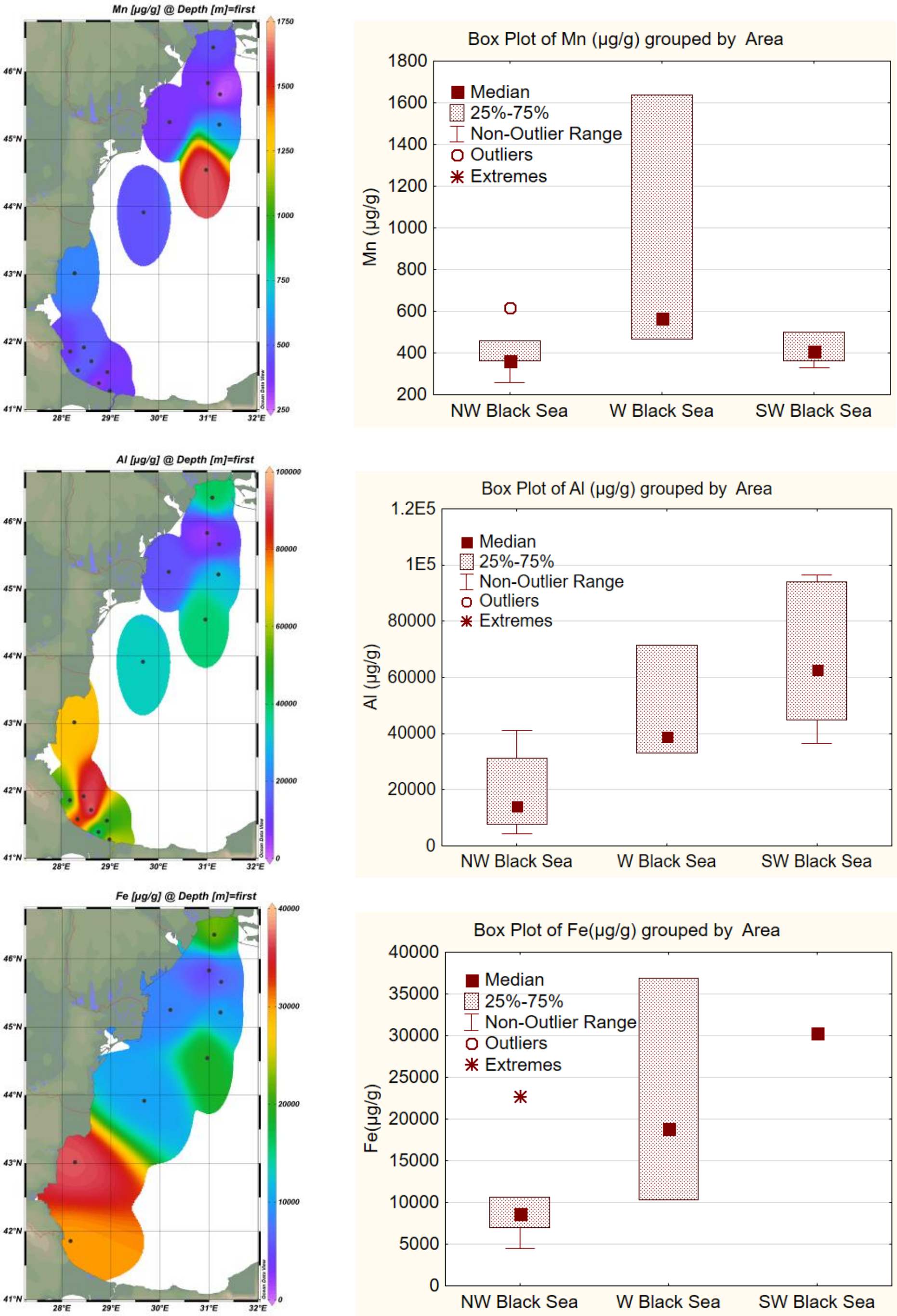


Figure 3.7 - Mn, Al and Fe concentrations in sediments samples from NW, W and SW Black Sea, 2019

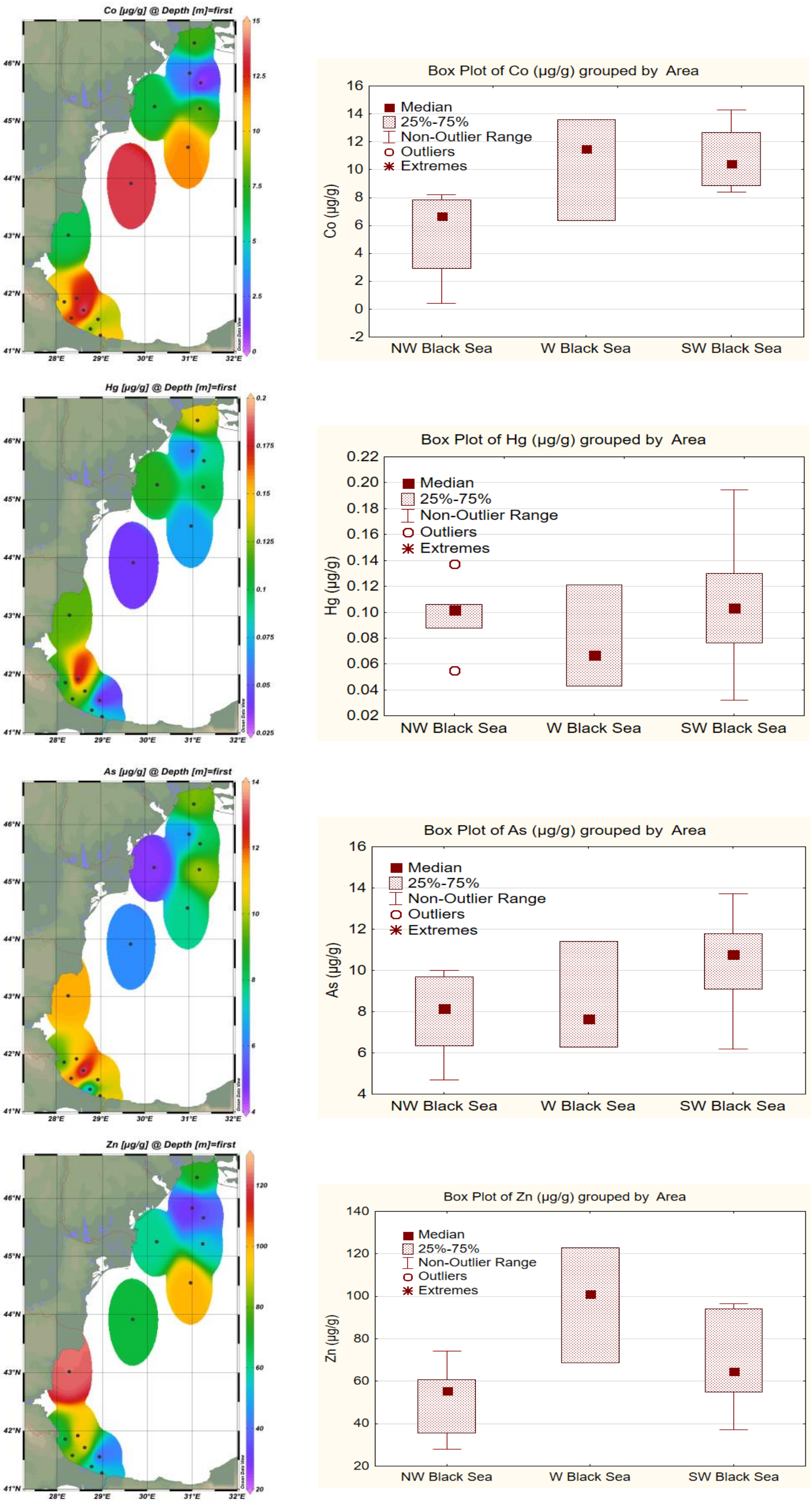


Figure 3.8 - Co, Hg, As and Zn concentrations in sediments samples from NW, W and SW Black Sea, 2019

Concentrations of metals in sediments were compared with Effects Range-Low (ERL) values, developed by the United States Environmental Protection Agency (US EPA) for assessing the ecological significance of sediment concentrations (Long et al., 1995). ERL is the lower tenth percentile of the data set of concentrations in sediments, which were associated with biological effects, resulted from a large database compiled from many studies. Adverse effects on organisms are rarely observed when concentrations fall below the ERL value.

For Cd and Zn, no value measured in 2019 surpassed ERL values (1.2 µg/g Cd, 150 µg/g Zn). For the other elements, the percentage of samples with concentrations higher than thresholds (ERL) ranged from 6% for Pb and Hg, to 16% for Cr, 56% for Cu and 90% for Ni (Figure 3.9).

Similar with previous studies (MISIS Report, 2014), Ni levels frequently exceeded ERL value (20.9 µg/g Ni). It was suggested that natural background level of Ni in Black Sea sediments could be higher than proposed criteria. Also, Ni abundance in the upper continental crust of the Earth is 47 µg/g Ni, in oceanic crust is 150 µg/g Ni, and in oceanic sediment 71 µg/g Ni (Rauch et al., 2009).

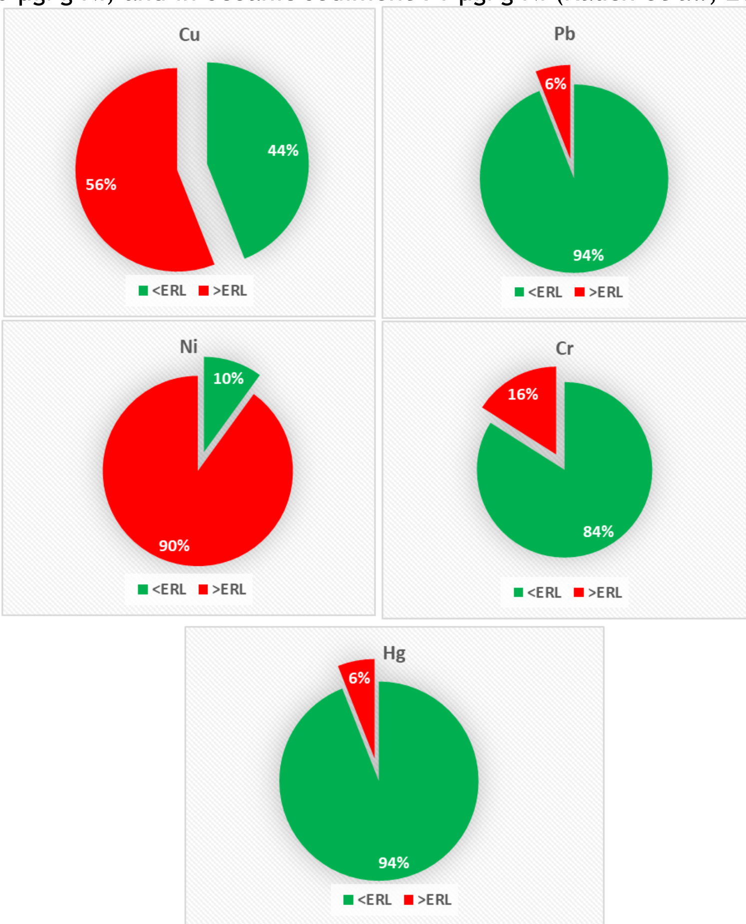


Figure 3.9 - Classification of sediments from NW, W and SW Black Sea, 2019, based on Effects Range-Low (ERL) values

These measurements from ANEMONE JC 2019 covering NW, W and SW Black Sea are generally included in similar variation ranges with heavy metals concentrations in sediments observed in 2013, during MISIS Joint Scientific Cruise (RO, BG and TR transects) (MISIS Report, 2014) (Table 3.5). ANEMONE included also UKR part of the Black Sea, and a different network of stations. New data obtained in 2019 could contribute to an improved assessment of the Black Sea region status with respect to contamination, adding new information from a wider area, new elements and first steps towards harmonized assessment methodologies.

Table 3.5 - Variation ranges of heavy metals concentrations in sediments in NW, W and SW Black Sea during 2013 and 2019 Joint Scientific Cruises

	ANEMONE JC, 2019			MISIS JC, 2013		
	Nr. samples	Minimum	Maximum	Nr. samples	Minimum	Maximum
Cu (µg/g)	18	8.810	55.07	13	13.76	50.31
Cd (µg/g)	19	0.024	0.610	13	0.057	0.386
Pb (µg/g)	19	5.370	57.74	13	9.21	45.18
Ni (µg/g)	19	9.230	70.42	13	15.70	57.80
Cr (µg/g)	19	7.670	97.88	13	38.00	88.00
Zn (µg/g)	15	28.000	123.00	13	26.10	85.60
Mn (µg/g)	15	257.000	1637.00	13	349.00	1425.00
V (µg/g)	7	55.131	112.50	13	5.00	91.00
Co (µg/g)	15	0.430	14.28	13	4.37	12.82

Biota

Marine organisms are continuously exposed to varying concentrations of metals in their environment. There is certain selectivity in metal accumulation, so there must be a distinction between essential and non-essential metals. Essential metals like copper, zinc, manganese, iron and cobalt are essential components of many enzymes and respiratory pigments. Therefore, marine organisms must provide sufficient metals for tissues to sustain metabolic and respiratory needs. Deficiency of these metals, but equally accumulation over certain levels might have harmful effects (White & Rainbow, 1985). Non-essential metals (lead, arsenic, mercury, cadmium) are highly toxic, even at very low levels, especially if accumulated in the metabolically active sites. Body is required to restrict non-essential metal accumulation or to pass them into the non-toxic forms (Depledge & Rainbow, 1990).

Thus, although metals are essential components of life, they become harmful when present in excess. Increasing levels of bioavailable metals in the marine environment is a problem for human health and marine ecosystems. Given the great diversity of biotic and abiotic factors governing metal bioaccumulation, like availability of food, hydro-chemical conditions, genetic differences, physiological status, etc., discrimination between changes in the level of accumulation in response to environmental contamination and those caused by natural variation of the physiology of the organism is sometimes difficult (UNEP, 1993).

Concentrations of heavy metals in *Mytilus galloprovincialis* samples collected in 2019 during Joint Cruise from W and SW Black Sea varied within the following limits: 1.04 - 2.29 µg/g Cu; 0.18 - 0.45 µg/g Cd; 0.04 - 0.37 µg/g Pb; 0.27 - 4.76 µg/g Ni; 0.11 - 0.45 µg/g Cr; 1.69 - 2.38 µg/g As; 0.006 - 0.012 µg/g Hg; 10.04 - 30.01 µg/g Mn; 0.18 - 0.21 µg/g Co; 40.92 - 60.91 µg/g Zn (Table 3.6**).**

In comparison with maximum admissible limits for human consumption from EC Regulation 1881/2006, all mussels samples had Cd, Pb and Hg concentrations below thresholds.

For mussels (*Mytilus edulis*) from North Sea and Baltic Sea the following threshold values, corresponding to normal background for metals, were proposed: Cu 2.0 µg/g; Cd 0.4 - 0.8 µg/g; Pb 0.4 - 1.0 µg/g; Ni 0.8 - 1.0 µg/g; Cr 0.4 - 0.6 µg/g (EPA, 2002). In reference to these values, concentrations of heavy metals in mussels investigated in 2019 were included in their variation range, except for nickel.

Table 3.6 - Heavy metals concentrations in Mytilus galloprovincialis samples from NW, W and SW Black Sea, 2019

	No. of observations	Mean	Median	Minimum	Maximum	Std.Dev.
Cu (µg/g ww)	7	1.597	1.382	1.038	2.290	0.546
Cd (µg/g ww)	7	0.290	0.276	0.184	0.451	0.097
Pb (µg/g ww)	7	0.139	0.067	0.037	0.370	0.137
Ni (µg/g ww)	7	1.796	1.134	0.273	4.760	1.739
Cr (µg/g ww)	7	0.249	0.240	0.111	0.454	0.132
As (µg/g ww)	7	2.041	2.041	1.698	2.384	0.484
Hg (µg/g ww)	7	0.009	0.009	0.006	0.012	0.003
Mn (µg/g ww)	7	20.022	20.022	10.038	30.007	14.120
Co (µg/g ww)	7	0.199	0.199	0.186	0.212	0.018
Zn (µg/g ww)	7	50.912	50.912	40.915	60.908	14.136

Generally, slightly higher concentrations for most elements were noticed in RO and TR samples, in comparison with mussels from BG stations (Figure 3.10**)**.
In comparison with heavy metals concentrations ranges determined in *Mytilus galloprovincialis* (6 samples) investigated in July 2013 during MISIS JC (MISIS Report, 2014) from RO, BG and TR areas: 1.13 - 4.48 µg/g Cu; 0.21 - 1.12 µg/g Cd; 0.002 - 0.216 µg/g Pb; 0.97 - 6.00 µg/g Ni; 0.07 - 1.31 µg/g Cr, in 2019 similar variation ranges were noticed, with maximum values being slightly smaller in 2019, except for Pb.

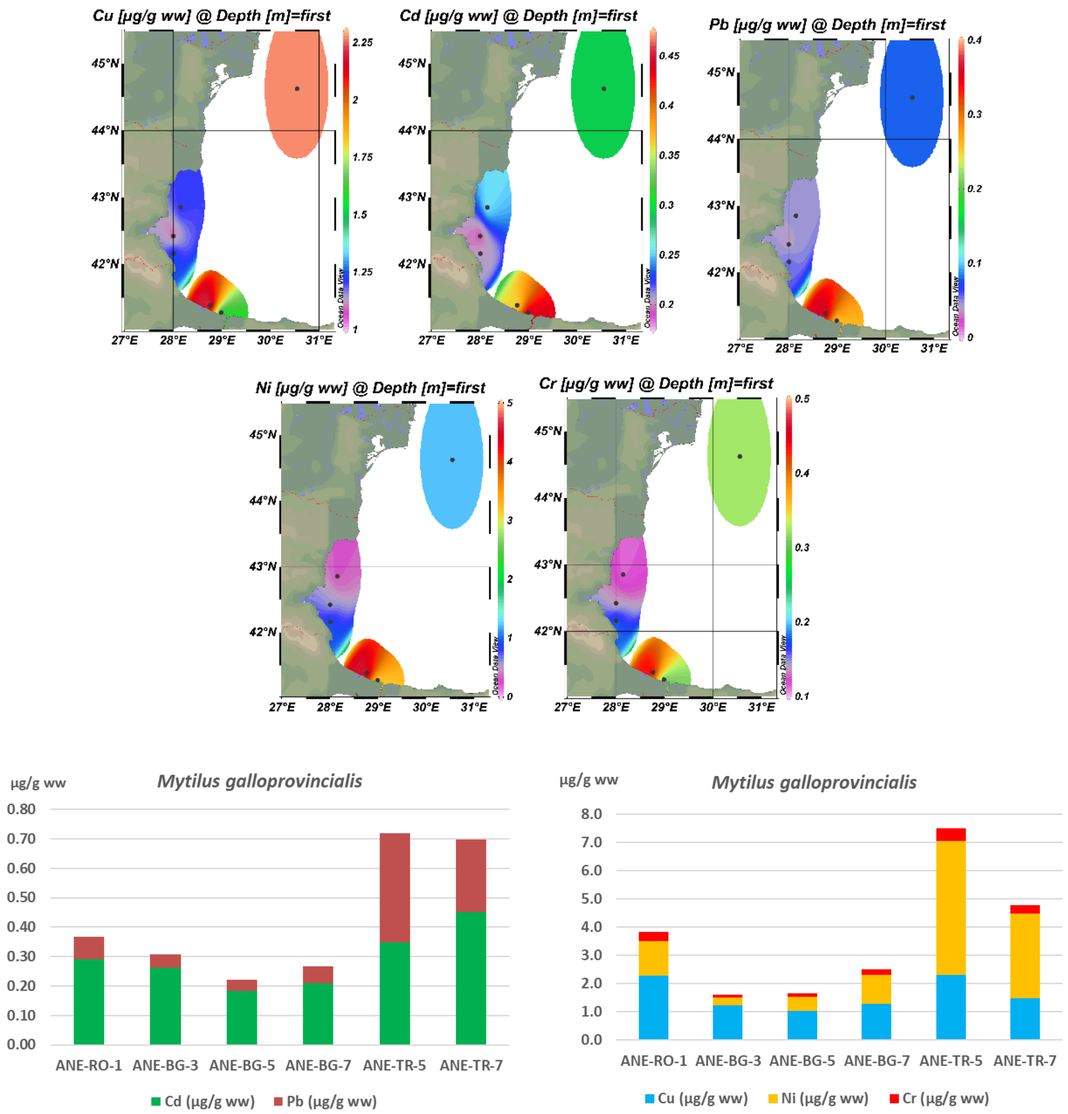


Figure 3.10 - Heavy metals concentrations in biota samples from NW, W and SW Black Sea, 2019

Conclusions

These measurements from ANEMONE JC, 2019 indicated a low-level trace metal pollution of marine waters, concentrations of cadmium, mercury, lead and nickel being much below recommended MAC-EQS from European Legislation (Directive 2013/39/EU).
A comparison with available sediment quality guidelines (ERLs) showed that the concentrations of Cd and Zn were below thresholds. For the other elements, the percentage of samples with concentrations higher than thresholds (ERL) ranged from 6% for Pb and Hg, to 16% for Cr, 56% for Cu and 90% for Ni. Frequent exceeding of the limits identified for Ni were most probably the result of a higher natural background.
In comparison with maximum admissible limits for human consumption from EC Regulation 1881/2006, all *Mytilus galloprovincialis* samples had Cd, Pb and Hg concentrations below thresholds.

3.4 Total petroleum hydrocarbons (TPH) and Polycyclic aromatic hydrocarbons (PAH)

Polycyclic aromatic hydrocarbons (PAHs) comprise a group of similar organic compounds comprised of at least two benzene rings. They typically result from the incomplete combustion of organic material (such as coal, petrol, diesel, and wood). PAHs are toxic and carcinogenic. The degree of toxicity and carcinogenicity is dependent on which type (or congener) of PAH it is. PAHs typically occur in mixtures, and it is therefore difficult to establish the risk that the mixture may pose.
The main sources of emission of technogenic PAHs into the environment are enterprises of the energy complex, road transport, chemical and oil refining industries. Almost all technogenic sources of PAHs are based on thermal processes associated with the combustion and processing of organic raw materials: oil products, coal, wood, garbage, food, tobacco, etc.
In the work devoted to the biological and biochemical control of PAHs when exposed to the population, the following is noted: PAHs are ubiquitous carcinogens that humans are exposed to in the environment and in certain workplaces. Therefore, health risk assessment is of great professional medical and environmental-medical importance.
In the work, it is noted that PAHs are formed during incomplete combustion of organic material, for example, in the form of components of cigarette smoke, in processed gases and in smoke from forest fires.

Water

Table 3.7 shows the minimum, average, maximum concentrations of 16 priority polyaromatic hydrocarbons (PAHs) and calculated from these indicators the sum of PAHs (PAHs total), toxic equivalency factor (TEFs) and sum carcinogenic PAHs (Σ carcinogenic PAHs) in surface water for regions Black Sea (BS): Ukrainian (5 stations), Romania (6 stations), Turkey (7 stations). In the Black Sea region near Bulgaria, PAHs in sea water have not been researched.
The PAH concentrations were compared with the maximum available concentration according to the EU Directive 2013/39/EU (MAC-EQS). As can be seen from Table 3.7, the minimum PAH concentrations in all regions did not exceed the permissible concentrations. Average PAH concentrations exceeded the permissible values in the Ukrainian part of BS for benzo(g,h,i)perylene by 1,03 times. The maximum PAH concentrations exceeded the permissible values in the Ukrainian part of BS for benzo[b]fluoranthene by 1.31 times and for benzo(g,h,i)perylene by 2.76 times.
For the rest of PAHs in the Ukrainian part of BS, no excess of threshold was recorded.
In the Romanian and Turkish parts of the Black Sea, PAH concentrations in seawater did not exceed the threshold.
In Figure 3.11 shows distribution PAHs total, TEFs, Σ carcinogenic PAHs in surface water for NW, W and SW regions of the Black Sea.
As follows from Figure 3.11, the highest PAHs total values are found in the Romanian part of BS (869,4 ng/L), but the TEFs and Σ carcinogenic PAHs are at a low level, which indicates low concentrations of highly toxic PAHs in their total community. In the Ukrainian part of BS PAHs total, on the contrary, is much lower (106.8 ng/L) than in the Romanian part, but the TEFs and Σ carcinogenic PAHs indicate high concentrations of highly toxic PAHs in the Ukrainian part of the BS.
High concentrations of PAHs in the surface layers of seawater in the Ukrainian and Romanian parts of BS can be explained by the influence of rivers carrying PAHs in their fresh waters.
In the Turkish part, BS PAHs total, TEFs, and Σ carcinogenic PAHs are at a low level, which indicates a low input of PAHs into the surface layer of seawater.
Figure 3.12 shows the distribution gradient of PAHs total and TEFs in the surface layer of seawater in the areas of research in the BS.

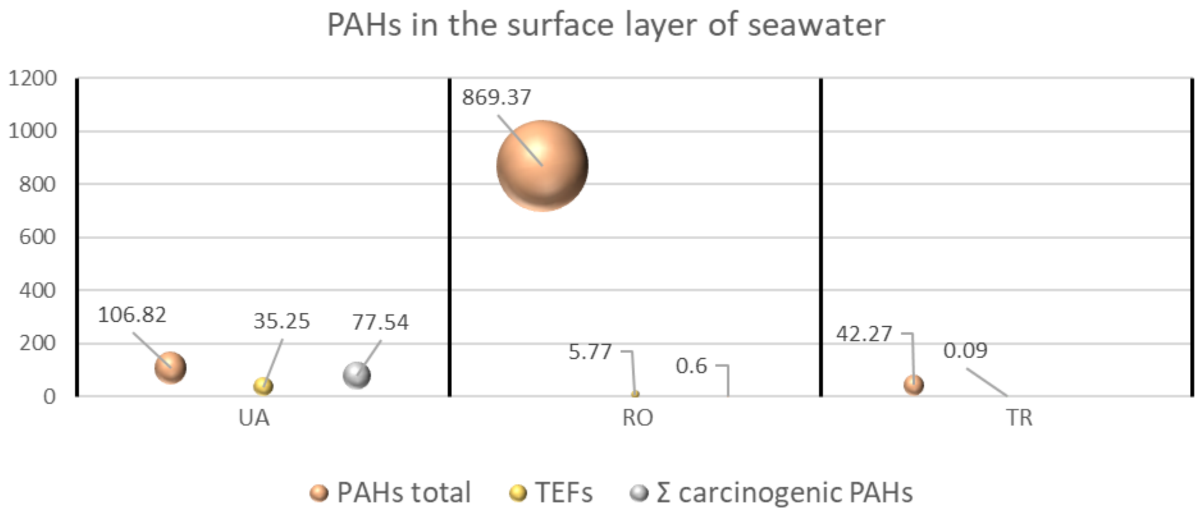


Figure 3.11 - Distribution PAHs total, TEFs, Σ carcinogenic PAHs in surface water

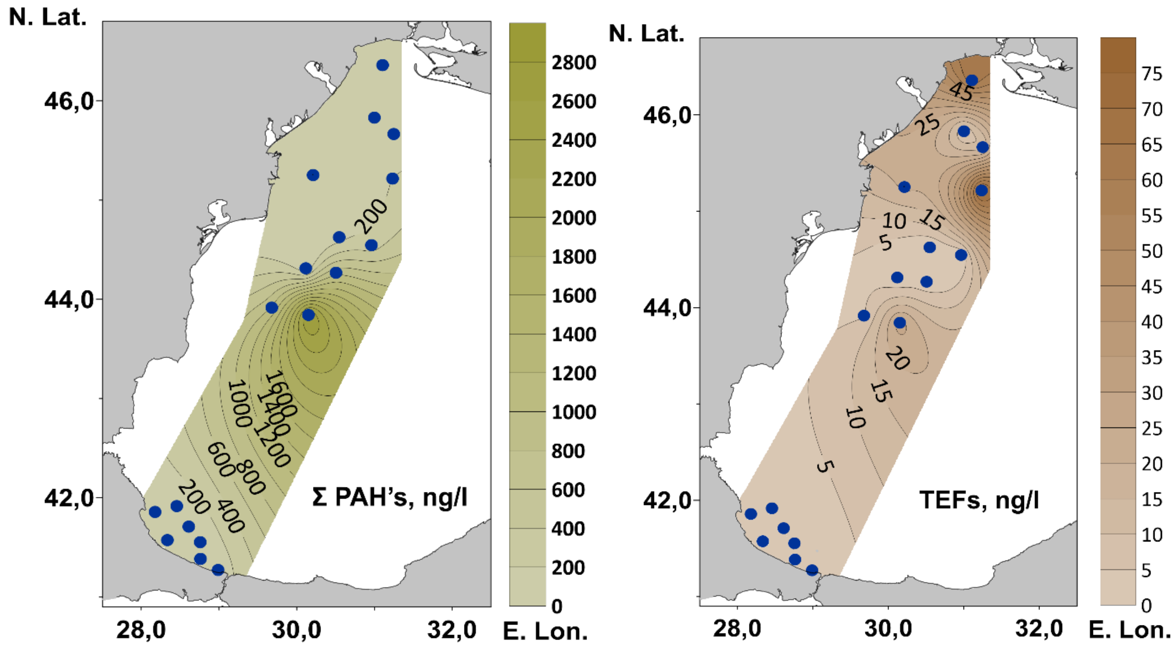


Figure 3.12 - Gradient distribution of PAHs total, TEFs, in surface water

Table 3.7 - Minimum, average, maximum concentrations PAHs and PAHs total, TEFs, Σ carcinogenic PAHs in surface water

Indicators	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene	Anthracene	Fluoranthene	Pyrene	Benzo[a]anthracene	Crysene	Benzo[b]fluoranthene	Benzo[k]fluoranthene	Benzo[a]pyrene	Benzo (g,h,i)perylene	Dibenzo(a,h)anthracene	Indeno(1,2,3-c,d)pyrene	PAH total	TEFs	Σ carcinogenic PAHs
	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
2013/39/EU AA-EQS Marine	130000					100	120				17	17	27	0.82					
RO																			
MIN	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	1.6	0.26	0.6
AVER	308	517	0.1	0.1	0.1	0.1	0.1	43.3	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	869	5.77	0.6
MAX	946	2734	0.1	0.1	0.1	0.1	0.1	236	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	3918	28.8	0.6
TR																			
MIN	11.4	0	0	0	0	0	0	10.6	0	0	0	0	0	0	0	0	22	0.02	0
AVER	22.0	4.89	0	0	0	0	0	15.4	0	0	0	0	0	0	0	0	42.27	0.09	0
MAX	43.8	34.2	0	0	0	0	0	21.5	0	0	0	0	0	0	0	0	99.5	0.41	0
UA																			
MIN	1.42	0	1.57	1.2	0	0	5.32	1.91	0.6	0	0.96	0	0	0	0.71	3.76	17.5	4.89	11.8
AVER	6.97	0.22	3.2	11.03	9.32	0.19	19.7	8.7	7.46	7.35	10.6	0.08	0.14	0.85	1.59	19.4	107	35.2	77.5
MAX	22.2	0.58	5.91	22.78	22.45	0.41	42.15	17.78	14.6	18.2	22.3	0.21	0.42	2.26	3.18	39.2	235	75.3	163

Sediment

Table 3.12 shows the minimum, average, maximum concentrations PAHs and calculated from these indicators the PAHs total, TEFs and Σ carcinogenic PAHs in bottom sediment for regions Black Sea (BS): Ukrainian (5 stations), Romania (6 stations), Bulgaria (1 station), Turkey (7 stations).
In Figure 3.13 shows distribution average PAHs total, TEFs, Σ carcinogenic PAHs in bottom sediment for regions Black Sea Ukrainian, Romania, Turkey.

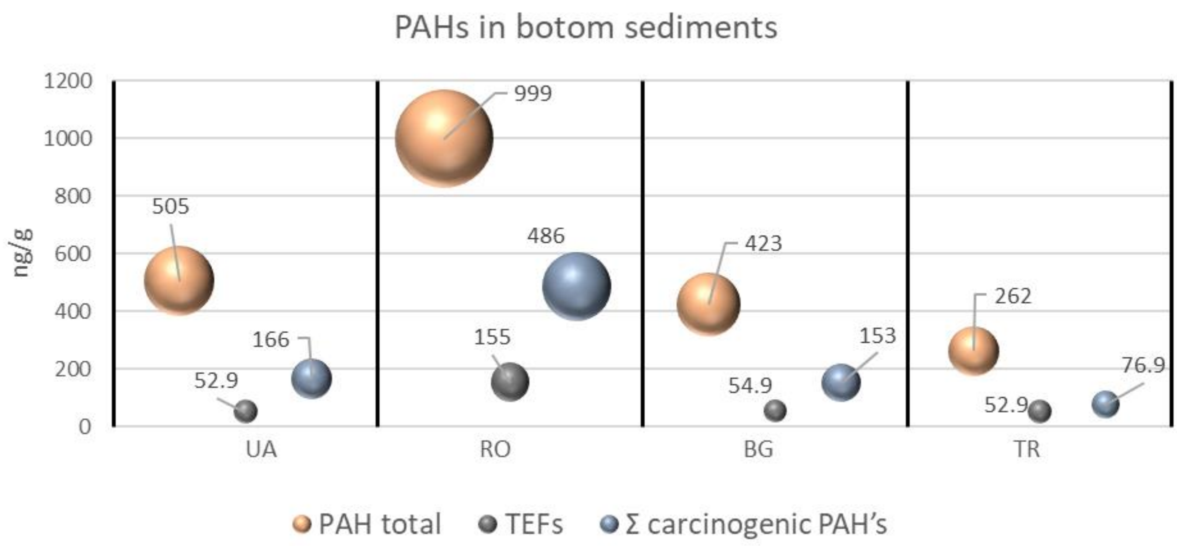


Figure 3.13 - Distribution PAHs total, TEFs, Σ carcinogenic PAHs in bottom sediments

As can be seen from Table 3.12 and Figure 3.13, the PAHs concentration in bottom sediments is significantly higher than in seawater, which is due to their low solubility in water. However, the accumulation of PAHs in bottom sediments can lead to secondary pollution of sea water and biological objects.
The highest levels of PAHs content in bottom sediments were recorded in the Romanian part of BS: PAHs total 999 ng/g, in the Ukrainian and Bulgarian parts of BS PAH concentrations are lower: PAHs total 505 ng/g and 423 ng/g, respectively, in the Turkish part of BS concentration PAHs are the lowest: PAHs total 262 ng/g.
TEFs in the Ukrainian, Bulgarian and Turkish parts of BS are at the same level 52.9 – 54.9 ng/g, in the Romanian part of BS TEFs have the highest rate 155 ng/g, which correlates with PAHs total.
Σ carcinogenic PAHs exceed the TEFs value in all BS regions, which characterizes the accumulation of chemical compounds in bottom sediments, which, entering a biological object can cause irreversible changes in the genetic apparatus.

Table 3.8 - Minimum, average, maximum concentrations PAHs and PAHs total, TEFs, Σ carcinogenic PAHs in sediments

		Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene	Anthracene	Fluoranthene	Pyrene	Benzo[a]anthracene	Crysene	Benzo[b]fluoranthene	Benzo[k]fluoranthene	Benzo[a]pyrene	Benzo (g,h,i)perylene	Dibenzo(a,h)anthracene	Indeno(1,2,3-c,d)pyrene	PAH total	TEFs	Σ carcinogenic PAHs
		ng/g dry sed																		
	RO																			
MIN		0.10	0.10	0.10	0.00	0.10	0.10	0.10	142	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	143.4	0.394	0.600
AVER		0.10	0.10	0.10	21.2	48.9	24.6	118	151	109	16.8	92.9	103	60.5	133	62.4	58.6	1000.2	165.128	486.4
MAX		0.10	0.10	0.10	127	147	147	143	154	153	100	141	141	122	270	188	119	1952.3	393.814	864.0
	BG																			
BG-1		26.7	26.8	0.60	2.33	43.2	8.31	45.5	58.4	21.6	22.9	28.9	17.2	25.8	34.9	17.5	42.2	422.84	56.966	153.2
	TR																			
MIN		3.44	1.46	0.21	1.00	9.64	0.56	6.62	5.83	2.16	3.27	4.31	1.73	2.62	2.83	0.44	2.56	48.68	4.331	13.82
AVER		19.2	2.98	0.95	8.60	53.9	3.74	29.1	29.3	9.83	16.6	22.3	9.92	13.1	21.25	3.65	18.2	262.62	24.170	77.0
MAX		28.1	5.24	1.72	29.6	186	7.11	62.2	58.3	17.8	29.4	38.5	18.3	22.9	37.95	6.91	32.5	582.53	43.830	136.91
	UA																			
MIN		5.73	13.6	1.75	2.63	39.9	4.11	16.1	15.1	6.81	7.39	9.75	6.49	8.29	10.63	6.02	14.3	168.6	19.022	51.660
AVER		36.3	34.8	4.73	12.6	85.6	13.3	52.4	46.3	25.6	23.7	32.8	21.6	27.6	29.48	12.7	45.8	505.31	55.394	166.1
MAX		149	71.8	11.0	39.6	196	34.2	88.5	81.8	57.0	44.9	65.9	48.1	50.2	59.82	29.0	92.6	1119.42	111.519	342.8

Figure 3.14 shows the distribution gradient of PAHs total and TEFs in the bottom sediments across regions BS.

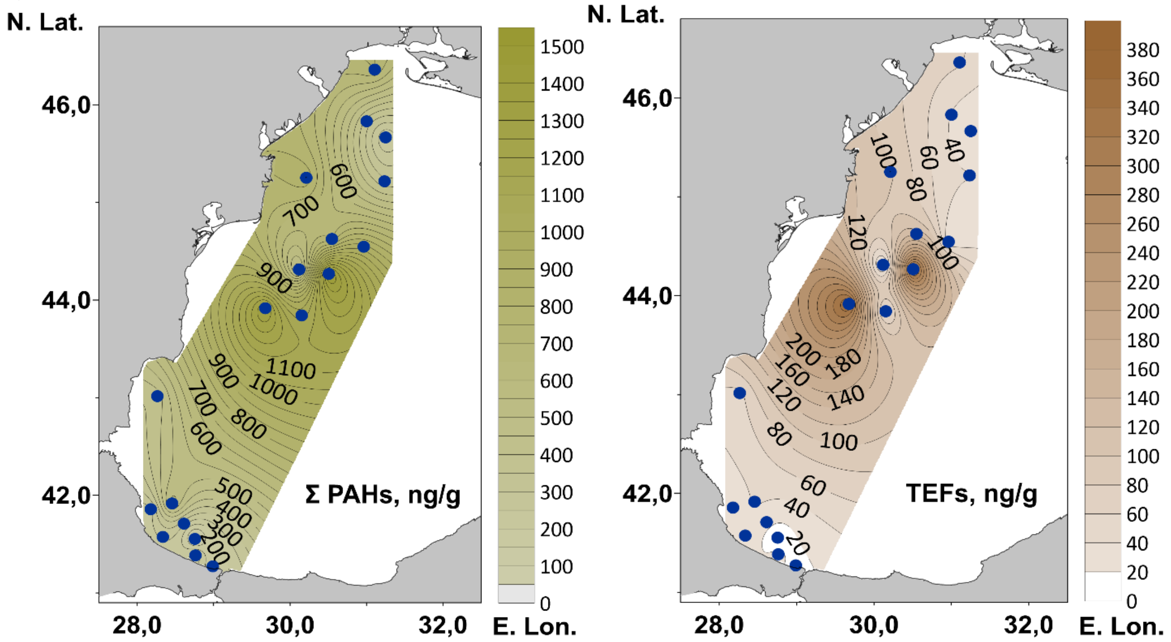


Figure 3.14 - Gradient distribution PAHs total, TEFs, in bottom sediments

According to the recommendations (Traven, 2008), bottom sediments can be classified into 3 categories depending on the total PAH content:

- <250 ng/g - slightly contaminated.
- 250 - 500 ng/g - contaminated.
- >500 ng/g - highly contaminated.

Table 3.9 shows that, based on total PAH content, the following categories were observed:

- at stations NMS-UA-1, NMS-UA-15, RO-1, RO-2, RO-4, RO-5, RO-6, TR-2, sediments are characterized as highly contaminated.
- at stations NMS-UA-2, NMS-UA-16, RO-3, BG-1, TR-3, TR-4, TR-5, sediments are characterized as contaminated.
- at stations NMS-UA-17, TR-1, TR-6, TR-7, sediments are characterized as slightly contaminated.

Sources of pollution in bottom sediments, based on geochemical markers, were identified as follows:

- in the Ukrainian part of BS, in the bottom sediment samples taken at stations NMS-UA-1, NMS-UA-2, NMS-UA-16 and NMS-UA-15, pyrogenic PAHs obtained as a result of combustion of petroleum hydrocarbons prevail, whereas at station NMS- UA-17 it is possible intake of petrogenic PAHs as a result of oil products handling.
- in the Romanian and Bulgarian parts of BS, in the samples of bottom sediments there is a mixed type of pollution of both pyrogenic and petrogenic nature.
- in the Turkish part of BS, at the TR-4 station, in the sample of bottom sediment, pollution is of a pyrogenic nature, mainly combustion of petroleum hydrocarbons. At the rest of the stations there are mixed pollution, both pyrogenic and petrogenic.

Table 3.9 - Nature of surfactant pollution and probable sources of pollution in the Black Sea in each station

Station	Bot. Depth (m)	Source of surfactant pollution, assessment by geochemical marker						Classification of samples by contamination
		Ph/An (Probability 66.7%)	(Py+Flu)/(Chr+Ph) (Probability 70.8%)	An/178 (Probability 50%)	Flu/Py (Probability 50%)	Flu/Flu+Py (Probability 79.2%)	BaA/228 (Probability 66.7%)	
UA-1	25	pyrogenic PAHs	pyrogenic PAHs	diesel oil, shale oil, coal and some samples of crude oil	pyrogenic PAHs burning coal	burning kerosene and grass, most coal and wood; creosote	pyrogenic PAHs	very dirty
UA-2	53							contaminated
UA-16	24.8							contaminated
UA-17	40.4	petrogenic PAHs		lignite, diesel emissions and fuel oil				slightly contaminated
UA-15	25	pyrogenic PAHs		diesel oil, shale oil, coal and some samples of crude oil				very dirty
RO-1	78	pyrogenic PAHs	pyrogenic PAHs	diesel oil, shale oil, coal and some samples of crude oil	petrogenic PAHs	petrogenic PAHs (most TPHs)	pyrogenic PAHs	very dirty
RO-2	106	petrogenic PAHs		lignite, diesel emissions and fuel oil	pyrogenic PAHs wood burning			very dirty
RO-3	76							contaminated
RO-4	103	pyrogenic PAHs		diesel oil, shale oil, coal and some samples of crude oil				very dirty
RO-5	67							very dirty
RO-6	103							very dirty
BG-1	43	pyrogenic PAHs	pyrogenic PAHs	diesel oil, shale oil, coal and some samples of crude oil	wood burning	petrogenic PAHs (most TPHs)	pyrogenic PAHs	contaminated
TR-1	75	pyrogenic PAHs	pyrogenic PAHs	diesel oil, shale oil, coal and some samples of crude oil	wood burning	petrogenic PAHs (most TPHs)	pyrogenic PAHs	slightly contaminated
TR-2	90	petrogenic PAHs		lignite, diesel emissions and fuel oil	burning coal	burning kerosene and grass, most coal and wood; creosote		very dirty
TR-3	71	pyrogenic PAHs		diesel oil, shale oil, coal and some samples of crude oil	wood burning	petrogenic PAHs (most TPHs)		contaminated
TR-4	86				burning coal	burning kerosene and grass, most coal and wood; creosote		contaminated
TR-5	77	petrogenic PAHs		lignite, diesel emissions and fuel oil	wood burning	petrogenic PAHs (most TPHs)		contaminated
TR-6	88				burning coal	burning kerosene and grass, most coal and wood; creosote		slightly contaminated
TR-7	35	pyrogenic PAHs		diesel oil, shale oil, coal and some samples of crude oil	wood burning	petrogenic PAHs (most TPHs)		slightly contaminated

The analysis of TPHs was carried out only in bottom sediments at stations located in the Romanian and Turkish parts of the BS.
Figure 3.15 shows the results of the analysis of total petroleum hydrocarbons (TPHs) in the sediment samples at the stations of the Romanian and Turkish parts of BS.

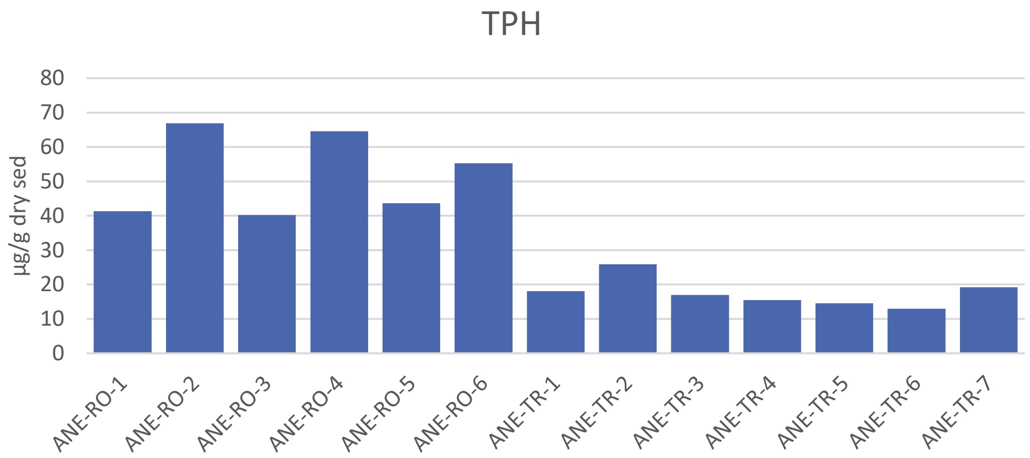


Figure 3.15 - Results of analysis of total petroleum hydrocarbons (TPH) in sediment samples

Figure 3.15 shows that the level of pollution of bottom sediments TPH in the Romanian part of BS is higher than in the Turkish one.
Comparing the obtained results with the "Environmental Standards for the Quality of the Marine Environment", developed at UkrSCES, it can be noted that in the Romanian part of the Black Sea bottom sediments correspond to a satisfactory quality class, and in the Turkish part - to a good quality class.

Biota

The threshold of pollutants for the assessment of biological samples were taken from DIRECTIVE 2013/39/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 12 August 2013 and from Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.
Table 3.10 shows the concentrations of PAHs, PAHs total, TEFs, Σ carcinogenic PAHs in biological samples caught at sampling stations.
Table 3.10 shows that an excess of 1.99 times relative to the threshold, was recorded in *Mytilus galloprovincialis* at the TR-7 station, this is comparable to the indicators of pollution of bottom sediments in the area of this station.
In *Mytilus galloprovincialis* caught in the Romanian and Bulgarian parts of BS, the threshold was not exceeded. The obtained results of the analysis of the biological object at station RO-1 are not comparable with the assessment of bottom sediments in the area of this station (bottom sediments RO-1 are very dirty), perhaps in the area of this station there is no secondary water pollution accumulated in bottom sediments of PAHs.
In Mytilus galloprovincialis at TR-7, the levels of TEFs and Σ carcinogenic PAHs indicate the accumulation of carcinogenic chemical compounds in biological objects.
Biological samples caught at sampling stations can generally be characterized as low-contaminated PAHs.

Table 3.10 - Concentrations of PAHs, PAHs total, TEFs, Σ carcinogenic PAHs in biota

Station	Species	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene	Anthracene	Fluoranthene	Pyrene	Benzo[a]anthracene	Crysene+Triphenylene	Crysene	Benzo[b]fluoranthene	Benzo[k]fluoranthene	Benzo[a]pyrene	Benzo (g,h,i)perylene	Dibenzo(a,h)anthracene	Indeno(1,2,3-c,d)pyrene	PAH total	TEFs	Σ carcinogenic PAHs
		ng/g wet weight																			
2013/39/EU AA-EQS Marine								**30**							**5**						
RO-1	*Mytillus galloprovincialis*	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1		0.1	0.1	0.1	0.1	0.1	0.1	0.1	1.6	0.25	0.6
BG-3	*Mytillus galloprovincialis*	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1		0.1	0.1	0.1	0.1	0.1	0.1	0.1	1.6	0.25	0.6
BG-5	*Mytillus galloprovincialis*	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1		0.1	0.1	0.1	0.1	0.1	0.1	0.1	1.6	0.25	0.6
BG-7	*Mytillus galloprovincialis*	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1		0.1	0.1	0.1	0.1	0.1	0.1	0.1	1.6	0.25	0.6
TR-7	*Mytilus galloprovincialis*	35.6	2.55	0.29	10.1	102	2.51	59.8	55.8	2.47	7.34		4.97	1.84	0.91	2.69	0.48	1.79	291	3.28	12.5

Conclusions

The average concentration of PAHs in the surface layer of seawater exceeded the permissible values in the Ukrainian part of BS by benz (g,h,i)perylene by 1.03 times.
For the rest of PAHs in the Ukrainian part of BS, no excess of permissible concentrations was recorded.
In the Romanian and Turkish parts of the Black Sea, PAH concentrations in seawater did not exceed the threshold.
PAHs total have the highest value in the Romanian part of BS (869.4 ng/L), but the TEFs and Σ carcinogenic PAHs are at low levels, which indicates low concentrations of highly toxic PAHs in their total community. In the Ukrainian part, BS PAHs total, on the contrary, is much lower (106.8 ng/L) than in the Romanian part, but the TEFs and Σ carcinogenic PAHs indicate high concentrations of highly toxic PAHs. In the Turkish part, BS PAHs total, TEFs, and Σ carcinogenic PAHs are at a low level, which indicates a low input of PAHs into the surface layers of seawater.
High concentrations of PAHs in the surface layers of sea water in the Ukrainian and Romanian parts of BS, in the bottom sediments of the Ukrainian, Romanian and Bulgarian parts of BS can be explained by the influence of rivers carrying PAHs in their fresh waters both dissolved in water and precipitated on fine particulate matter. The development of hydrocarbon deposits on the BS shelf also affects the level of PAHs pollution.
The highest PAH content in bottom sediments was recorded in the Romanian part of BS PAHs total 999 ng/g, in the Ukrainian and Bulgarian parts of BS PAH concentrations are lower than PAHs total - 505 ng/g and 423 ng/g, respectively, in the Turkish part of BS concentration PAHs are the lowest - PAHs total 262 ng/g.
In bottom sediments, TEFs in the Ukrainian, Bulgarian and Turkish parts of BS are at the same level 52.9 - 54.9 ng/g, in the Romanian part BS TEFs have the highest value - 155 ng/g, which correlates with the PAHs total.
Σ carcinogenic PAHs exceed the value of TEFs in all BS regions, this characterizes the accumulation of chemical compounds in bottom sediments, which, when entering into biological object, can cause irreversible changes in the genetic apparatus.
Sources of PAH contamination in BS:

- in the Ukrainian part of BS, pyrogenic PAHs predominate at all sampling stations, obtained as a result of combustion of petroleum hydrocarbons, at station NMS-UA-17, petrogenic PAHs may be supplied as a result of oil product spills;
- in the Romanian and Bulgarian parts of BS at all sampling stations there is mixed pollution of both pyrogenic and petrogenic nature.
- in the Turkish part of BS at the TR-4 sampling station, the pollution is of a pyrogenic nature, mainly the combustion of petroleum hydrocarbons. At the rest of the stations there are mixed pollution, both pyrogenic and petrogenic.

Biological samples caught at sampling stations can generally be characterized as lightly contaminated with PAHs. The only excess of the threshold by 1.99 times was recorded in *Mytilus galloprovincialis* at the TR-7 station, which is comparable to the values of pollution of bottom sediments in the area of this station. Also, in *Mytilus galloprovincialis* at TR-7 station, the levels of TEFs and Σ carcinogenic PAHs indicate the accumulation of carcinogenic chemical compounds in biological objects, possibly due to secondary pollution of seawater with PAHs accumulated in bottom sediments.

3.5 Organochlorine pesticides

Organochlorine pesticides (OCPs) are persistent and toxic chemicals, belonging to the group of persistent organic pollutants (POPs). Although these compounds were widely used in the 1940s in large quantities, they were banned in developed countries in the 1970s because of their high persistence in the environment and their harmful effects in human health (Karasali & Maragou, 2016). Organochlorine pesticides are more persistent in the environment than most other synthetic organic pesticides. Their persistence, both in humans and in the environment, has caused public concern, since, in certain situations, they may pose health and environmental problems. For this reason, and because some insects have developed resistance to this class of compounds, their use is steadily decreasing (Pleština, 2003). Although the environmental levels of organochlorine insecticides are now slowly declining, most of them are classified as endocrine disrupting chemicals (EDCs) because of their hormone-like effects on the endocrine systems of wildlife and humans, and persistent organic pollutants (POPs) under the Stockholm Convention (Tsai, 2014).
Stockholm Convention on Persistent Organic Pollutants is an international environmental treaty, signed in 2001 and effective from May 2004, that aims to eliminate or restrict the production and use of persistent organic pollutants (POPs). In most countries of the Black Sea the use of this POPs has been banned or restricted, Bulgaria, Georgia and Romania have signed and ratified this convention, while the remaining three Black Sea countries are signatories. Public health use of DDT is allowed under the Stockholm Convention, but only for the control of mosquitoes (the malaria vector) (BSC, 2008a).
The chlorinated pesticides investigated were mainly those included in the Stockholm Convention "dirty dozen": aldrin, dieldrin, endrin, heptachlor, hexachlorobenzenes (HCBs), DDT (inclusive its metabolites DDE and DDD). Additionally, the isomers of hexachlorocyclohexane (HCH) were investigated. Lindane, also known as gamma-hexachlorocyclohexane (γ-HCH) was investigated in all stations. α-Hexachlorocyclohexane (α-HCH) and ß-Hexachlorocyclohexane (ß-HCH) which are byproducts of the production of the lindane were investigated mainly in the NW area.

Water

The investigated compounds had concentration below detection limit, except for HCB, ß-HCH, lindane and p,p' DDT (Table 3.11).
Most of the investigated compounds were observed in the northwestern area where it was highlighted low to moderate concentrations of ß-HCH (0.00009 - 0.00996 µg/L), lindane (0.00005 -0.00027 µg/L) and p,p' DDT (0.0064 – 0.032 µg/L) (Figure 3.16). The presence of heptachlor and p,p' DDE was noticed, occasionally. Also, some high values were reported in the western part of the Black Sea for HCB (14.48 µg/L- RO-2, 3.86 µg/L - ANE-RO-3, 1.47 µg/L - RO-5) and lindane (17.76 µg/L - RO-2, 4.77 µg/L - RO-2) surpassing the threshold values proposed for water in order to define good ecological status (according to Directive 2013/39/EU).
The predominance of p.p' DDT in comparison with its metabolites (Figure 3.17) suggest a recent and ongoing water pollution in northwest part of the Black Sea.

Table 3.11 - Concentrations of organochlorine pesticides in surface waters, October 2019

Station	HCB (µg/L)	α-HCH (µg/L)	ß-HCH (µg/L)	Lindane (µg/L)	Heptachlor (µg/L)	Aldrin (µg/L)	Dieldrin (µg/L)	Endrin (µg/L)	p,p'DDE (µg/L)	p,p'DDD (µg/L)	p,p'DDT (µg/L)
RO-1	<0.004	-	-	<0.003	<0.003	<0.003	<0.002	<0.003	<0.002	<0.002	<0.002
RO-2	14.482	-	-	17.759	<0.003	<0.003	<0.002	<0.003	<0.002	<0.002	<0.002
RO-3	3.868	-	-	<0.003	<0.003	<0.003	<0.002	<0.003	<0.002	<0.002	<0.002
RO-4	<0.004	-	-	<0.003	<0.003	<0.003	<0.002	<0.003	<0.002	<0.002	<0.002
RO-5	1.473	-	-	4.768	<0.003	<0.003	<0.002	<0.003	<0.002	<0.002	<0.002
RO-6	<0.004	-	-	<0.003	<0.003	<0.003	<0.002	<0.003	<0.002	<0.002	<0.002
TR-1	<0.0002	-	-	<0.00025	<0.00025	<0.00067	<0.0005	<0.0025	<0.00005	<0.00005	<0.00005
TR-2	<0.0002	-	-	<0.00025	<0.00025	<0.00067	<0.0005	<0.0025	<0.00005	<0.00005	<0.00005
TR-3	<0.0002	-	-	<0.00025	<0.00025	<0.00067	<0.0005	<0.0025	<0.00005	<0.00005	<0.00005
TR-4	<0.0002	-	-	<0.00025	<0.00025	<0.00067	<0.0005	<0.0025	<0.00005	<0.00005	<0.00005
TR-5	<0.0002	-	-	<0.00025	<0.00025	<0.00067	<0.0005	<0.0025	<0.00005	<0.00005	<0.00005
TR-6	<0.0002	-	-	<0.00025	<0.00025	<0.00067	<0.0005	<0.0025	<0.00005	<0.00005	<0.00005
TR-7	<0.0002	-	-	<0.00025	<0.00025	<0.00067	<0.0005	<0.0025	<0.00005	<0.00005	<0.00005
UA - 1	<0.00005	<0.00005	0.00364	<0.00005	<0.00005	<0.00005	<0.00005	-	<0.00005	<0.00005	0.02603
UA - 2	<0.00005	<0.00005	0.00277	0.00015	<0.00005	<0.00005	<0.00005	-	<0.00005	0.00029	0.03969
UA-16 surface	<0.00005	<0.00005	0.00153	0.00012	<0.00005	<0.00005	<0.00005	-	<0.00005	<0.00005	0.01185
UA-16 bottom	<0.00005	<0.00005	0.00543	0.00016	<0.00005	<0.00005	<0.00005	-	<0.00005	<0.00005	0.01251
UA-17 surface	<0.00005	<0.00005	0.00283	0.00027	<0.00005	<0.00005	<0.00005	-	<0.00005	<0.00005	0.00727
UA-17 bottom	<0.00005	<0.00005	0.00333	0.00009	<0.00005	<0.00005	<0.00005	-	<0.00005	<0.00005	0.00636
UA-15 surface	<0.00005	<0.00005	0.00386	0.00016	<0.00005	<0.00005	<0.00005	-	<0.00005	<0.00005	0.01754
UA-15 bottom	<0.00005	<0.00005	0.00996	<0.00005	0.248	<0.00005	<0.00005	-	<0.00005	<0.00005	0.03164

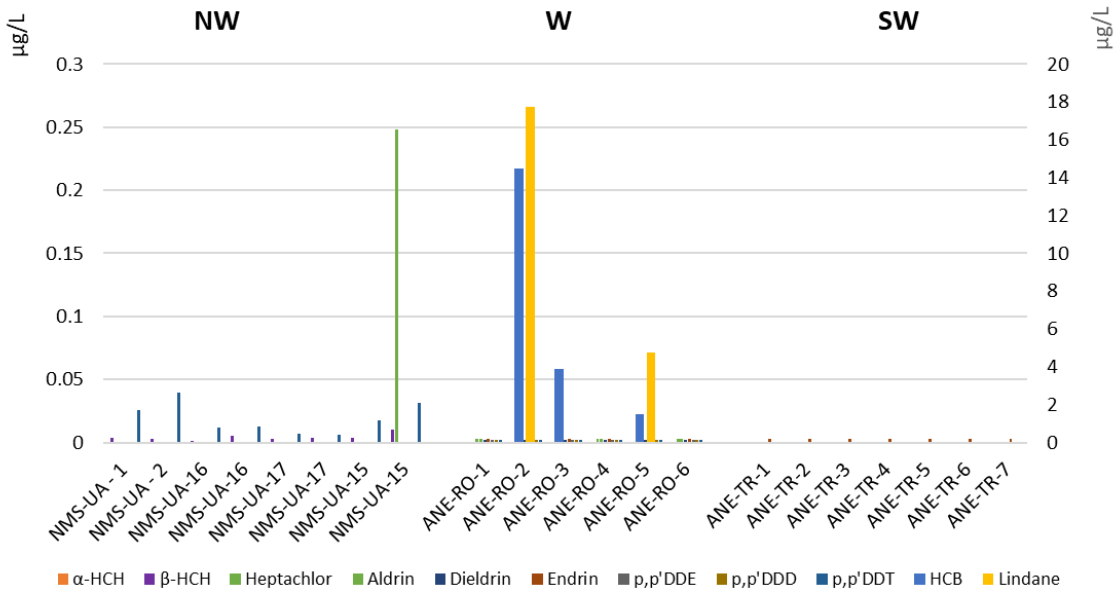


Figure 3.16 - Distribution of organochlorine pesticides in Black Sea surface waters, October 2019

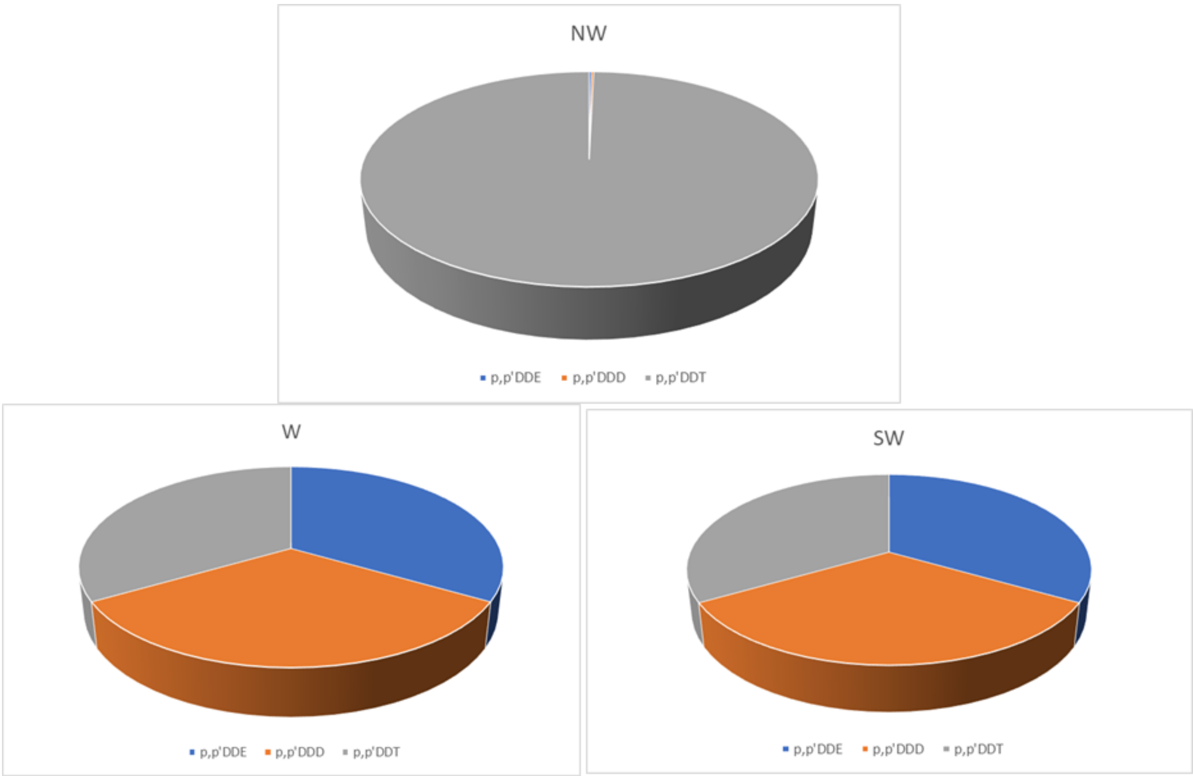


Figure 3.17 - Share of DDT and its metabolites concentrations in NW, W and SW surface waters, 2019

In relation with European environmental quality standards (EQS) for water (Directive 2013/39/CE), concentrations of hexachlorobenzene, lindane, DDT, DDT total and sum of cyclodiene in surface waters complied in general with threshold values (Table 3.12). The assessment was not done for heptachlor as detection limits are higher than the threshold value.

Table 3.12 - Percentage of observations exceeding threshold values stipulated by Directive 2013/39/CE, in surface waters, 2019

	Environmental Quality Standard Directive 2013/39/CE	Number of observations	Percentage of observations surpassing EQS
HCB	MAC: 0.05 µg/L	21	14%
Lindane	MAC: 0.02 µg/L	21	10%
Sum Cyclodiene	AA: 0.005 µg/L	21	0%
DDT	AA: 0.01 µg/L	21	29%
Total DDT	AA: 0.025 µg/L	21	29%

Sediment

The level of organochlorine pesticides fluctuated in a large range from detection limit to 56.48 ng/g dw HCB, 0.33 ng/g dw α-HCH , 14.60 ng/g dw ß-HCH , 7.83 ng/g dw heptachlor, 7.43 ng/g dw lindane, 5.55 ng/g dw aldrin, 4.06 ng/g dw dieldrin, 10.53 ng/g dw endrin, 9.35 ng/g dw p,p' p,p' DDE, 6.33 p,p'DDD and 24.10 ng/g dw p,p' DDT (Table 3.13)

Heptachlor and p,p' DDT represented the dominant compounds in the northwestern part, HCB, endrin and p,p' DDE in the western part and p,p' DDE, p,p' DDD and ß-HCH in the south-western part. Highest OCPs values were recorded in the western area, especially for HCB (56.48 ng/g dw - RO-4 and 24.57 ng/g dw - RO-2) (Figure 3.18).

In relation with sediment quality criteria (ERL: Effects Range – Low and EAC: Environmental Assessment Criteria), percentages of observations surpassing ERL and EAC values varied, depending on the element, between 5% and 42% (Table 3.14). Environmental Assessment Criteria (EACs) was proposed by OSPAR as a means for assessing the significance of concentrations of hazardous substances in the marine environment and if EACs were not available with Effects Range Low (ERLs) developed by the United States Environmental Protection Agency for assessing the ecological significance of sediment concentrations (OSPAR Commission, 2008). EACs (lower) are concentrations below which it is reasonable to expect that there will be an acceptable level of protection of marine species from chronic effects from specific hazardous substances. Concentrations below the ERL rarely cause adverse effects in marine organisms.

Table 3.13 - Concentrations of organochlorine pesticides in surface sediments, October 2019

Station	HCB (ng/g dry sed)	α-HCH (ng/g dry sed)	ß-HCH (ng/g dry sed)	Lindane (ng/g dry sed)	Heptachlor (ng/g dry sed)	Aldrin (ng/g dry sed)	Dieldrin (ng/g dry sed)	Endrin (ng/g dry sed)	p,p'DDE (ng/g dry sed)	p,p'DDD (ng/g dry sed)	p,p'DDT (ng/g dry sed)
RO-1	4.94	-	-	<0.3	<0.2	5.26	2.25	6.37	3.63	3.32	<0.2
RO-2	24.57	<0.05	14.60	<0.3	<0.2	2.68	2.88	4.65	9.35	4.49	<0.2
RO-3	15.25	-	-	<0.3	<0.2	5.55	<0.2	4.16	2.35	3.96	<0.2
RO-4	56.48	-	-	<0.3	<0.2	<0.2	2.04	10.53	6.36	<0.2	<0.2
RO-5	6.12	<0.05	<0.05	<0.3	<0.2	<0.2	4.06	3.70	0.20	<0.2	<0.2
RO-6	15.14	-	-	7.83	<0.2	<0.2	1.89	<0.3	6.60	<0.2	<0.2
BG-1	<0.05	<0.05	8.72	<0.05	0.32	0.37	<0.05	-	<0.05	<0.05	5.64
TR-1	<0.05	<0.05	5.62	<0.05	7.43	0.30	<0.05	-	<0.05	<0.05	3.98
TR-1	-	0.18	0.49	0.09	<0.05	<0.05	<0.05	<0.05	2.05	3.98	0.31
TR-2	-	0.31	0.94	0.18	<0.05	<0.05	<0.05	<0.05	3.78	6.33	0.82
TR-3	-	0.33	0.74	0.14	<0.05	<0.05	<0.05	<0.05	2.30	4.44	0.42
TR-4	-	0.28	0.74	0.11	<0.05	<0.05	<0.05	<0.05	1.73	3.16	0.34
TR-5	-	0.21	0.42	0.08	<0.05	<0.05	<0.05	<0.05	1.69	2.75	0.39
TR-6	-	<0.05	0.22	0.26	<0.05	<0.05	<0.05	<0.05	0.24	0.37	0.25
TR-7	-	0.18	0.49	0.21	<0.05	<0.05	<0.05	<0.05	1.12	2.03	0.33
UA - 1	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	-	<0.05	<0.05	3.18
UA - 2	<0.05	<0.05	<0.05	<0.05	<0.05	0.08	<0.05	-	<0.05	0.22	2.73
UA-16	<0.05	<0.05	11.61	0.30	6.75	0.39	<0.05	-	<0.05	<0.05	8.27
UA-17	<0.05	0.23	<0.05	<0.05	5.18	0.34	<0.05	-	<0.05	<0.05	2.32

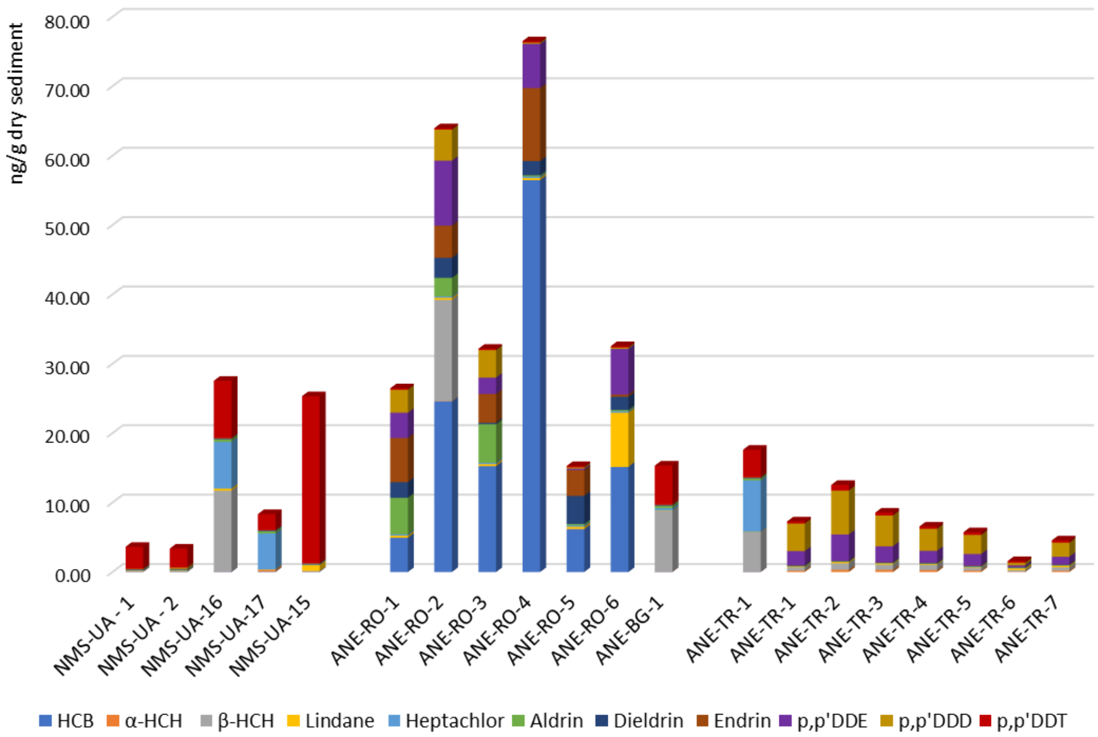


Figure 3.18 - Distribution of organochlorine pesticides in surface sediments, October 2019

Table 3.14 - Percentage of observations surpassing sediment quality criteria (EAC, ERL), in surface sediments, 2019

	Sediment Quality Criteria (ERL, EAC)	Number of observations	Percentage of observations surpassing ERL / EAC
HCB	ERL: 20 ng/g	19	11%
Lindane	ERL: 3 ng/g	19	5%
p.p' DDE	ERL: 2 ng/g	19	42%
Dieldrine	ERL: 2.2 ng/g	19	16%

Biota

Data for persistent organic pollutants in biota is poor, as only five mussels (*Mytilus galloprovincialis*) samples were collected, mainly in the western part of the Black Sea. Individual organochlorine pesticides varied between detection limit and 116.08 ng/g ww (Table 3.15). Highest concentrations were recorded, in the western part, for HCB (18.37 to 116.08 ng/g ww), lindane (2.63 to 24.11 ng/g ww) and heptachlor (0.88 to 21.12 ng/g ww) (Figure 3.19).

Table 3.15 - Concentrations of organochlorine pesticides in Black Sea Mytilus galloprovincialis, October 2019

Station	RO-1	BG-3	BG-5	BG-7	TR-7
HCB (ng/g wet tissue)	116.08	64.32	14.85	18.37	-
α-HCH (ng/g wet tissue)	-	-	-	-	0.02
β-HCH (ng/g wet tissue)	-	-	-	-	0.54
Lindane (ng/g wet tissue)	8.3	2.63	4.76	24.11	0.01
Heptachlor (ng/g wet tissue)	21.12	15.67	0.88	6.34	<0.007
Aldrin (ng/g wet tissue)	0.38	<0.045	<0.045	<0.045	<0.007
Dieldrin (ng/g wet tissue)	0.23	5.77	2.02	23.22	-
Endrin (ng/g wet tissue)	<0.060	3	0.8	<0.060	-
p,p'DDE (ng/g wet tissue)	<0.0300	<0.0300	<0.0300	<0.0300	2.21
p,p'DDD (ng/g wet tissue)	7.05	4.98	1.98	9.9	1.69
p,p'DDT (ng/g wet tissue)	3.1	3.4	<0.0300	<0.0300	0.17

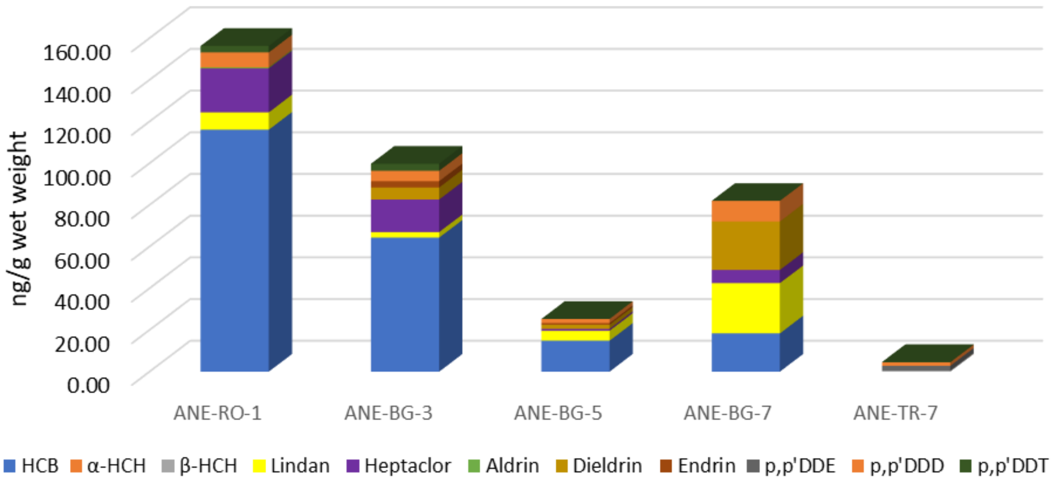


Figure 3.19 - Distribution of organochlorine pesticides in Black Sea Mytilus galloprovincialis, October 2019

In relation with European environmental quality standards (EQS) for biota (Directive 2013/39/CE), concentrations of hexachlorobenzene and heptachlor exceeded the threshold values in 100%, respectively 80% of the samples (Figure 3.20). European regulations don't establish maximum admissible levels of organochlorine pesticides in biota for human consumption, so the results were not evaluated in respect with D9.

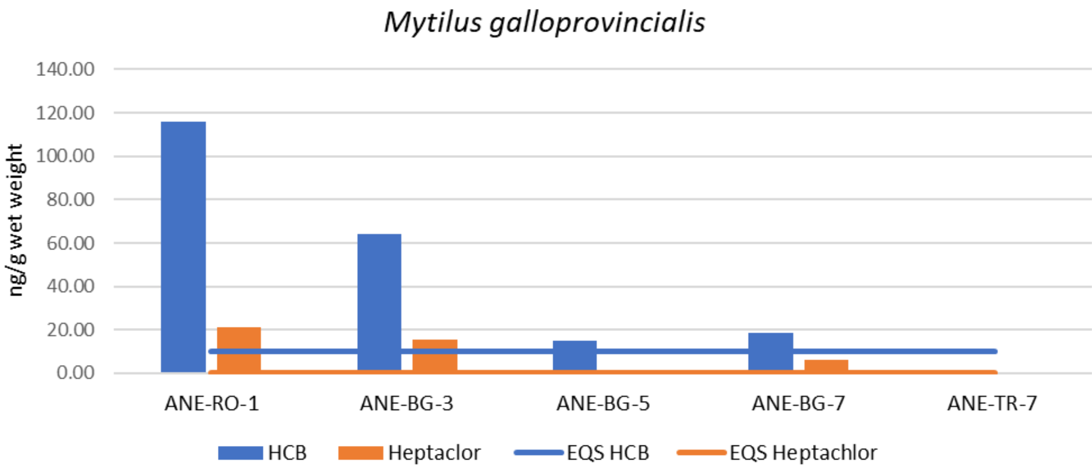


Figure 3.20 - HCB and heptachlor concentrations in Black Sea Mytilus galloprovincialis, in relation to threshold values stipulated by Directive 2013/39/CE, October 2019

Conclusion

Most of the organochlorine pesticides concentration were below detection limit in seawater. The levels of detected compounds, HCB, lindane and p,p' DDT, indicated a moderate level of organic pollution, as they exceeded threshold values in 10 to 29% of the samples.

In sediment, the level of organochlorine pesticides fluctuated in a large range from detection limit to 56.48 ng/g, the highest OCPs values being recorded in the western area. The percentages of observations surpassing threshold values varied, depending on the element, between 5% and 42%.

Highest levels of organochlorine pesticides in biota, were recorded, in the western part, where HCB and heptachlor concentrations exceeded the threshold values in 100%, respectively 80% of the samples.

3.6 Polychlorinated Biphenyls (PCBs)

Polychlorinated biphenyls (PCBs), as persistent organic pollutants (POPs), have been extensively studied during the last 30 years in view of their extensive production and usage, their long-range transport capability within the environment, and their bioaccumulation, persistence, and impact on both ecosystems and human health (Y. Xing et al., 2005). Because of their chemical stability and heat resistance, PCBs were once widely used as additives in heat exchange fluids, components for transformers and large capacitors, carbon-free copy paper, as well as paints and plastics, before their persistence and toxicity were examined and revealed (X. Cui et al., 2020). In general, PCB 28, 52, 101, 118, 138, 153, and 180 are often used as indicators of environmental pollution (EFSA, 2010). The production and use of PCBs.

have been discontinued in most countries since a ban on their manufacturing, processing and distribution was introduced in 1985 (UNEP 2001), but large amounts remain in electrical equipment, plastic products, and buildings. Studies have shown that PCBs are still present in the global environment, because of their long-range transport feature, despite the bans on PCB production in developed countries that entered into force more than three decades ago (Breivik et al., 2007). The estimated results suggest that almost 97% of the global historical use of PCBs have occurred in the Northern Hemisphere (Breivik et al., 2002). In 2002 the European Commission prescribed a list of actions to further reduce the presence of dioxins and dioxin-like PCBs and later introduced action and maximum levels with random monitoring by Member States (EFSA 2010).

EU MSFD aims to reach good environmental status in marine waters of Europe. Under the MSFD the level of the contaminants needed to be under a certain level not to pose a risk to the marine ecosystem and their trends should not be increased (MSFD 2008 and Com. Dec. 2017). The threshold levels for the selected contaminants are listed in EQS Directive related with the EU WFD (2006), where the chemical status of surface waters is assessed using EQSs for a list of priority substances. Although PCB's are concerned as emerging contaminant, their threshold values (for the water and sediment matrices) are not yet included in the priority substances list yet. But it is needed to collect and evaluate the availability of marine data for those substances to understand potential marine monitoring and to select most relevant contaminants (Lohman et al., 2007; Tornero et al., 2019).

Scientific knowledge about the current contamination status with persistent organ halogenated pollutants (POPs) of the Black Sea marine and coastal area are very scare and local. PCBs and OCPs were measured in sediments collected in 2000 from the mouth of the Danube Delta and it was found that the Danube River is a potential source of contamination to the Black Sea (Fillmann et al., 2002 and). Organochlorine contamination in eggs of aquatic birds from the Danube Delta was investigated for the first time in 1982 (Fossi et al., 1984), followed by a more recent investigation conducted in 1997 in the same area (Aurigi et al., 2000). Results of the recent studies about the Black Sea marine environment (MISIS project, 2012-2014) showed that concentrations of organochlorine compounds in water were higher or comparable with those reported in the Black Sea region in previous expeditions. Furthermore, it was found that except PCB 52, the values measured for other PCBs compounds were low or under detection limit.

In this study, the PCBs contents of water, sediment, and biota samples collected from the western Black Sea shelf area were determined as a part of the ongoing ANEMONE project. The set of 6 indicator PCBs IUPAC No 28, 52, 101, 138, 153, 180 (Indicator PCBs) was considered as recommended by the European Union for assessing the pollution by PCBs (EC, 1999).

Water

The concentrations of polychlorinated biphenyls (PCBs) (7 congeners) in the 21 sea water samples (in 18 surface water) from Ukraine, Romanian and Turkish area are presented in Table 3.16. Sum of the 7 conceners concentrations were between 0.002-0.039 µg/l. Generally, most of the PCBs concentrations in seawater have been measured below detection limits especially in TR and RO stations. It sholud be considered that the PCB values below the detection limits were not considered as zero but included in calculation as a real value in percentile calculation (Table 3.16). PCB118 was the dominant compounds detected in UA waters.

Table 3.16 - Concentrations of individual PCBs (µg/L) in water samples from the Ukraine, Romanian, Bulgarian and Turkish area

Station	Date	Bot. Depth (m)	Depth (m)	PCB28 (µg/L)	PCB52 (µg/L)	PCB101 (µg/L)	PCB118 (µg/L)	PCB138 (µg/L)	PCB153 (µg/L)	PCB180 (µg/L)
RO-1	1.10.2019	78	0	<0.004	<0.006	<0.006	<0.004	<0.007	<0.009	<0.003
RO-2	1.10.2019	106	0	<0.004	<0.006	<0.006	<0.004	<0.007	<0.009	<0.003
RO-3	1.10.2019	76	0	<0.004	<0.006	<0.006	<0.004	<0.007	<0.009	<0.003
RO-4	1.10.2019	103	0	<0.004	<0.006	<0.006	<0.004	<0.007	<0.009	<0.003
RO-5	2.10.2019	67	0	<0.004	<0.006	<0.006	<0.004	<0.007	<0.009	<0.003
RO-6	2.10.2019	103	0	<0.004	<0.006	<0.006	<0.004	<0.007	<0.009	<0.003
TR-1	5.10.2019	75	0	< 0.00004	< 0.00017	< 0.00013	< 0.00008	< 0.0002	< 0.0002	< 0.003
TR-2	6.10.2019	90	0	< 0.00004	< 0.00017	< 0.00013	< 0.00008	< 0.0002	< 0.0002	< 0.003
TR-3	5.10.2019	71	0	< 0.00004	< 0.00017	< 0.00013	< 0.00008	< 0.0002	< 0.0002	< 0.003
TR-4	5.10.2019	86	0	< 0.00004	< 0.00017	< 0.00013	< 0.00008	< 0.0002	< 0.0002	< 0.003
TR-5	6.10.2019	77	0	< 0.00004	< 0.00017	< 0.00013	< 0.00008	< 0.0002	< 0.0002	< 0.003
TR-6	6.10.2019	88	0	< 0.00004	< 0.00017	< 0.00013	< 0.00008	< 0.0002	< 0.0002	< 0.003
TR-7	6.10.2019	35	0	< 0.00004	< 0.00017	< 0.00013	< 0.00008	< 0.0002	< 0.0002	< 0.003
UA -1	29.07.2019	28	0	0.00189	0.0006	0.00205	0.00468	0.00362	0.00085	0.00123
UA - 2	30.07.2019	53	0	< 0.00005	0.00114	0.00371	0.00540	0.00320	0.00177	0.00030
UA-16	31.08.2019	25	0	< 0.00005	0.00048	0.00072	0.00120	0.00090	0.00032	0.00020
UA-16	31.08.2019	25	23	< 0.00005	0.00057	0.00122	0.00245	0.00127	0.00034	0.00006
UA-17	31.08.2019	41	0	< 0.00005	0.00040	0.00044	0.00094	0.00047	< 0.00005	0
UA-17	31.08.2019	41	40	< 0.00005	0.00031	0.00031	0.00071	0.00065	< 0.00005	0
UA-15	3.09.2019	25	0	0.00025	0.00098	0.00118	0.00266	0.00281	0.00015	0
UA-15	3.09.2019	25	24.5	0.00019	0.00109	0.00239	0.00500	0.00276	0.00073	0.00008
Percentile-75th (valid N:21)				0.004	0.006	0.006	0.004	0.007	0.0001	0.003

Sediment

The concentrations of polychlorinated biphenyls (PCBs) (as sum of 7 congeners) in coastal sediments from Ukraine, Romanian, Bulgarian and Turkish area are presented in Table 3.17. All surface sediment PCB ranged from 0.19 to 367.05 ng/g with an average value of 36.34 ng/g, with higher total PCB values measured in Romanian surface sediments (Figure 3.22). PCB28, PCB52, PCB101 and PCB118 contents of the Romanian surface sediments and PCB28 contents of the Bulgarian surface sediments were measured above the Environmental Assessment Criteria (EAC) values (Figure 3.21 and Table 3.17). Mean values of the dominant PCBs (28, 52, 101 and 118) in different MRU stations have been assessed as all mean PCB contents of the UA and TR sediment samples were found below the EAC levels. Similarly, all PCBs detected in the BG sediment samples were found below the EAC level except the mean PCB28 concentrations. Mean contents of the dominant PCBs detected in the RO sediments were found above the EAC levels. Highest concentrations of PCBs recorded in the Romanian area is probably due to the influence of discharges from the River Danube (MISIS Project, 2012-2014).

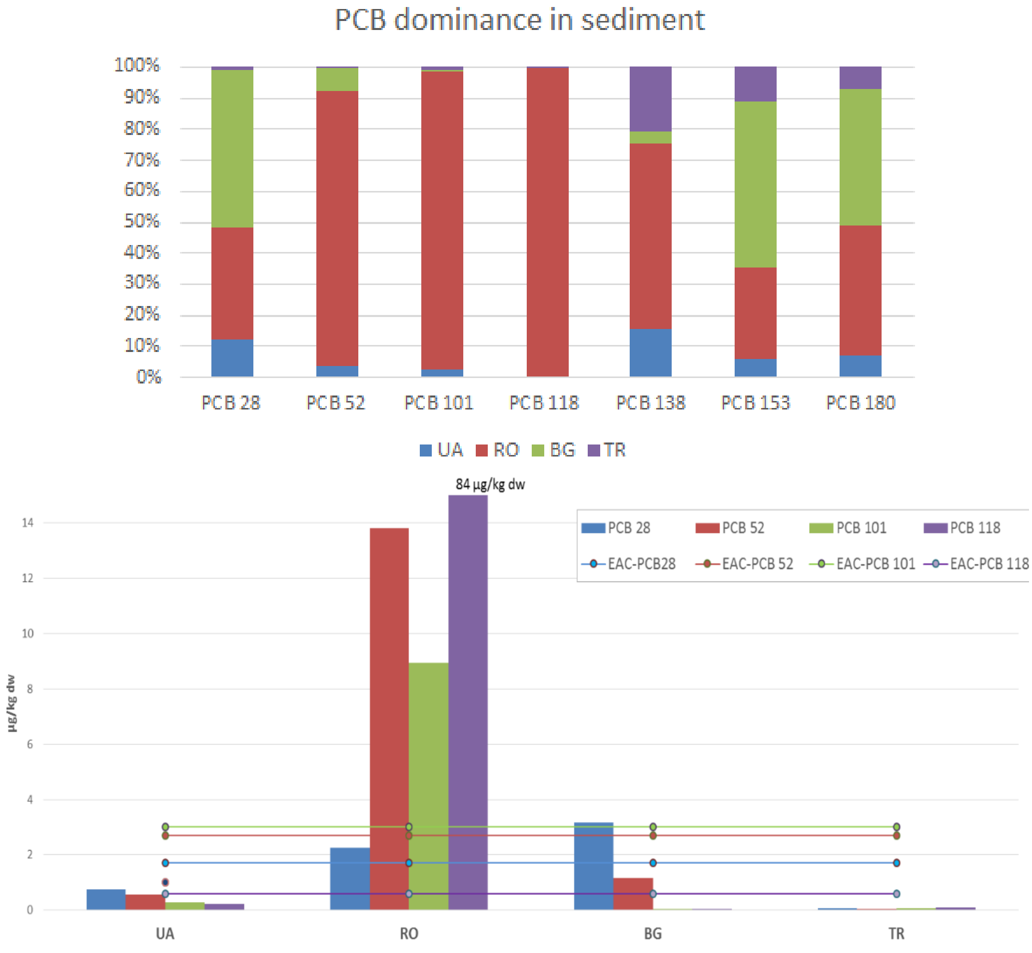


Figure 3.21 - Dominant PCBs in the surface sediment and Comparison with threshold values. PCB dominances in sediment matrix (up), PCB's compared with the threshold values (down)

Table 3.17 - Concentrations of individual and total PCBs (ng/g dry sed.) in surface sediment samples

Station	Date	Bot. Depth (m)	PCB28 (ng/g)	PCB52 (ng/g)	PCB101 (ng/g)	PCB118 (ng/g)	PCB138 (ng/g)	PCB153 (ng/g)	PCB180 (ng/g)	Total PCB (ng/g)
RO-1	1.10.2019	78	6.7	16.9	7.3	5.2	<0.7	<0.6	0.9	38.29
RO-2	1.10.2019	106	<0.4	16.8	13.7	<0.4	<0.7	<0.6	1.0	33.59
RO-3	1.10.2019	76	<0.4	16.0	8.8	4.3	<0.7	<0.6	<0.3	31.09
RO-4	1.10.2019	103	<0.4	24.8	11.6	326.9	<0.7	<0.6	2.1	367.05
RO-5	2.10.2019	67	2.4	8.1	5.0	167.3	<0.7	<0.6	0.5	184.58
RO-6	2.10.2019	103	3.3	<0.3	7.2	<0.4	<0.7	<0.6	<0.3	12.81
BG-1	3.10.2019	43	3.16	1.17	<0.05	<0.05	<0.05	1.09	0.90	6.32
TR-1	5.10.2019	75	0.07	0.038	0.057	0.099	0.242	0.237	0.145	0.89
TR-2	6.10.2019	90	0.10	0.050	0.099	0.169	0.475	0.439	0.349	1.68
TR-3	5.10.2019	71	0.09	0.046	0.078	0.128	0.299	0.273	0.152	1.07
TR-4	5.10.2019	86	0.08	0.046	0.074	0.107	0.231	0.212	0.126	0.88
TR-5	6.10.2019	77	0.07	0.035	0.058	0.086	0.188	0.198	0.112	0.75
TR-6	6.10.2019	88	0.03	0.018	0.026	0.024	0.041	0.036	0.022	0.19
TR-7	6.10.2019	35	0.05	0.032	0.063	0.082	0.212	0.194	0.118	0.75
UA -1	29.07.2019	25	< 0.05	0.08	< 0.05	0.17	0.11	< 0.05	< 0.05	0.36
UA - 2	30.07.2019	53	0.30	< 0.05	1.13	0.76	0.66	0.14	0.09	3.08
UA-16	31.08.2019	25	0.69	0.33	< 0.05	< 0.05	< 0.05	0.26	0.18	1.46
UA-17	31.08.2019	40	2.70	< 0.05	< 0.05	< 0.05	< 0.05	0.12	0.13	2.95
UA-15	3.09.2019	25	< 0.05	2.32	< 0.05	< 0.05	< 0.05	< 0.05	0.28	2.60
*Environmental Assessment Criteria (EAC) values			1.7	2.7	3.0	0.6	7.9	40	12	-
Min.			0.027	0.018	0.026	0.024	0.041	0.036	0.022	0.19
Max.			6.713	24.822	13.721	326.875	0.700	1.090	2.079	367.05
Mean			1.108	4.587	2.911	26.650	0.361	0.363	0.409	36.34
Std. Deviation			1.775	7.858	4.524	82.120	0.282	0.279	0.505	90.53
Percentile-75th (valid N:19)			2.359	8.124	7.189	0.760	0.700	0.600	0.542	21.95

***EAC- Environmental Assessment Criteria represent the contaminant concentration in the environment below which it can be assumed that no chronic effects will occur in marine species, including the most sensitive species (OSPAR Commission 2008).**

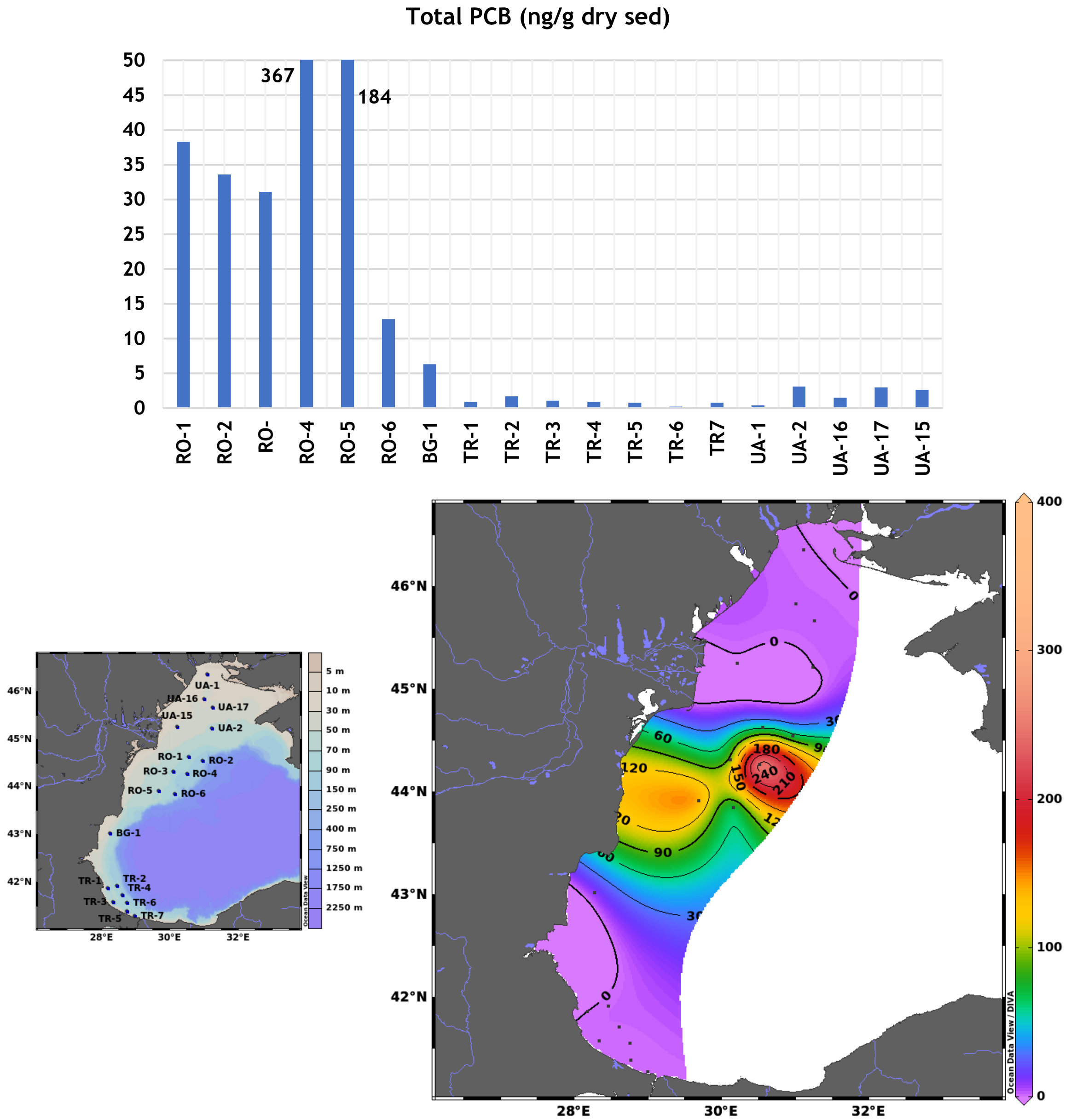


Figure 3.22 - Spatial distribution of total PCB (as sum of 7 congeners) concentrations in surface sediment. Total PCB distributions (up), Total PCB distribution map (down)

Biota

The polychlorinated biphenyls concentrations determined in the tissues of the *Mytilus galloprovincialis* with relevance for human consumption are presented in Table 3.18. A total of 5 *Mytillus galloprovincialis* samples were analyzed, but 2 biota samples could not be analyzed because there was not enough sample.

Total concentrations of the polychlorinated biphenyls varied from 0.5 to 331.6 ng/g ww. Higher levels were measured for PCB 101, PCB 118, PCB 153 and especially PCB 52 and PCB 180 in some samples (Figure 3.23).

The EU Marine Strategy Framework Directive – Descriptor 9 requires that “contaminants in fish and other seafood for human consumption do not exceed levels established by Community legislation or other relevant standards”. The Commission Regulation (EC) no. 1259/2011 sets a maximum concentration of 75 ng/g wet weight for sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 in muscle meat of fish and fishery products and products thereof. The *Mytilus galloprovincialis* samples from Romanian and Bulgarian waters exceeded the regulated levels for PCBs (Table 3.18).

Table 3.18 - Concentrations of individual and total PCBs (ΣPCBs) (ng/g wet tissue) in biota samples, October 2019

Station	Species	Date	Depth (m)	PCB 28	PCB 52	PCB 101	PCB 118	PCB 138	PCB 153	PCB 180	PCB28, PCB52, PCB101, PCB138, PCB153, PCB180 (Sum of 6PCBs)
RO-1	*Mytillus galloprovincialis*	1.10.2019	78	34.6	60.0	84.7	99.6	0.1	0.1	60.7	339.9
BG-3	*Mytillus galloprovincialis*	3.10.2019	57	0.1	85.9	9.3	156.8	65.0	56.5	0.1	373.4
BG-5	*Mytillus galloprovincialis*	4.10.2019	49	0.1	0.1	91.4	44.6	124.4	106.5	9.2	376.2
BG-7	*Mytillus galloprovincialis*	4.10.2019	48	0.1	0.1	29.2	158.0	68.6	62.9	0.1	318.8
TR-1	*Merlangius merlangus euxinus*	5.10.2019	75	-	-	-	-	-	-	-	-
TR-5	*Mytilus galloprovincialis*	6.10.2019	77	-	-	-	-	-	-	-	-
TR-7	*Mytilus galloprovincialis*	6.10.2019	35	0.02	0.04	0.06	0.09	0.18	0.19	0.01	0.6
COMMISSION REGULATION (EC) No 1881/2006* Maximum levels				-	-	-	-	-	-	-	75
Min.				0.018	0.036	0.063	0.088	0.117	0.100	0.014	0.5
Max.				34.63	85.88	91.39	157.95	124.43	106.50	60.74	331.6
Mean				6.97	29.20	42.92	91.80	51.66	45.23	14.00	190.0
Median				0.067	0.050	29.186	99.627	64.966	56.469	0.050	216.7
Std. Deviation				15.46	40.95	42.59	69.46	52.60	45.44	26.42	122.5
Percentile-75th (valid N:5)				17.35	72.92	88.05	157.36	96.52	84.70	34.95	240.3

***5 samples of mollusks - *Mytillus galloprovincialis*: 1 from the Romanian waters (RO-1), 3 from the Bulgarian waters (BG-3, BG-5, BG-7), and 1 from the Turkish waters (TR-7)**

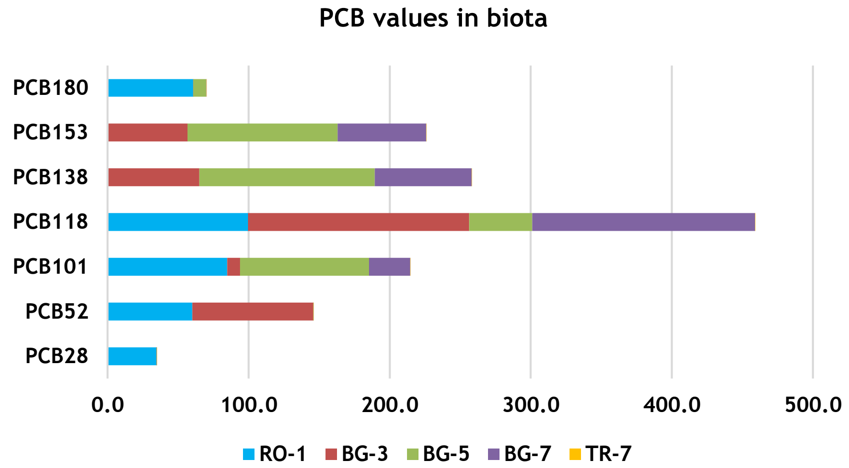


Figure 3.23 - Individual PCBs levels (ng/g wet weight tissue) in mollusks, October 2019

Conclusions

Generally, most of the PCBs concentrations in seawater have been measured below or close the detection limits especially in TR and RO stations. This is related with the current method's capability for water matrix. PCB contents of the surface sediments ranged from 0.19 to 367.05 ng/g with an average value of 36.34 ng/g, with highest total PCB values measured in RO surface sediments. Contents of PCB28, PCB52, PCB101 and PCB118 in the RO surface sediments and PCB28 in the BG surface sediments were measured above the Environmental Assessment Criteria (EAC) values. Higher PCB levels were measured in the biota samples (mollusks) collected from Bulgarian coastal area. The *Mytilus galloprovincialis* samples from Romanian and Bulgarian waters exceeded the regulated levels for PCBs.

3.7 Integrated assessment - all contaminants - CHASE tool

The HELCOM Chemical Status Assessment Tool (CHASE) (Andersen et al., 2016) integrates data on hazardous substances in water, sediments and biota as well as bio-effect indicators and is based on a substance- or bio-effect-specific calculation of a **'contamination ratio' (CR)** being the ratio between an observed concentration and a threshold value. **Values <1.0** indicate areas potentially 'unaffected', while **values >1.0** indicate areas potentially 'affected'. These ratios are combined within matrices, i.e. for water, sediment and biota and for biological effects. The overall assessment used a 'one out, all out principle' with regard to each matrix. The CHASE tool can in combination with temporal trend assessments of individual substances be advantageous for use in remedial action plans and, in particular, for the science-based evaluation of the status and for determining which specific substances are responsible for a status as potentially affected.

Assessments of the environmental health of marine environments with regard to hazardous substances have traditionally been carried out on a substance-by-substance basis, focusing on thresholds for toxic effects, background concentrations and temporal trends (OSPAR 2010; EEA 2011).In Europe, following recent EU legislation, member states are required to carry out integrated assessments of 'chemical status' (Water Framework Directive) and 'contamination status' (Marine Strategy Framework Directive).

In the framework of an integrated thematic assessment of hazardous substances in the Baltic Sea (HELCOM 2010) it was developed a tool for integrated assessment of chemical status. The rationale for this new tool was twofold. Firstly, the tool should enable comparison between areas with differences in monitoring activities. Secondly, the new tool would fall in line with the HELCOM approach to develop and use indicator-based assessment tools for assessing eutrophication, hazardous substances and biodiversity. The prototype tool was named the HELCOM Chemical Status Assessment Tool (CHASE). In the implementation of the MSFD, EU member states are required to assess 'good environmental status' of marine waters. For this purpose, CHASE was further developed, where substances are combined under four themes: (1) contaminants in water, (2) contaminants in sediments, (3) contaminants in biota and (4) biological effects of contaminants. CHASE tool provides a unique approach to data-driven integrated assessments.

The benefit of using integrative tools is that they give a larger picture of the assessed elements by using numerous indicators and allowing inclusion of different substances, matrices, species and analytical methods to a single assessment (Andersen et al., 2016). There are four elements in the CHASE tool—water, sediment, biota and biological effects. The elements 'water' and 'sediment' include concentrations in the environment which reflect short term and long-term pollution, respectively. The elements 'biota' and 'biological effects' show the levels accumulated in organisms. All four elements combined provide a broad picture of the status of environmental contamination. The four groups are first assessed separately, and the final status is defined as the lowest status of the four elements. Thus, this status is based on the 'one out, all out principle' (OO-AO), which was considered appropriate as the four elements represent different aspects of the contamination status. Moreover, the approach adopted gives equal weight to all the elements because contamination in any of the four groups is seen as potentially equally harmful to the ecosystem.

The integrated assessment provides a final status for an assessment unit (i.e., a spatial unit), placing it in one of five classes: bad, poor, moderate, good and high. The classifications of bad, poor and moderate status indicate an environmental state which is '**affected**' (i.e., affected by hazardous substances). The classifications of good and high status indicate an environmental state '**unaffected**' (i.e., unaffected by hazardous substances). Thus, this classification system is essentially binomial (unaffected vs. affected) and is distinguished by a threshold value. The other classes are based on defined deviations from the unaffected/affected boundary.

In CHASE, each indicator is assessed against a specific threshold level and the results of the indicators are then combined to obtain the status for each element. For each of the indicators (n) at an assessment unit, the **contamination ratio (CR)** of the measured concentration (C_m) to a relevant assessment criterion for good environmental status ($C_{Threshold}$) is calculated. Integration of the CRs of the indicators within an element could be done in different ways: (1) the arithmetic mean of indicator CR values, (2) the root mean square (RMS) of CR values, (3) a contamination score (CS) and (4) the pollution level index. The **contamination score (CS)** it is considered the most appropriate for CHASE tool, as this minimizes the problem of 'dilution' of high values when several substances from an area are analyzed (Andersen et al., 2016).

Generally, more reliable results are produced if data from both abiotic and biotic environment are incorporated and if indicator selection is more harmonized in the assessment areas. It was noticed that the number of elements in CHASE affected the assessment result. If an assessment unit had only few data from one matrix, it is more likely to end up with a positive status result. The CHASE assessment comprises two abiotic matrices (water and sediment), that represent contamination of habitats, and two biotic matrices (concentrations in biota and effects observed in biota), that provide a direct link to marine life (i.e. populations, communities, food web) (Andersen et al., 2016).

CHASE was applied for ANEMONE Joint Cruise contaminants data in order to assess the status across assessment units/ stations and to identify what hazardous substances poses the higher risk for not achieving good environmental status.

In order to make monitoring results more comparable within Black Sea region, a common set of contaminants (heavy metals, polycyclic aromatic hydrocarbons, organochlorinated pesticides, polychlorinated biphenyls) was determined in seawater, sediments and mussels samples. Overall, 18 seawater samples, 19 sediments and 6 mussels from Black Sea region were investigated for hazardous substances presence, allowing the status assessment of 22 stations/assessment units. For Ukraine: water and sediments from 5 stations, and 0 biota samples were included; Romania: 6 stations – water and sediments, 1 biota sample; Bulgaria: 3 biota samples and 1 sediment were analyzed; Turkey: 7 stations, water and sediments, 2 biota samples.

The CHASE assessment tool was tested in the Black Sea with Joint Cruise contaminants data set and the assessment results were produced, as overall scores related to assessment units (stations and regions), and matrix /water, sediments, biota related scores. Generally, results could be influenced by the number of samples and type of matrices investigated in the assessment units, number of indicators, and thresholds that were used (Figure 3.24).

There were evinced sub-regional differences in the status results (overall scores), with worse status predominating in the northwestern part of the Black Sea and better status in the southern part of the Black Sea (Figure 3.24).

Across the investigated stations, the CHASE overall test assessment (that were influenced by water scores being the worst) showed a range of status results from bad to good, the majority of them (50%) being in the bad state, followed by 45.45% in moderate state, whereas the remaining 4.54% were in good status) (Figure 3.25).

In comparison, CHASE scores for sediment matrix evinced a higher variability, most samples being in moderate state (73.68%), followed by 10.53% in good state and the same percentage in bad state, whereas remaining 5.26% were in poor state (Figure 3.26).

In order to enable back-tracking of the integrated result to the substance results, the CHASE tool shows the indicators behind the assessment results, and these can be used to identify sources of pollution or substances that potentially cause the greatest harm to environment (Andersen et al., 2016).

For the ANEMONE Joint Cruise contaminants data, the hazardous substances with the highest contamination ratio (CR>1) in seawater are ranked as follows: heptaclor (in all samples) (in this particular case, EQS is lower than limit of detection), benzo(a)perylene (in 39% of seawater samples), sum of cyclodiene pesticides (33%), heavy metals (Zn, Cu, Cd) (28%), PCB (PCB101, PCB118, PCB153) (28%), benzo(a)pyrene (28%), ppDDT (22%), PCB138 (22%), Pb (17%), HCB (17%), whereas anthracene, lindane, DDTtotal and PCB180 presented lower frequencies of occurence of CR>1 (in 11% of seawater samples). (Figure 3.27).

For the ANEMONE Joint Cruise contaminants data, the hazardous substances with the highest contamination ratio (CR>1) in sediments are ranked as follows: Ni (in 74% of sediments samples) (for Ni proposed EQS might be lower than natural background level for Black Sea), dieldrin (53%), Cu (37%), endrin (37%), As (32%), PCB101 (32%), ppDDE, ppDDD, PCB28, PCB52 and PCB118 (all with frequency of 26%), whereas Cr, benzo(a)perylene, HCB, lindane, Pb, Hg, fluorene, anthracene presented lower frequencies of occurence of CR>1 values (between 5-16% of sediment samples). (Figure 3.28).

Table 3.19 - CHASE Overall Score, and CHASE Score per matrix, contaminants data, 2019

Station	CHASE Overall Score	CHASE Water	CHASE Sediment	CHASE Biota
UA-1	5	5	3	
UA-2	5	5	2	
UA-3	5	5	2	
UA-4	5	5	3	
UA-5	5	5	3	
RO-1	5	5	4	4
RO-2	5	5	3	
RO-3	5	5	3	
RO-4	5	5	5	
RO-5	5	5	5	
RO-6	5	5	3	
BG-1	3		3	
BG-3	3			3
BG-5	2			2
BG-7	3			3
TR-1	3	3	3	
TR-2	3	3	3	
TR-3	3	3	3	
TR-4	3	3	3	
TR-5	3	3	3	2
TR-6	3	3	3	
TR-7	3	3	3	2

1-High
2-Good
3-Moderate
4-Poor
5-Bad

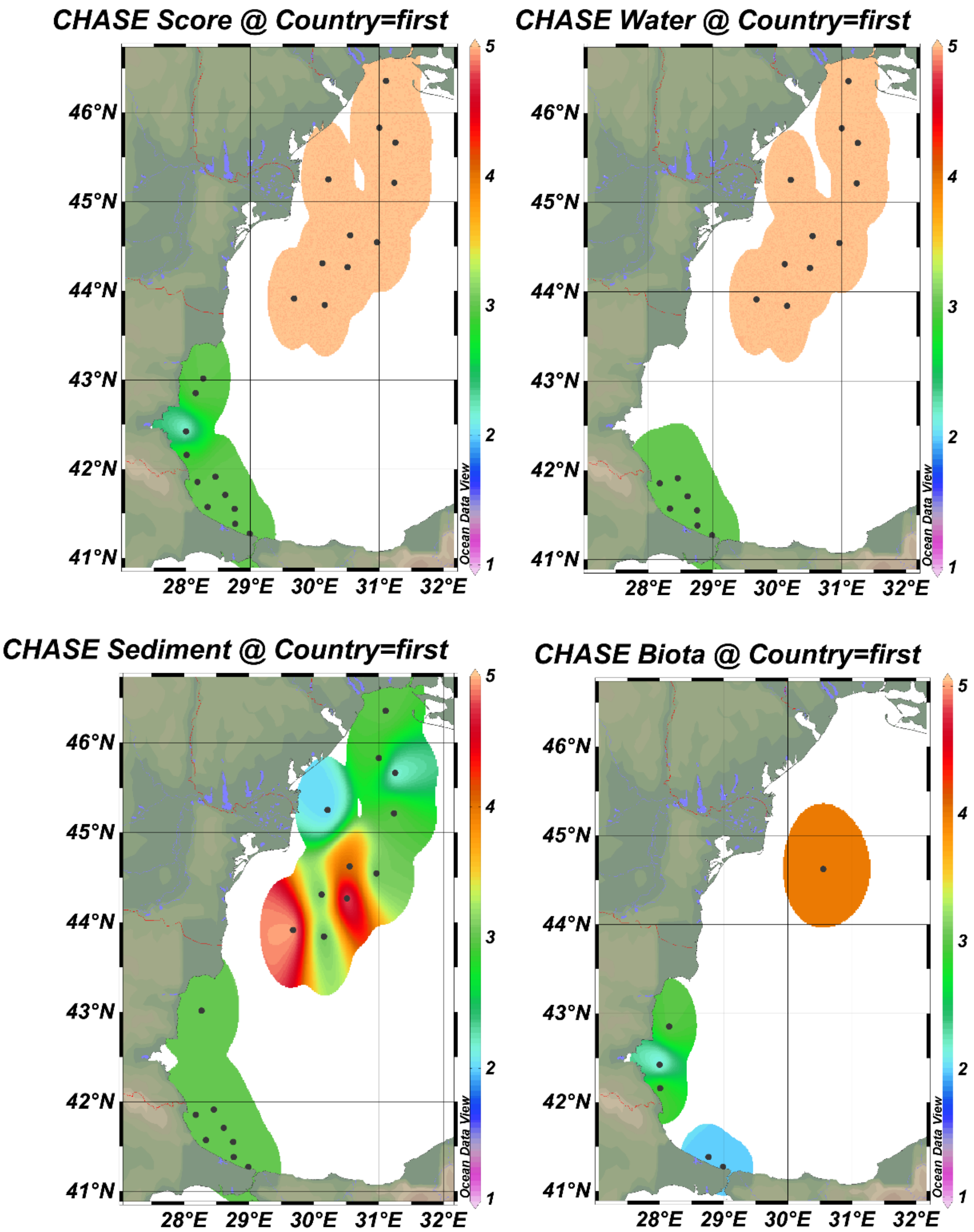


Figure 3.24 - CHASE Overall Score, and CHASE Score per matrix, contaminants data, 2019

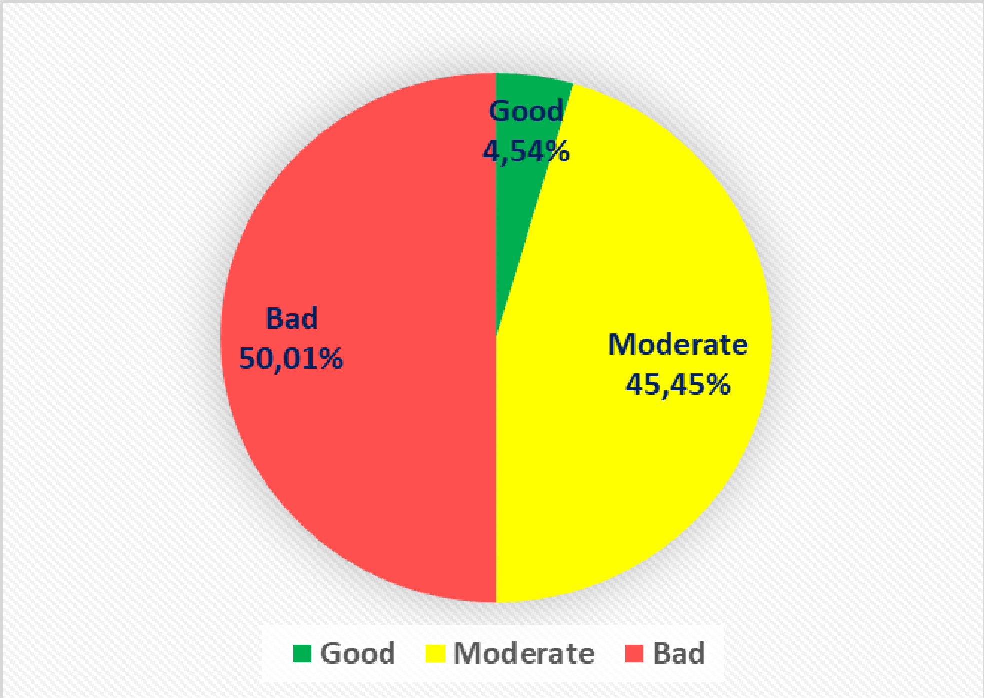


Figure 3.25 - Quality status of the assessment units/stations, based on CHASE Overall Score, contaminants data, 2019

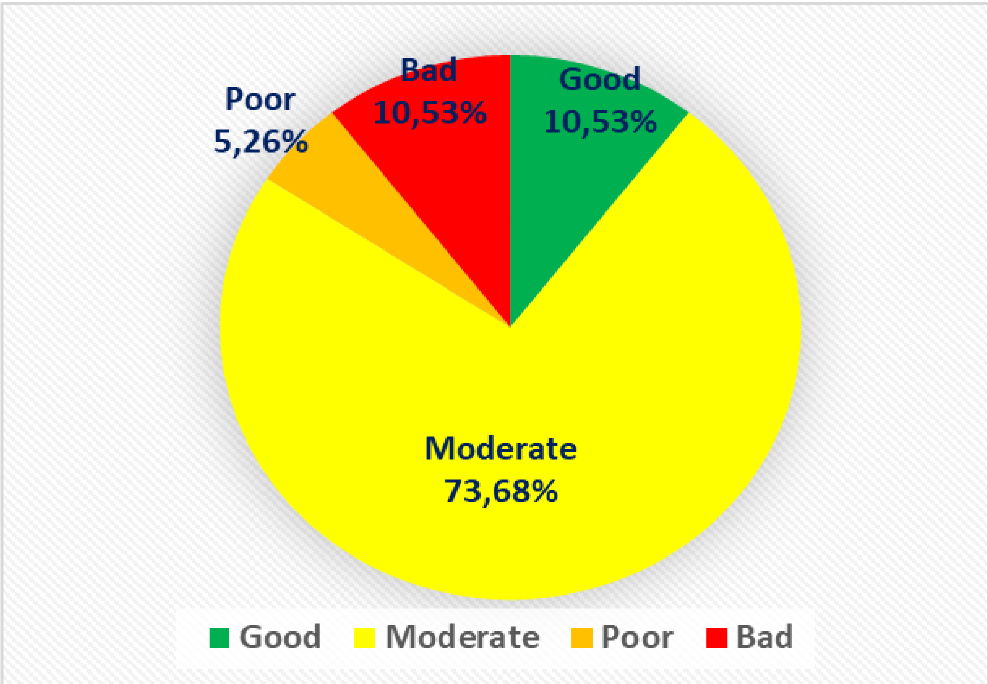


Figure 3.26 - Quality status of the assessment units/stations, based on CHASE Matrix Score/Sediments, contaminants data, 2019

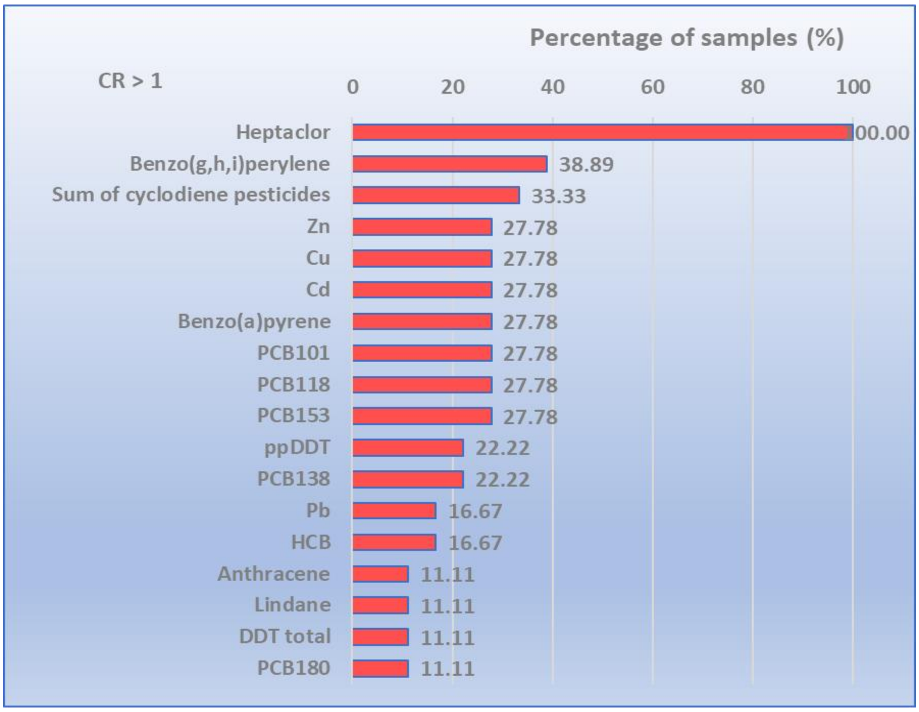


Figure 3.27 - Frequency of occurrence of hazardous substances with contamination ratio (CR) > 1 in seawater samples, 2019

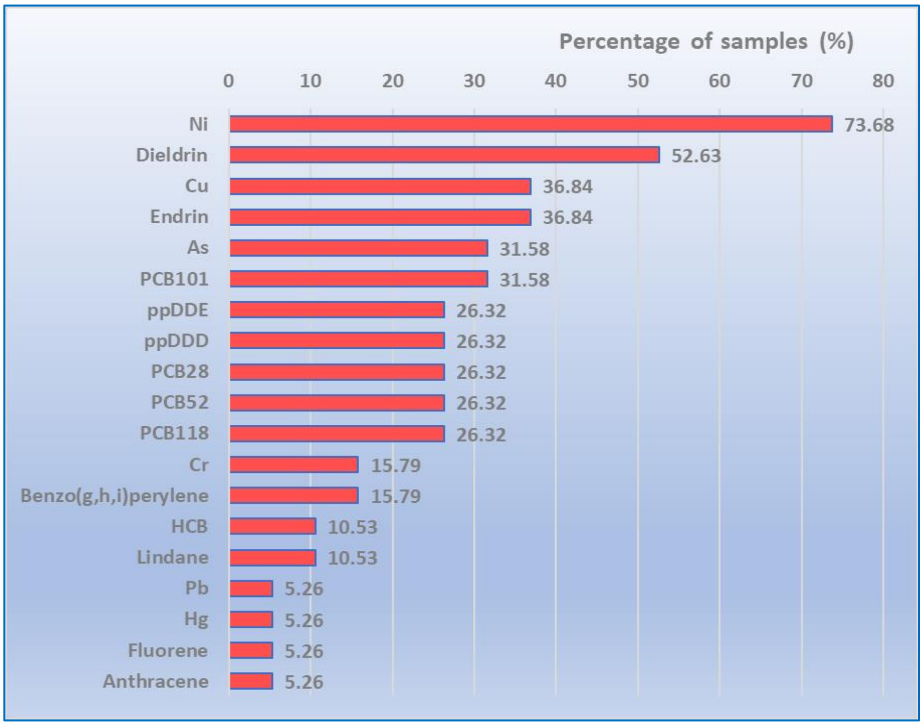


Figure 3.28 - Frequency of occurrence of hazardous substances with contamination ratio (CR) > 1 in sediment samples, 2019

3.8 Conclusions

New data on a wide range of contaminants in seawater, sediments and biota from the NW, W and SW Black Sea were obtained following ANEMONE Joint Cruise, 2019, thus contributing to further integrated assessments of the Black Sea state of environment.
CHASE was applied for ANEMONE JC contaminants data in order to assess the status across assessment units and to identify what hazardous substances poses the higher risk for not achieving good environmental status.
There were evinced sub-regional differences in the status results (overall scores), with worse status predominating in the northwestern part of the Black Sea and better status in the southern part of the Black Sea.
Across the investigated stations, the CHASE overall test assessment (that were influenced by water scores being the worst) showed a range of status results from bad to good, most of them (50%) being in the bad state, followed by 45.45% in moderate state, whereas the remaining 4.54% were in good status).
In comparison, CHASE scores for sediment matrix evinced a higher variability, most samples being in moderate state (73.68%), followed by 10.53% in good state and the same percentage in bad state, whereas remaining 5.26% were in poor state.
In order to enable back-tracking of the integrated result to the substance results, the CHASE tool shows the indicators behind the assessment results, and these can be used to identify substances that potentially cause the greatest harm to environment.
For the ANEMONE Joint Cruise contaminants data, the hazardous substances with the highest contamination ratio (CR>1) in seawater are ranked as follows: heptaclor (in all samples) (EQS is lower than limit of detection), benzo(a)perylene (in 39% of seawater samples), sum of cyclodiene pesticides (33%), TM (Zn, Cu, Cd) (28%), PCB (PCB101, PCB118, PCB153) (28%), benzo(a)pyrene (28%), ppDDT (22%), PCB138 (22%), Pb (17%), HCB (17%), whereas anthracene, lindane, DDTtotal and PCB180 presented lower frequencies of occurrence of CR>1 (in 11% of seawater samples).
For the ANEMONE Joint Cruise contaminants data, the hazardous substances with the highest contamination ratio (CR>1) in sediments are ranked as follows: Ni (in 74% of sediments samples) (proposed EQS might be lower than natural background level of Ni for Black Sea), dieldrin (53%), Cu (37%), endrin (37%), As (32%), PCB101 (32%), ppDDE, ppDDD, PCB28, PCB52 and PCB118 (all with frequency of 26%), whereas Cr, benzo(a)perylene, HCB, lindane, Pb, Hg, fluorene, anthracene presented lower frequencies of occurrence of CR>1 values (between 5-16% of sediment samples).
With respect to Marine Strategy Framework Directive, Descriptors 8 and 9, results from the ANEMONE Joint Cruise will promote further work toward common understanding of good environmental status and will contribute to the assessment of the Black Sea environmental state in a regionally harmonized approach.

3.9 Gaps and recommendations

The purpose of assessments under Descriptor 8 is to determine whether this aspect of GES is being achieved within assessment region. The approach involved (measurements of contaminant concentrations and effects, followed by comparisons against targets) needs additional research, for a better understanding of the underlying fundamental principles and for the further development of monitoring approaches (JRC, 2010). MSFD GES target setting implies understanding of the processes affecting contaminant cycling and availability, the responses of marine organisms to contaminants, the identification of sources and the availability of appropriate monitoring tools. Scientific knowledge of the functional relationships between pressures and impacts, and the consequent responses contains significant gaps. The implementation of measures to ensure the Good Environmental Status as described under Descriptor 8 requires a combination of several assessment tools which need to be developed.

Identified gaps in knowledge that need addressing in future marine research areas across European seas correspond to the following topics:

Understanding of the ecosystem responses to pollution

Research could contribute to a better understanding of the relationship between pressures, their effects and impacts on the marine environment. Hazardous substances, especially synthetic chemicals, occur in the environment as mixtures. The mixtures and their combined effect on organisms and the ecosystem are currently unknown, but this should be subject to ongoing work and research for understanding of causal relationships and of processes between contaminants and their effects on biota and to quantify the effect and impact of contaminants at the population level and higher levels of biological organization. Especially important is to assess the effects of complex mixtures of inorganic and organic pollutants upon organisms and ecosystems.
Levels of pollution effects on the ecosystem components, having regard to the selected biological processes and taxonomic groups where a cause/effect relationship is established should be monitored. Implementing of biological effects techniques used in environmental health assessment, like assays for specific inhibition of enzymes, induction of proteins, pollutant metabolites, DNA microarrays, immunotoxicity, physiological responses and pathology, is needed. Introducing an ecotoxicology monitoring program will allow integration of chemical and biological effects measurements. Combination of biological effects and chemical measurements will provide an improved assessment due to the ability to address effects that are potentially caused by a wide range of contaminants as well as those that are more clearly linked to specific compounds or groups of compounds (Vethaak et al., 2017).

Linking sources, pathways and environmental status: biogeochemistry of substances

Little is known on the relationship between the mechanisms of entry of pollutants (riverine, atmospheric, land-based and sea-based sources) into marine waters and their availability and potential effects on organisms and ecosystems. Research is need on long time series that relate pollutant exposure and cycling to effects to organisms and ecosystem functioning (HELCOM, 2010).
Data for better quantification of contaminants fluxes and inputs into marine environment and their sea/air and water/sediments interfaces exchanges is lacking. Monitoring programmes would need to be designed to allow tracing back chemicals from the environment via their pathways to the sources in order to allow the appropriate development of programmes of measures to achieve good environmental status and assess progress being made.
Monitoring programme should allow the combination of the data covering waterborne and atmospheric inputs, environmental concentrations, and biological effects of hazardous substances.

Climate change

Warming of the atmosphere in response to climate change may increase the tendency for atmospheric transport of certain substances, more rain and floods can result in higher run-off from land and increased storminess may lead to additional remobilization of contaminants from marine sediments. Change in sea water temperature and other possible biological impacts of climate change add to the stress on organisms and coupled with pollution effects may make marine organisms more vulnerable to chemical contamination (Morton, 2016). An improved understanding of these processes may lead to the need for a regular review of assessment criteria.

Knowledge on the marine food webs with regard to contaminants

The transfer of contaminants through the food chain needs to be better understood, and also the possibility of additive, synergistic and antagonistic effects. The toxic effects of chemical contaminants on marine organisms are dependent on bioavailability and persistence, the ability of organisms to accumulate and metabolize contaminants, their interference with specific metabolic or ecological processes. Little is known about contaminant uptake in the first trophic levels (plankton), and how different biogeochemical statuses of marine ecosystems favor the bioaccumulation and cycling of contaminants (Chouvelon et al., 2019).

Aggregation of information on substances

There is a multitude of chemicals (and effects of them) in the environment and methods for a sound aggregation of information from monitoring should be addressed in an integrated assessment framework for contaminants and biological effects.

4 Marine litter

"Properties and quantities of marine litter do not cause harm to the coastal and marine environment."

4.1 Overview of Marine litter at Black Sea basin level

In December 2019, Black Sea Commission prepared the "Guidelines on Monitoring of Marine Litter in the Black Sea Environment" as a support document for the Black Sea Marine Litter Action Plan. This document represents a vision how to build Marine Litter Monitoring Programme(s) for the Black Sea region. This document includes a very extensive and up to date analysis of marine litter monitoring efforts in the region and covers all riparian countries. In order to have a synchronized approach and to integrate the work done to gather all data, the part dedicated to the overview of the marine litter monitoring efforts is quoted below, in this sub-chapter.

Marine litter, either originating from the vessels or from the shores or rivers, is a persistent pollution problem along the coasts of the Black Sea, in the sea and on the bottom of the sea. Marine litter is also a transboundary problem in this enclosed sea basin which displays a very dynamic current system, enabling transportation of any matter from a given location in the basin to almost any coastal area. A great portion of the marine litter in this region is of a non-biodegradable nature, and is not an aesthetic problem simply, but might threaten the biodiversity of the basin. As indicated by Topçu et al (2013), the Black Sea is an enclosed sea surrounded by industrializing countries, an important maritime route, an intensive fishery area and tourism attraction (Simeonova et al., 2017), and has not received sufficient attention regarding coastal litter pollution. It is an almost totally enclosed sea whose unique connection with the Mediterranean Sea is the narrow passage of the Turkish Straits System.

In the report of "Marine litter in the Black Sea Region: A review of the problem" this issue was considered one of the most urgent and difficult environmental problems in the region (BSC, 2007). However, not all the neighboring countries hold effective management strategies and regulations despite all these protection measures and conventions (Vişne & Bat, 2015). In its report on global inputs of plastics by countries, Jambeck (2015) indicated that Black Sea countries were responsible for inputs up to respectively 67.3 tons every day, which means 24580 tons per year, just for land-based plastics (Table 4.1). As a consequence, the presence of marine litter in this basin has become a common situation, with plastic as the main component.

Table 4.1 - Plastic waste littered in the Black Sea[2]

Country	Coastal population*	Waste generation (kg/day)	Inadequately managed plastic waste (kg/day)	Mismanaged plastic waste in 2010	Mismanaged plastic waste in 2025	Plastic littered (kg/day) 2010
Bulgaria	1 002 695	1 283 450	48 273	18 739	25 770	3057
Georgia	1 124 249	1 899 981	38 149	14 472	24 532	1501
Romania	875 170	910 177	9 172	3 610	8 261	719
Russia	1 08 12 53**	1 055 659	197 22	80 75	128 94	2403
Turkey	7 740 493***	13 696 158	26 978	110 453	179 620	32733
Ukraine	6 812 799	5 382 111	338 841	128 765	233 388	13940
Total	10 896 166	10 531 378	481 135	284 105	484 466	67353

***Based upon a 50 km coastal buffer created in GIS with global population densities. ** estimated at 1/10 of the total Russian coastal population ***22.73% of the total coastal population of Turkey (PAP/RAC, 2005)**

The drainage basin of the Black Sea covers 2.1 million km^2 with more than 160 million people living in the region, embracing Austria, Hungary, former Yugoslavia, Moldavia and in a lesser extent southern Germany, Belarus and the Slovak republic when considering riverine inputs.

Three of the larger European rivers discharge into the Black Sea, including the Danube, Dnieper and Dniester. On top of the consequences, the basin is the receptacle for the industrial and municipal wastes of over one third of Europe, part of it untreated or partially treated. In addition to this, the

[2] Data were compiled from the Spreadsheet containing Data from 192 Countries, as published by Jambeck et al., 2015.

Black Sea has a very dynamic current system allowing cross-border transportation of waste materials (Topçu & Öztürk, 2010), which in turn makes this enclosed sea very vulnerable to marine litter. As a consequence of the circulation, coastal litter pollution in the Black Sea is a typical trans-boundary problem. The main sources are land-based, but in some areas, up to half of labeled stranded litter are seaborne debris, also due to international shipping (Topçu et al., 2013).

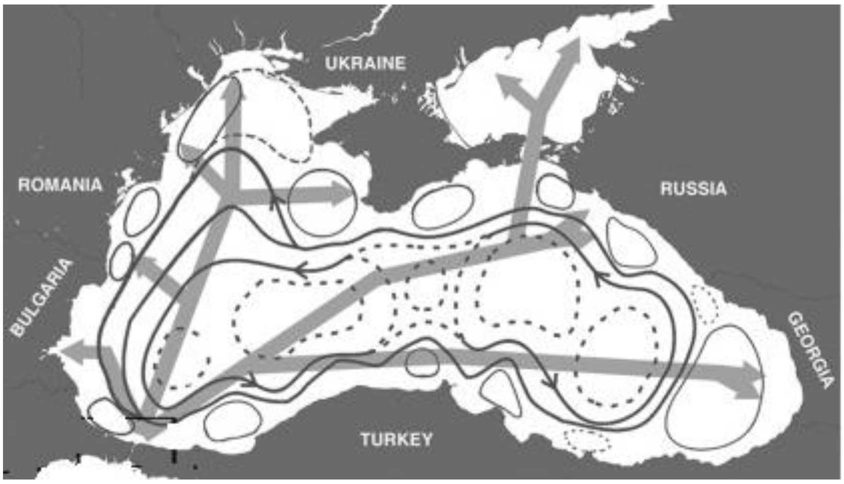


Figure 4.1 - Major currents of the upper layer circulation and main shipping routes (thick lines) in the Black Sea (after Topçu et al., 2013)

There is no extensive listing of hot spots of accumulation. As indicated by experts (BSC, 2013), litter accumulation was described in many places around the Black Sea: in coastal cities including seaside resort complexes, ports, navigation routes, industrial zones, wild beaches and estuaries of rivers. Cities with typical "hots spots" for marine litter were described off Bourgas, Varna, Batumi, Poti, Kobuleti, Constantza and Mangalia, Sochi, Tuapse, Samsun, Zonguldak and Giresun. Touristic areas around Bourgas, Varna and Sinop and river mouths and basins of the Danube, Dnieper, Bough, Chorokhi and the Yeşilırmak delta were also listed as accumulation areas. As a consequence, litter has become an issue, also from an economic and social point of view, changing the perception related to the cleanliness of the sites and beaches. In Bulgaria for example, two thirds of Bulgarians (66%) who participated in a discrete marine litter survey still considered the beach in question as somewhat clean (Brouwer et al., 2017) however cigarette butts, plastic bottles and bags were mentioned by beach users as an important concern.

4.1.1 Beach litter

Detailed data on marine litter is not common for the Black Sea, with only a few studies dedicated to this issue. To support implementation of monitoring, more data from northern part of the basin will be reported soon, as obtained within the Joint Black Sea Survey in May-June 2016 and the National Pilot Monitoring Surveys, coordinated within the EMBLAS project[3].

In the Report of the Black Sea Commission on Marine Litter in the Black Sea Region (2009), importance of plastic was clearly demonstrated by the data of random studies at the Black Sea coast and in coastal waters. In sporadic studies in Crimea, predominance of plastic ML (80–98% of the recorded pieces) has been determined in different coastal areas and seasons in comparison with glass ML (2–20%) represented mainly by broken and unbroken bottles. The density of pollution by plastic items (films, bags and bottles, etc.) varied on the beaches from 2698 to 55000 pieces/km^2, while the density of glass bottles ashore varied between 280 and 1455 pieces/km^2. The ML weighting indices varied from 333 to 6250 kg/km^2 (plastics) and from 222 to 1455 kg/km^2 (glass). The average overall values of ML quantity on the Crimean unorganized beaches were then estimated as 16348 ± 5076 pieces/km^2 for plastic objects (1910 ± 612 kg/km^2) and 674 ± 107 pieces/km^2 for glass objects (552 ± 96 kg/km^2). More results were published recently in other areas from the basin. Estimations from surveys on 10 beaches of the Turkish western Black Sea Coast (Topçu et al., 2013) indicated densities ranging from 0.085 to 5.058 items/m^2. Debris was mainly composed of unidentifiable small size (2–7 cm) plastic

[3] http://emblasproject.org

pieces and beverage-related litter such as bottles and bottle caps. Fishing related debris seemed to have a small share in stranded marine litter. About half of the labeled litter was of foreign origin, including 25 different countries, 23% of which are in the Black Sea region. The south-eastern Black Sea coast in Turkey was also evaluated more recently for marine litter composition and density covering nine beaches during four seasons along the southeastern Black Sea coasts (Terzi et al., 2017). The marine litter (> 2 cm in size), was collected from the coast and categorized into material and usage categories. The data analysis showed that plastic was the most abundant litter (≥ 61.65%) by count and weight followed by styrofoam and fabric. The marine litter density ranged from 0.03 to 0.58 with a mean (± SD) of 0.16 ± 0.02 items/m^2 by count. Based on weight, it varied between 0.44 g/m^2 and 14.74 g/m^2 with 3.35 ± 1.63 g/m^2. The east side had a higher marine litter density than the west side with significant differences between beaches. The variations due to different seasons were not significant for any beach. In the Turkish Cilician Basin of the Mediterranean, the average litter density on 13 beaches was 0.92 ± 0.36 items/m^2 (Aydn et al., 2016). Litter items resulting from convenience food consumption and smoking made up more than half of the total litter collected, while agricultural, industrial, fishing activities together contributed only 6% of the total number of items. Plastic items on average constituted more than 80% of the dominant material type. Percentages of the litter transported with currents from neighbouring countries (transboundary litter) varied from 0 – 4.23% between beaches. Direct deposition from land was identified as the main route for transport of items to the coastal environment.

In Romania, an extensive study on beaches between 2011 and 2014 (Galgani et al., 2016), indicated that most waste items localized and identified on land were represented by plastic packaging, paper, wood, glass etc., textile, medical waste, chemicals thrown by tourists on the beaches during the summer season and also abandoned in the sea by commercial and fishing vessels. Also, a considerable amount of waste is brought from the Danube River, especially in seasons with heavy rains.

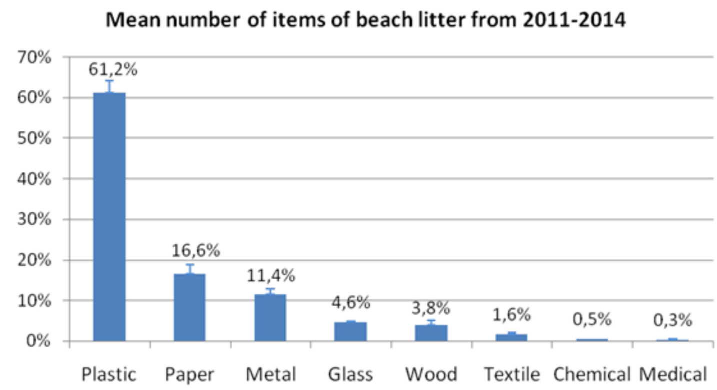


Figure 4.2 - Composition percentages (%) of identified waste along the Romanian Coast during the period 2011-2014

ONG Mare Nostrum performed beach litter monitoring since 1999 through different methodologies and programs. Since 2016 was applied a monitoring methodology according to the “Guidance on Monitoring of Marine Litter in European Seas” produced by the European Union Task Group on Marine Litter (TSG ML) and the UNEP MAP IMAP. Macrolitter of more than 2.5 cm, was surveyed on the beach area during 2 monitoring session each year (Spring and Autumn) for 8 sampling areas, each of 100m length.

As a main conclusion is was established that there is a significant difference between Spring and Autumn, especially in years with high density of tourists. The number of items surveyed doubles or even triples during summers with high number of tourists. There is also a high increase in small pieces of litter comparative with large items.

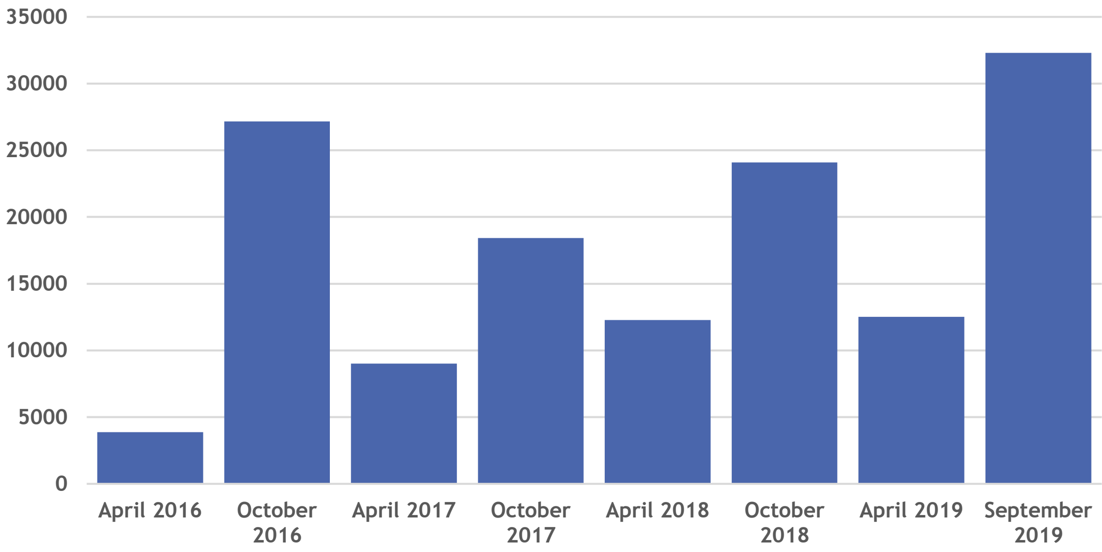


Figure 4.3 - Trends in number of litter items surveyed on 8 monitoring samples distributed from Vama Veche until Vadu, during 2016-2019

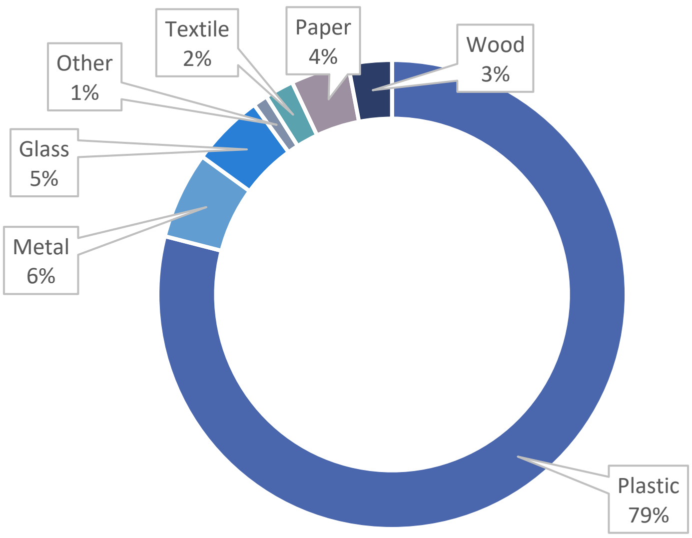


Figure 4.4 - Litter categories percentage distribution surveyed on 8 monitoring samples distributed from Vama Veche until Vadu, during 2016-2019

Different kind of plastic items have a high occurrence and the cigarette but is the most common litter in all the samples surveyed.
In a more detailed study using the Marine Litter App. developed by the European Environment Agency[4] and combining citizen participation to assess debris on beaches from Romania enabled to demonstrate that the main wastes identified were cigarette butts and items related to plastic containers (Figure 4.5).

[4] https://www.eea.europa.eu/themes/coast_sea/marine-litterwatch

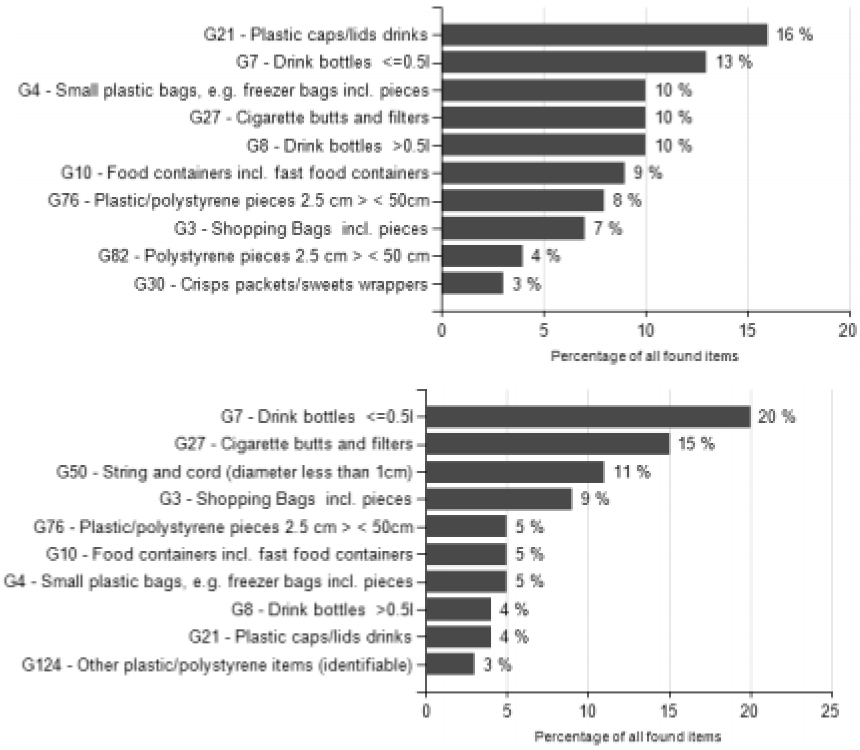


Figure 4.5 - Top 10 litter items (and G1 to N: corresponding TGML master list categories) on Mamaia (up, January 2015) and Vama veche (down, June 2015) beaches (Romania) (Golumbeanu et al., 2017)

This was confirmed by an evaluation of top ten items after cleaning operations (ocean coastal clean-up, 2017) where cigarette butts were the most abundant items found (42 %) followed by bottles caps (21.2) and plastic bottles (10.6).

In Bulgaria (Simeonova et al., 2017) marine litter surveys conducted in 8 beaches along the coastline using OSPAR (TG ML compatible) protocol exhibited predominance of artificial polymer materials - 84.3%. ML densities ranged from 0.0587±0.005 to 0.1343±0.008 n/m^2, highest on the urban beaches. The seasonal dynamics of most top 10 ML showed highest quantities in summer than other seasons, as the differences are of high statistical significance ($0.001 \leq P \leq 0.05$). Top 1 ML item for most of the beaches was cigarette butts and filters reaching 1008±10.58 nos. in summer and from 19±3.41 to 89±7.81 nos. during the rest of the seasons ($P<0.001$). For the pronounced seasonality contributed the recreational activities, increased tourist flow and the wild camping.

4.1.2 Floating Litter

The Black Sea is not exempt from the global invasion of floating debris; however, data are still lacking and a basin-wide survey is urgently needed to identify accumulation areas and develop regionally effective solutions to the problem. The occurrence of marine litter in the Black Sea region is poorly known and even less data has been reported on the abundance of floating debris. Results from a ship-based visual survey carried out in the northwestern part of the Black Sea (Suaria et al., 2016), provided the first preliminary data on the characteristics of floating debris in Romanian waters. High litter densities peaking to 135.9 items/km^2 were found in the study area (mean 30.9 ± 7.4 items/km^2). Probably due to the proximity of the Danube delta, natural debris were on average, much more abundant than anthropogenic litter in most surveyed locations (mean 141.4 ± 47.1 items/km^2, max 1131.3 items/km^2). Most of the 225 objects we sighted consisted of pieces of wood and other riparian debris (75.5%), however plastic items remained undoubtedly the most abundant type of litter, representing 89.1% of all sighted man-made items.

4.1.3 Sea Floor Litter

A study (Moncheva et al., 2016) described the results of a pilot assessment of bottom ML in the Black Sea during the MISIS Project Joint Black Sea Cruise (22–31 August 2013) along 3 transects in the NW Black Sea in front of Romania, Bulgaria and Turkey. The aim of the study was a pilot quantitative assessment of sea floor litter using both trawling and a Remotely Operated Vehicle (ROV). Marine debris densities ranged from 304 to 20 000 items/km^2 with a mean density of - 6359 items/km^2. The number of items decreased from north to south with maximum in front of the Romanian coast, considerably in front of Bulgaria (9598 items/km^2) and Turkey (7956 items/km^2). In coastal areas (<40m depth), the abundance of ML was generally much higher than on the continental shelf, except in Bulgaria. In all samples, fishing and tourism related activities obviously contributed significantly to littering of the seafloor. By material the most frequent and abundant debris were plastics, constituting ~ 68 %. The nature of the ML suggested mainly shipping/fishing.
NIMRD conducted research surveys between 2011 and 2014 (Galgani et al., 2016) with sampling trawl (bottom trawl) for demersal fish stock assessment, enabling the collection of litter on the seabed during operation for analysis of litter density, composition, and sources. A 21/22-34 m bottom trawl was employed in southern, central and northern part of Romania), at depths ranging between 15 - 90 m. During the monitoring period 2011-2014, a total number of 168659 items of various types have been identified and inventoried. In 2012, most of hardly biodegradable materials (plastic) were found in the close vicinity of the Constanța and Mangalia harbors [approx. 96.61%, 259.39 kg) of the total amount of such wastes collected from the seabed (268.48 kg)], where vessel traffic is also the busiest. In 2014, 27 hauls (1625 km^2) were operated, collecting 329.18 kg (420 items) of waste, with plastic representing 27% of weight (48 % by number). Hauls duration was 60 min, trawl speed was at 2.5 kts and horizontal opening of the tool was 13 m, in which case the surface covered during research hauls was 60.190 m^2 (0.06019 km^2). For the period, the largest amounts of metal and plastic were located in the areas around the ports of Constanta, Cape Midia and Mangalia where is recorded a heavy naval traffic. Nearly in the majority of hauls were identified plastic items (bags, bottles, bags, buckets, cans, linoleum, etc.). Also, a considerable contribution in bringing into the sea a large quantity of plastic waste has the Danube River through its three discharge mouths (Chilia, Sulina, Sf. Gheorghe). Many fragments were from fishing gear (seines, trawl, purse, etc) lost or abandoned, also from other countries because of illegal fishing. Litter, including wood were brought from the three arms of the Danube and carried by the currents along the shoreline in both offshore and shallow waters areas.

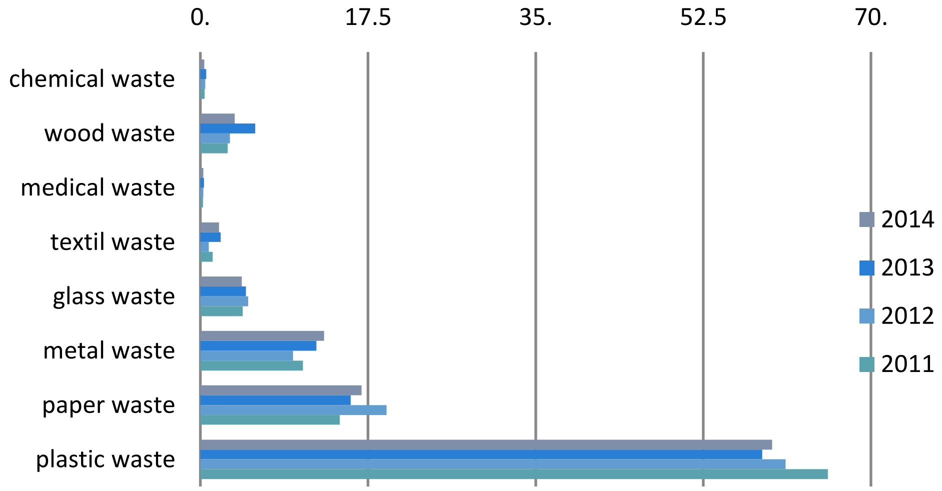


Figure 4.6 - Typology (%) of sea floor litter along the Romanian Coast during the period 2011-2014 (Galgani et al., 2016).

Abundance, spatial distribution and qualitative composition of benthic marine litter have been also investigated in a study area of Constanta Bay (Ioakemidis et al., 2014) after 16 trawling tows (9 m width, 20 mm mesh) and 76 km sampling. Plastic accounted for 45.2 ± 4.8 % of total litter (14.3 and 12.9 for bottles and bags) and 22% of debris were of metal. in Constanta Bay the highest density (1068 items/km^2) were recorded in front of the Danube mouth, whereas no consistent distribution pattern in relation to depth was observed on the shelf (<60 m), except for metals that seems to accumulate in the deeper parts of the surveyed areas. Overall, the results highlighted the importance

of the Danube River and fishing activities in the area but also the presence of significant amount of small sized items showing the importance of marine litter fragmentation.
Also, the activities have continued until now; in 2019 at the Romanian Black Sea coast, during the assessment activities for demersal fish populations also was carried out the collection of litter on the seabed, at depths between 14 and 64 m.
Two expeditions were carried out with the bottom trawl of 10 days each, from all 81 trawls, only in 48 trawling operations it was identified marine litter.
During bottom trawling operations, the area covered by the 48 trawls was 2133135 m^2 and the total amount of waste collected was 883 kg (of which 74% were metal objects or in pieces) or 549 copies (of which 26% represented metal objects); with an average amount per square meter of approximately 0.41 g/m^2 and 0.0003 copies/m^2 of waste.
Analyzing the categories, the waste was represented by metal objects, plastics, lost or abandoned fishing nets, bottles, and textile fabrics. The percentage situation of this waste from the total quantity by categories in kg and number of pieces is presented below:

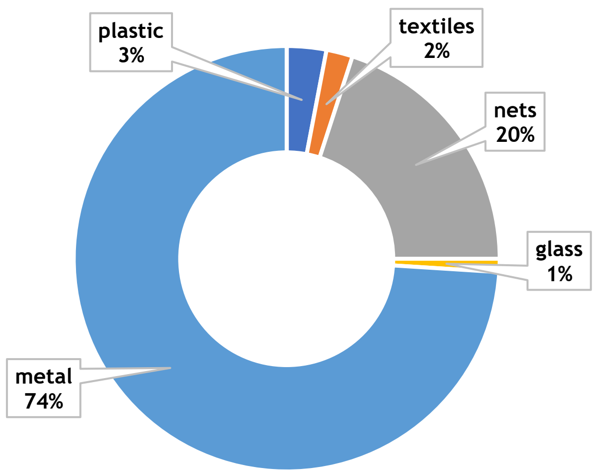


Figure 4.7 - Typology (%) of sea floor litter along the Romanian Coast in 2019 (Source NIMRD)

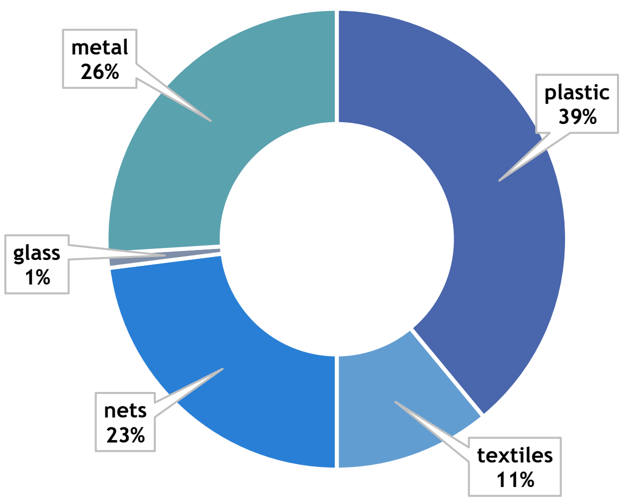


Figure 4.8 - Number of items (%) of sea floor litter along the Romanian Coast in 2019 (Source NIMRD)

Compared to previous years, except for metal, the other categories showed low values (g/m^2) with low oscillations from one year to another.

4.1.4 Microplastics

A two-year survey (2010, 2012) using stationary driftnets detected mean plastic abundance (n = 17349; mean ± S.D: 316.8 ± 4664.6 items per 1000 m^{-3}) and mass (4.8 ± 24.2 g per 1000 m^{-3}) in the Danube River (Lechner et al., 2014). Industrial raw material (pellets, flakes and spherules) accounted for substantial parts (79.4%) of the plastic debris. The plastic input via this main river into the Black Sea was then estimated at 4.2 t per day.
At sea, this is only recently (Aytan et al., 2016) that for the first time, the occurrence and distribution of microplastics has been reported for the Black Sea. Microplastics were assessed from zooplankton samples taken during two cruises along the southeastern coast of the Black Sea in the November of 2014 and February of 2015. In each cruise neuston sample were collected at 12 stations using 200 µm mesh. Microplastics (0.2–5 mm) were found in 92% of the samples. The primary shapes were fibers (49.4%) followed by plastic films (30.6%) and fragments (20%), and no micro beads were found. Average microplastic concentration in November ($1.2 \pm 1.1 \times 10^3$ par. m^{-3}) was higher than in February ($0.6 \pm 0.55 \times 10^3$ par.m^{-3}), possibly caused by increased mixing. The highest concentrations of microplastics were observed in offshore stations during November sampling. The heterogeneous spatial distribution (0.2×10^3–3.3×10^3 par. m^{-3} for all samples) and accumulation in some stations could be associated to transport and retention mechanisms linked with wind and the dynamics of the rim current, as well by different sources of plastic. There were no statistically significant differences in MP concentration between sampling stations and sampling periods. Overall, the results indicated that Black Sea is a hotspot for microplastic. This first study also provides a good scientific and technical background for future monitoring.
There is no regular monitoring of marine litter but some pilot's scales studies, providing a good scientific and technical background for implementing the BSIMAP. Most protocols used for beach, floating, sea floor, microplastics and ingested litter are UNEP / RSCs and TGML compatible and may rely on some existing infrastructures and initiatives such as cleaning operation, regular fish stocks assessments for both benthic and pelagic fishes, and stranding networks. Most of the approaches have been tested locally, also including the use of Smartphone approaches for beach litter or ROVs for sea floor litter. Capacity building, harmonization, common protocols, common data management, and quality insurance will be the next step to support the implementation of monitoring.

4.2 Marine Litter assessment at MSFD descriptor level

The Marine Strategy Framework Directive (MSFD), adopted in June 2008, commits Member States to adopt an ecosystem approach to manage the marine environment. By this directive, member states aim to achieve good environmental status (GES), described by 11 descriptors of its marine waters by 2020.
The MSFD applies to the marine area over which a Member State exercises jurisdictional rights in accordance with the United Nations Convention on the Law of the Sea (UNCLOS).
Monitoring, assessing its environmental status and managing coastal area through the implementation of knowledge on ecosystems biodiversity, functions and services are crucial actions to ensure the long-term sustainability.
According to the COM DEC 848/2017 laying down criteria and methodological standards on good environmental status of marine waters and specifications and standardized methods for monitoring and assessment, and repealing Decision 2010/477/EU, for Descriptor 10 – Marine litter the following criteria and methodological standards where identified.
Properties and quantities of marine litter do not cause harm to the coastal and marine environment.

Relevant pressure: Input of litter

Table 4.2 - Criteria, including criteria elements, and methodological standards according to MSFD

Criteria elements	Criteria	Methodological standards
Litter (excluding micro-litter), classified in the following categories (13): artificial polymer materials, rubber, cloth/textile, paper/cardboard, processed/worked wood, metal, glass/ceramics, chemicals, undefined, and food waste. Member States may define further sub-categories.	D10C1 — Primary: The composition, amount and spatial distribution of litter on the coastline, in the surface layer of the water column, and on the seabed, are at levels that do not cause harm to the coastal and marine environment. Member States shall establish threshold values for these levels through cooperation at Union level, taking into account regional or subregional specificities.	Scale of assessment: Subdivisions of the region or subregion divided where needed by national boundaries. Use of criteria: The extent to which good environmental status has been achieved shall be expressed for each criterion separately for each area assessed as follows: (a) the outcomes for each criterion (amount of litter or micro-litter per category) and its distribution per matrix used under D10C1 and D10C2 and whether the threshold values set have been achieved. (b) the outcomes for D10C3 (amount of litter and micro-litter per category per species) and whether the threshold values set have been achieved. The use of criteria D10C1, D10C2 and D10C3 in the overall assessment of good environmental status for Descriptor 10 shall be agreed at Union level. The outcomes of criterion D10C3 shall also contribute to assessments under Descriptor 1, where appropriate.
Micro-litter (particles < 5mm), classified in the categories 'artificial polymer materials' and 'other'.	D10C2 — Primary: The composition, amount and spatial distribution of micro-litter on the coastline, in the surface layer of the water column, and in seabed sediment, are at levels that do not cause harm to the coastal and marine environment. Member States shall establish threshold values for these levels through cooperation at Union level, taking into account regional or subregional specificities.	
Litter and micro-litter classified in the categories 'artificial polymer materials' and 'other', assessed in any species from the following groups: birds, mammals, reptiles, fish or invertebrates. Member States shall establish that list of species to be assessed through regional or subregional cooperation.	D10C3 — Secondary: The amount of litter and micro-litter ingested by marine animals is at a level that does not adversely affect the health of the species concerned. Member States shall establish threshold values for these levels through regional or subregional cooperation.	
Species of birds, mammals, reptiles, fish or invertebrates which are at risk from litter. Member States shall establish that list of species to be assessed through regional or subregional cooperation.	D10C4 — Secondary: The number of individuals of each species which are adversely affected due to litter, such as by entanglement, other types of injury or mortality, or health effects. Member States shall establish threshold values for the adverse effects of litter, through regional or subregional cooperation.	Scale of assessment: As used for assessment of the species group under Descriptor 1. Use of criteria: The extent to which good environmental status has been achieved shall be expressed for each area assessed as follows: — for each species assessed under criterion D10C4, an estimate of the number of individuals in the assessment area that have been adversely affected. The use of criterion D10C4 in the overall assessment of good environmental status for Descriptor 10 shall be agreed at Union level. The outcomes of this criterion shall also contribute to assessments under Descriptor 1, where appropriate.

Specifications and standardized methods for monitoring and assessment

1. For D10C1: litter shall be monitored on the coastline and may additionally be monitored in the surface layer of the water column and on the seabed. Information on the source and pathway of the litter shall be collected, where feasible.
2. For D10C2: micro-litter shall be monitored in the surface layer of the water column and in the seabed sediment and may additionally be monitored on the coastline. Micro-litter shall be monitored in a manner that can be related to point-sources for inputs (such as harbours, marinas, waste-water treatment plants, storm-water effluents), where feasible.
3. For D10C3 and D10C4: the monitoring may be based on incidental occurrences (e.g. stranding of dead animals, entangled animals in breeding colonies, affected individuals per survey).

Units of measurement for the criteria:

- D10C1: amount of litter per category in number of items:
    - per 100 meters (m) on the coastline,
    - per square kilometer (km^2) for surface layer of the water column and for seabed,
- D10C2: amount of micro-litter per category in number of items and weight in grams (g):
    - per square meter (m2) for surface layer of the water column,
    - per kilogram (dry weight) (kg) of sediment for the coastline and for seabed,
- D10C3: amount of litter/micro-litter in grams (g) and number of items per individual for each species in relation to size (weight or length, as appropriate) of the individual sampled,
- D10C4: number of individuals affected (lethal; sub-lethal) per species.

4.2.2 Floating litter

The floating macro debris were recorded from the bow of the vessel by two observers looking at each side of the ship, approximately 9 m above sea level, within a 50 m observation strip on each side (i.e. a fixed-width strip transect of 100 m). The identification and categorization of items was restricted to Level 1 materials from the MSFD master list (EU MSFD TG10 "Guidance on Monitoring of Marine Litter in European Seas 2013)). All visual observations were carried out under both low wind speed conditions (< 3 Beaufort) and high winds speed conditions (4 and 5 Beaufort), using both eye and binoculars (7x50 WPC-CF Fujinon) for identification of debris. Tracks and coordinates were recorded, using the GPS navigator Garmin eTrex 30. The vessel speed was between 8-10 kts (14.81 – 18.52 km/h).
Two observers acted both as observers and data recorder, using voice recorders and individual sheets in order to reduce the gaps in observation period. The observers were on effort during the transit between the fixed stations of the mission and went off-effort when the ships arrived in the next station. The brakes in the station usually were for at least 2 hours which provided plenty of time for the observers to rest, during the work of the other scientist in the station.
Litter items were identified according to litter type and size. Three size classes were recorded (10-50 cm; 50-100 cm; > 100 cm). Classification of the floating debris items was done in 8 categories according to the type of the material (artificial polymer materials; rubber; cloth/textile; paper/cardboard; processed wood; metal; glass/ceramics and unidentified). The total surface of the surveyed area was estimated by multiplying the transect distance by the observation width. The litter density (items/km^2) was calculated by dividing the items count with the surveyed area surface. No specific methodology (Buckland et al., 1993) or correction factors (Ryan, 2013) regarding the effective strip width were applied.
During the ANEMONE Joint Cruise, a total of 19 transects were performed. A distance of 419.75 km was covered corresponding to 26 hours and 22 minutes of visual observations (Figure 4.9). Detailed information regarding the survey area and number of transect per country is presented on Table 3.

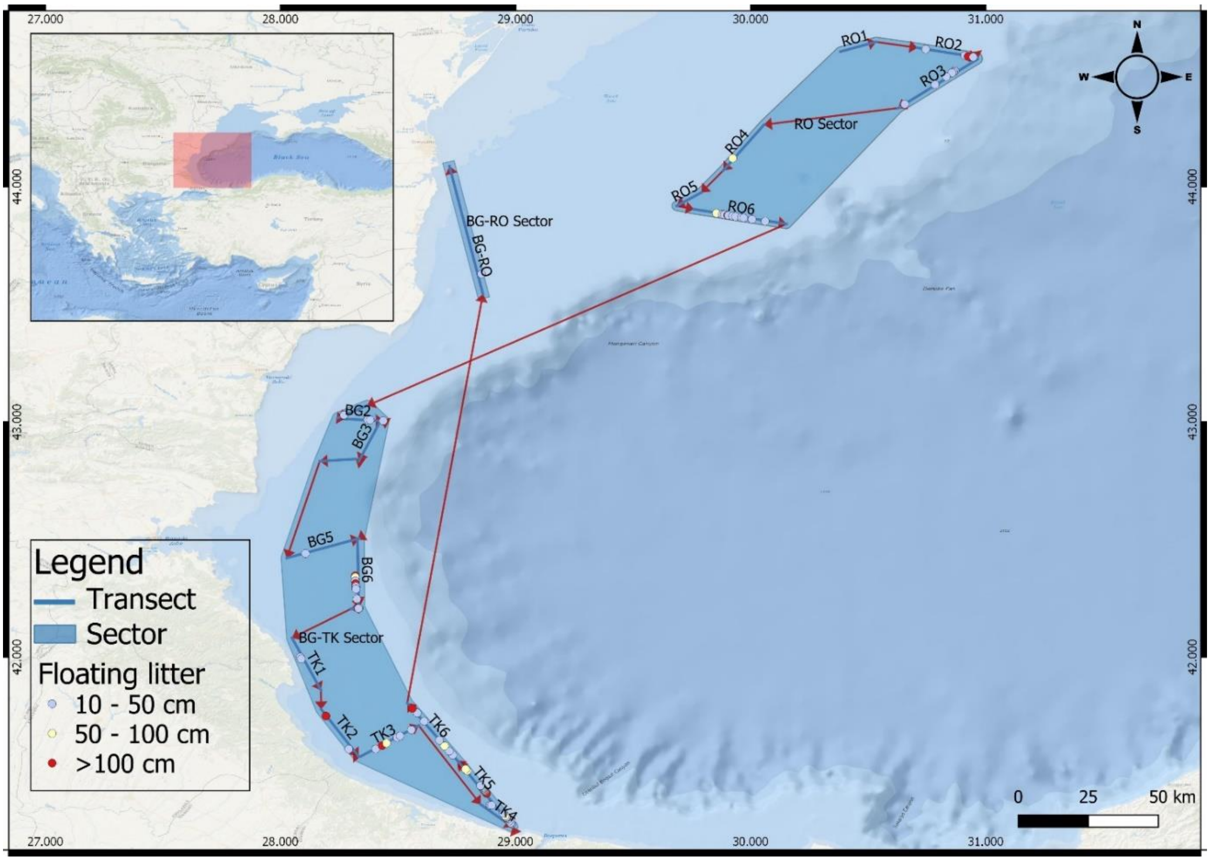


Figure 4.9 - Positions of the observational transects. In red, the direction of the vessel and passage sections

Table 4.3 - Summary of floating litter survey

Country	Number of transects	Area covered (km^2)
Bulgaria	7	11.68
Romania	6	13.79
Turkey	6	16.2

A total 79 marine litter items were identified, out of which 14 were found floating in Bulgarian waters, 21 and 44 respectively in Romanian and Turkish waters. Four litter-free transects were recorded (two in Bulgarian and two in Romanian waters). The number of debris per transect varied between 1 and 24, reaching 5.26± 5.93 items on average. Fifty three percent of the transects contained less then 5 items, and only 13% comprised more than 10 item.

Based on these results, the average density of floating macro-litter in Bulgarian waters was found 2.43 ± 2.4 items/km^2, 1.73 ± 1.24 items/km^2 in the Romanian waters and 2.43±2.17 items/km^2 in Turkish waters. The special distribution of litter abundances in the Black Sea is presented in Figure 4.10.

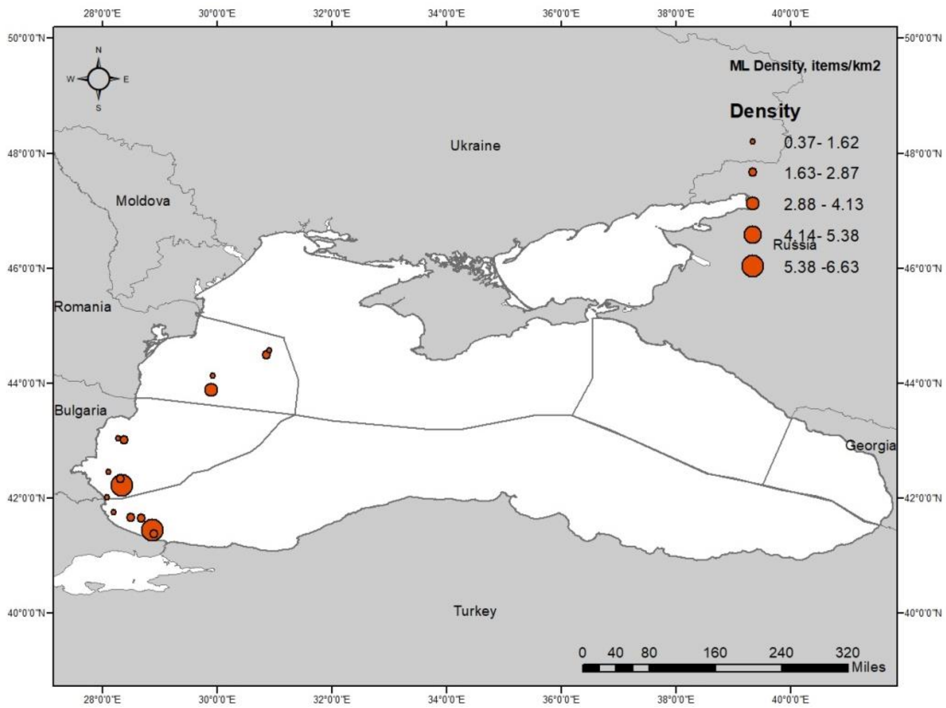


Figure 4.10 - Spatial distribution of floating litter densities for the 15 transects

The highest abundances were recorded in Turkish coastal waters (6.63 items/km^2), followed by the Bulgarian shelf waters (6.45 items/km^2).

All items identified during the cruise were classified in 4 out of the 8 floating litter Level 1 categories as described in the MSFD TG10 guidance document (Galgani et al., 2013). Plastic items were dominant (94.84% of total items), followed by processed wood (3.38%) materials. The items from categories glass/ceramics and cloth/textile contributed less than 1% of the total floating litter identified during the cruise (Figure 4.10).

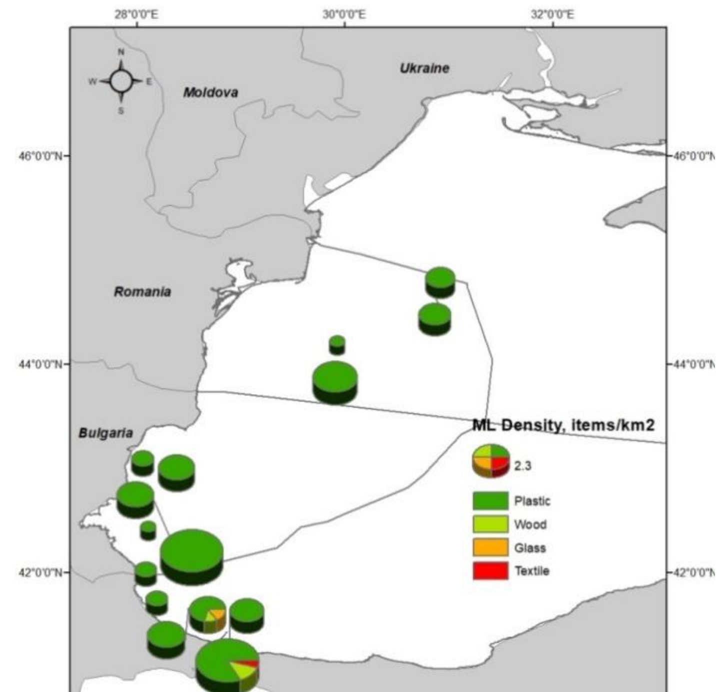


Figure 4.11 - Spatial distribution of floating litter densities per categories

Figure 4.12presents the percentage contribution of the 3 size classes. About 79% of surveyed litter items correspond to small-sized items ranging between 10 cm and 50 cm. Debris larger than 100 cm contribute only 8% of the total floating litter identified during the cruise.

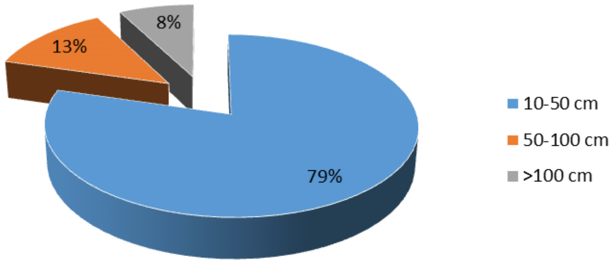


Figure 4.12 - Percentage contribution of the 3 size classes of floating litter

4.2.3Bottom litter

From all the methods assessed, trawling (otter trawl) has been shown to be the most suitable for large scale evaluation and monitoring (Goldberg, 1995, Galgani et al., 1995, 1996, 2000). Nevertheless, there are some restrictions in rocky areas and in soft sediments, as the method may be restricted and/or underestimate the quantities present.
The occurrence of international bottom trawls surveys such as IBTS (Atlantic), BITS (Baltic) and MEDITS (Mediterranean/Black Sea) provide useful and valuable means for monitoring marine litter.
For the Mediterranean Region, the protocol is derived from the MEDITS protocol (see the protocol manual, Bertan et al., 2007). The protocol is also a reference protocol for associated countries, including Romania and Bulgaria in the Black Sea. The hauls are positioned following a depth stratified sampling scheme with random drawing of the positions within each stratum. The number of positions in each stratum is proportional to the surface of these strata and the hauls are made in the same position from year to year. The following depths (10 – 50; 50 – 100; 100 – 200; 200 – 500; 500 - 800 m) are fixed in all areas as strata limits. The total number of hauls for the Mediterranean Sea is 1385; covering the shelves and slopes from 11 countries in the Mediterranean. The haul duration is fixed at 30 minutes on depths less than 200m and at 60 minutes at depths over 200m (defined as the moment when the vertical net opening and door spread are stable), using the same GOC 73 trawl with 20 mm mesh nets (Bertran et al, 2007) and sampling between May and July, at 3 knots between 20 and 800 m depth.
Procedure to collect litter data: On board the vessel, the litter collected is weighted as total and split into the categories and sub-categories as reported in the list below. It is mandatory to record or estimate total weight, regardless the categories and subcategories, and number of items for each main category: It is facultative to register weight by categories and number of items by sub-category. In case of large amount of litter in the catch, all big sized objects of litter must be recorded while a subsample could be analyzed for small sized litter (e.g. lids). Litter should be coded as total, by category and sub-category. Detailed data on total weight and litter composition must be reported in the specific form on litter.
Qualitative and quantitative data on the litter have to be connected to data regarding the characteristics of the haul (Date, code of haul, the GPS positions of the haul (start and end), trawled distance, average speed, characteristics of the haul (horizontal opening), depth of haul etc.), contained in file TA.
Data related to the fishing set and gear performance allows calculating the sampled surfaces for each haul and estimating a standardized index of total and by categories litter abundance per square kilometer.
There been made 3 hauls in Romanian waters, 3 in Bulgarian waters and 2 in Turkish waters at depths between 48 and 77 m deep and were highlighted different types of items.

Table 4.4 - Summary of bottom litter survey, 2019

Country	Depth (m)	Weight of litter (items/haul)
RO	69 - 77	0.2 – 1
BG	48 - 60	0.05 – 1.5
TR	70 -77	0.1 – 0.4

5 Nested Environmental status Assessment Tool (NEAT)

The holistic Nested Environmental status Assessment Tool (NEAT) was developed for supporting an integrated large-scale assessment of the ecological status of marine waters. Previous studies were dedicated to the assessment of the ecological status under Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive) (MSFD) criteria, but without an integrated approach for all descriptors. NEAT uses this ecosystem-based approach, excluding the "one-out, all-out" (OOAO) very restrictive principle. For the Joint Cruise assessment, we tested NEAT version 1.4.

NEAT evaluates the ecological status based on five classes adopted from the assessment scheme of the Directive 2000/60/EC of the European Parliament and the Council, establishing a framework for the Community action in the field of water policy Water Framework Directive (WFD). So, it should be noted from the outset that NEAT does not use two classes as established in MSFD. Each class is assigned to a specific colour in accordance with the ecological status and to WFD principles: High (blue); Good (green); Moderate (yellow); Poor (orange); Bad (red) (Marin et al., 2020).

In order to properly evaluate the ecological status of area submitted to evaluation during the Joint Cruise based on NEAT principles, some predefined steps were followed:

Firstly, **the assessment period was established**. For the current evaluation, NEAT was applied on Joint Cruise ANEMONE data from summer 2019.

The next step was to **define the Spatial Assessment Units (SAUs).** For Joint Cruise ANEMONE, various SAUs were considered within each country (Figure 5.1).

For evaluation purpose, the total area (expressed in square kilometers) was established for each SAU (Table 5.1).

Then, the identified habitats in each SAU were pre-defined as Pelagic habitats and Benthic habitats. For a more concise evaluation, for benthic habitats, four broad habitat types were identified, according to EUNIS marine habitat classification 2019: Circalittoral mud, Circalittoral mixed sediment, Offshore circalittoral mixed sediment and Offshore circalittoral mud.

The fifth step is considered to be the establishment of the ecosystem components submitted for evaluation.

For the current evaluation, the following elements were considered: biological components (benthic macroinvertebrates, phytoplankton, zooplankton, mesozooplankton), chemical data (nutrients, contaminants in water, sediments, and biota).

Choosing the descriptors with appropriate available data.

The current evaluation was performed under five out of the total eleven descriptors of MSFD: D1 (Biodiversity, including benthic habitats), D2 (Non-indigenous species/current evaluation performed only for non-native zooplankton species), D5 (Eutrophication), D8 (Contaminants), D9 (Contaminants in biota).

Establishing the appropriate ecological indicators.

For the Romanian Black Sea waters, the current evaluation was based on more than 60 indicators aggregated into the evaluation in a comparable and systematic way. Around 40 indicators were considered enough for adequate assessment of the ecological status (Borja et al., 2019). The indicators are considered the basis of the assessment; therefore, the establishment of threshold values is very important, the evaluation being exclusively based on these values. Each indicator is associated with an ecosystem component, so these indicators must be representative for the analyzed ecosystem components.

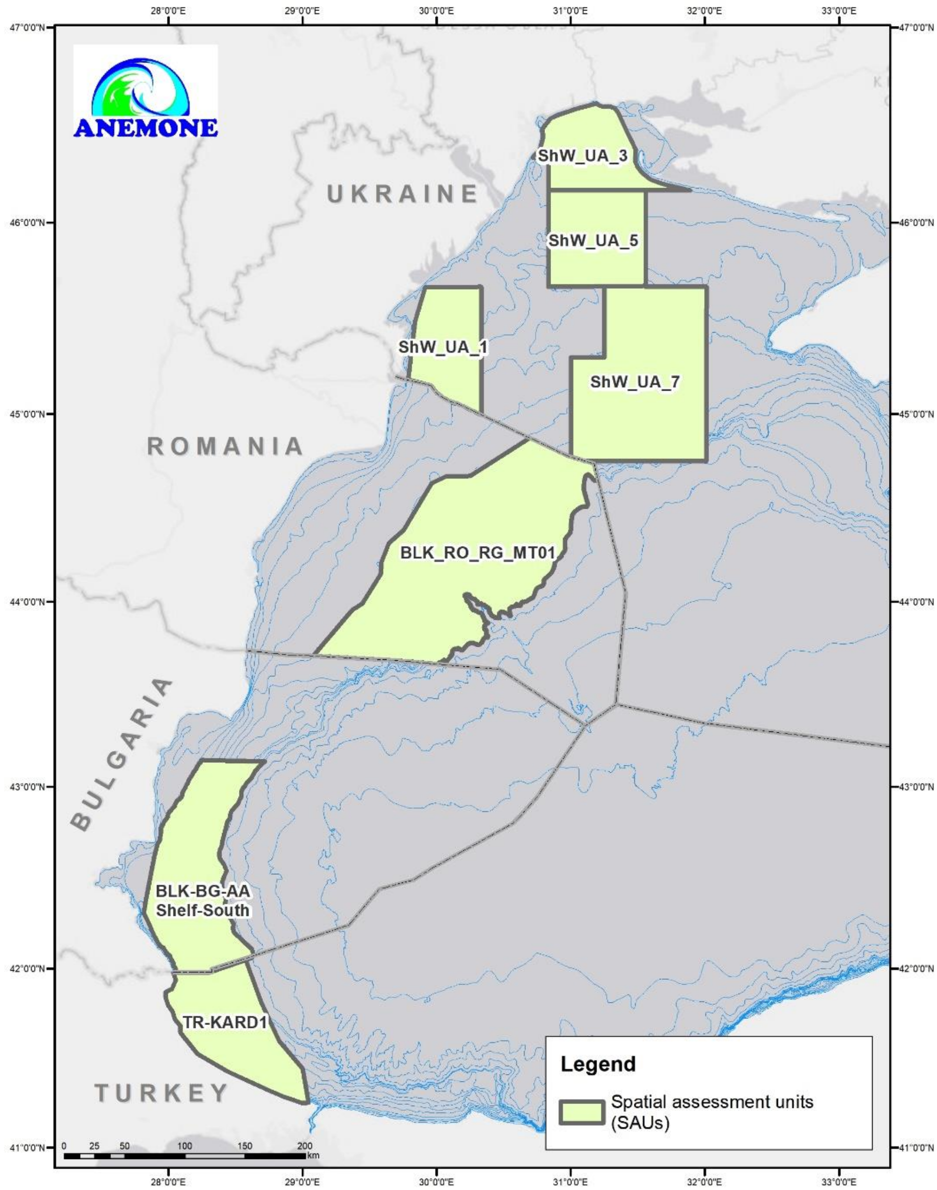


Figure 5.1 - Spatial assessment units (SAUs) for Ukraine, Romania, Bulgaria and Turkey

Table 5.1 - Surface of Spatial assessment units (SAUs) for Ukraine, Romania, Bulgaria and Turkey

SAU	Area (km^2)
UKRAINE	
ShW_UA_3	5065
ShW_UA_5	6481
ShW_UA_1	5066
ShW_UA_7	14640
ROMANIA	
BLK_RO_RG_MT01	21963
BULGARIA	
BLK-BG-AA-Shelf-South	10122
TURKEY	
TR-KARD1	7181

The assessed area is divided between SAUs (Figure 5.2) and countries (Figure 5.3), as follows:

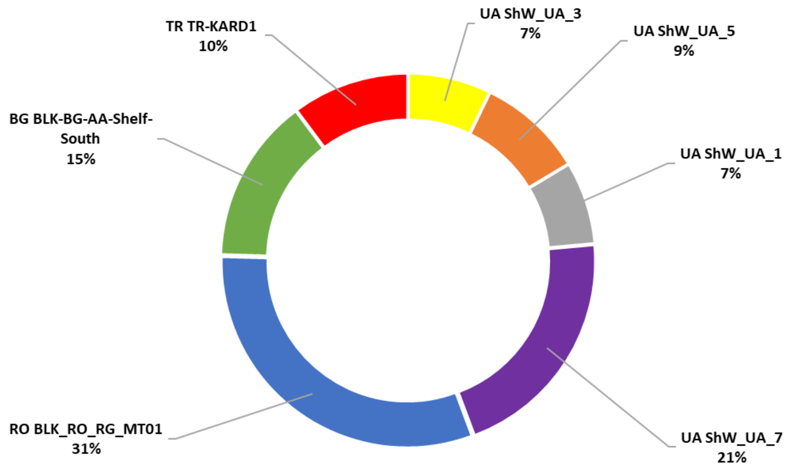


Figure 5.2 - Area proportion for each SAU

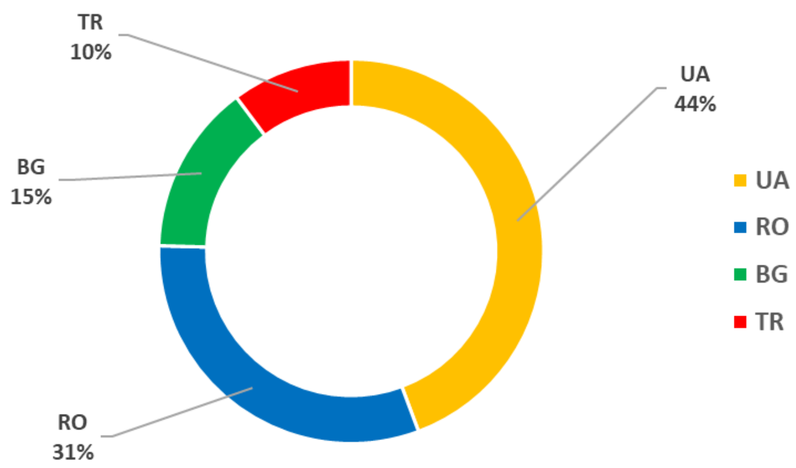


Figure 5.3-SAU area proportion by country

Results

The final NEAT assessment includes all ecosystem components and associated indicators to the equivalent Descriptor. The results are presented in Table 1.

For Descriptor 1, NEAT results showed a poor ecological status for the phytoplankton ecosystem component based on SAU evaluation for Ukraine, Romania and Turkey, while in Bulgaria the results showed a good ecological status. If we compare these results with the MSFD assessment based on SHL phytoplankton biomass, there are differences only for Ukraine, where two out of the 4 identified SAUs achieved GES. For Descriptor 5, NEAT results showed a moderate ecological status for phytoplankton (chlorophyll *a*) ecosystem component based on SAU evaluation for Ukraine and Turkey, while in Romania and Bulgaria the results showed a high ecological status. If we compare these results with the MSFD assessment based on SHL phytoplankton biomass, there are differences for Ukraine, where three out of the 4 identified MRU achieved GES and Turkey where the SAUs under consideration did not achieve GES.

Also, for Descriptor 1, the mesozooplankton community, based on NEAT assessment, recorded a good and a high status. The plankton exhibits variability on a range of spatial and temporal scales and the assemblage of species and populations of individual species are not fixed in time and space but are dynamic (Gowen et al., 2011). NEAT is using an integrative assessment; therefore, the result can be highly influenced by this approach. For the mesozooplankton's community, a number of four indicators were evaluated, the NEAT result integrating all these indicators. Thus, integrating the indicators can lead to a loss of information regarding some used indicators (Pavlidou et al., 2019).

After the integration of the assessment results for Descriptor 2 (evaluation performed only for the invasive zooplankton species - *Mnemiopsis leidyi*), for 3 out of the total 4 countries (Romania, Bulgaria and Turkey), the NEAT value shows “good” and “high” status, indicating a good ecological status for this environmental component. The indicator is based on average biomass values for *Mnemiopsis leidyi*, all these values being higher than the worst limits.
For Descriptor 1 and 5, NEAT values based on benthic macroinvertebrates showed “good” and “high” status for all SAUs in Ukraine, Romania, Bulgaria, and Turkey. Also, the state of benthic macroinvertebrates using M-AMBI*(n) showed EQR values over 0.68 which means that the state of benthic communities was in good status.
For Descriptor 5, indicators based on eutrophication showed “good” and “high” status for all SAU, which conflicts with the specific tool for the eutrophication assessment, BEAST. It is not the case for E-TRIX. The reason is that both NEAT and E-TRIX are not using reference values and accepted deviations in their evaluation as BEAST did. However, NEAT and E-TRIX used their scale – NEAT between worst and best values, and E-TRIX has two constants established for the Adriatic Sea to normalize the values. None of the tools is perfect, and for their improvement, we need more dedicated research on reference values establishment or other scales characteristics.
For Descriptor 8, indicators based on contaminants in water, sediments and biota showed “good” and “high” status, whereas the results obtained with the specific tool for the contamination assessment, CHASE, showed a wider variation range. Across the investigated stations, the CHASE overall test assessment (that were influenced by water scores being the worst) showed a range of status results from bad to good, the majority of them (50%) being in the “bad” state, followed by 45.45% in “moderate” state, whereas the remaining 4.54% were in “good” status. Both NEAT and CHASE are using thresholds values for contaminants in water, sediments, and biota, however NEAT uses also the scale between worst and best available values, that could influence the results. Also, for both tools, if an assessment unit has only few data from one matrix, it is more likely to end up with a positive status result.
NEAT is dedicated to the assessment of marine biodiversity status under the influence of various natural and anthropogenic pressures, contaminants being one factor that could affect or not ecosystem biotic components. With respect to contamination status, CHASE is more suitable, as it was developed as a specific tool for integrated assessment of chemical status. There are four elements in the CHASE tool—water, sediment, biota and biological effects. All four elements (if data available) combined provide a broad picture of the status of environmental contamination. The four groups are first assessed separately, and the final status is defined as the lowest status of the four elements. Of course, both tools need more data to be collected and processed, and more dedicated research on thresholds values establishment and harmonization.
For Descriptor 9, indicators based on contaminants in biota showed “high” status, whereas the results obtained with the specific tool for the contamination assessment, CHASE showed a range of status results from moderate to high, most of them (65%) being in the “moderate” state, followed by 17% in “high” state, 9% in “good” status and 9% in “poor” status. Even if both tools (NEAT and CHASE) are using thresholds values for contaminants in biota in respect with human consumption, NEAT also uses the scale between worst and best available values, that could influence the results. As CHASE is dedicated to contamination status, it is recommended to use it when intend to evaluate the chemical status, whereas when evaluate the ecosystem status NEAT is appropriate. NEAT evaluates contaminants as one of the factors that could put some pressure on the ecosystem and, in this way influence marine biodiversity status.
Anyway, it will be useful to investigate where do these differences come from and make adjustments in order to harmonize the tools results.

Table 5.2 - NEAT assessment results for all SAUs filtering by MSFD Descriptors; weighting by SAU surface

Colors indicate the ecological status: High - blue; Good - green; Moderate - yellow; Poor - orange; Bad - red

SAU	NEAT value	Status class	Confidence	Phytoplankton	Zooplankton	Benthic macroinvertebrates	Water column Nutrients	Sediments Contaminants	Biota Contaminants	Water column Contaminants	Mesozooplankton
D1 MSFD											
Black Sea - ANEMONE JOINT CRUISE	0.777	good	88.2	0.502	0.548	0.857					0.800
UKRAINE	0.703	good	100	0.368	0.651	0.765					0.799
ShW_UA_3	0.735	good	100	0.461	0.759	0.755					0.999
ShW_UA_5	0.731	good	100	0.483	0.735	0.782					0.744
ShW_UA_1	0.783	good	91.8	0.603	0.908	0.782					1.000
ShW_UA_7	0.652	good	100	0.204	0.487	0.755					0.685
ROMANIA	0.821	high	62	0.330	0.500	0.930					0.739
BLK_RO_RG_MT01	0.821	high	62	0.330	0.500	0.930					0.739
BULGARIA	0.824	high	59	0.708	0.611	0.890					0.836
BLK-BG-AA-Shelf-South	0.824	high	59	0.708	0.611	0.890					0.836
TURKEY	0.724	good	90.4	0.321	0.367	0.815					0.775
TR-KARD1	0.724	good	90.4	0.321	0.367	0.815					0.775
D2 MSFD (evaluation only for invasive zooplankton species - *Mnemiopsis leidyi*)											
Black Sea - ANEMONE JOINT CRUISE	0.829	high	69.2		0.829						
UKRAINE	n/a	n/a	n/a		n/a						
ShW_UA_3	n/a	n/a	n/a		n/a						
ShW_UA_5	n/a	n/a	n/a		n/a						
ShW_UA_1	n/a	n/a	n/a		n/a						
ShW_UA_7	n/a	n/a	n/a		n/a						
ROMANIA	0.805	high	50.6		0.805						
BLK_RO_RG_MT01	0.805	high	50.6		0.805						

SAU	NEAT value	Status class	Confidence	Phytoplankton	Zooplankton	Benthic macroinvertebrates	Water column Nutrients	Sediments Contaminants	Biota Contaminants	Water column Contaminants	Mesozooplankton
BULGARIA	0.777	good	51.9		0.777						
BLK-BG-AA-Shelf-South	0.777	good	51.9		0.777						
TURKEY	0.963	high	100		0.963						
TR-KARD1	0.963	high	100		0.963						
D5 MSFD											
Black Sea - ANEMONE JOINT CRUISE	0.838	high	89.4	0.786		0.857	0.831				0.673
UKRAINE	0.789	good	100	0.573		0.765	1.000				0.928
ShW_UA_3	0.782	good	100	0.523		0.755	1.000				0.989
ShW_UA_5	0.770	good	100	0.254		0.782	1.000				0.667
ShW_UA_1	0.826	high	100	0.920		0.782	1.000				0.997
ShW_UA_7	0.787	good	95.4	0.612		0.755	1.000				0.998
ROMANIA	0.918	high	97.7	1.000		0.930	0.960				0.540
BLK_RO_RG_MT01	0.918	high	97.7	1.000		0.930	0.960				0.540
BULGARIA	0.844	high	70.6	0.870		0.890	0.692				0.742
BLK-BG-AA-Shelf-South	0.844	high	70.6	0.870		0.890	0.692				0.742
TURKEY	0.791	good	50.2	0.597		0.815	0.875				0.423
TR-KARD1	0.791	good	50.2	0.597		0.815	0.875				0.423
D8 MSFD											
Black Sea - ANEMONE JOINT CRUISE	0.799	good	90.3					0.873	0.471	0.800	
UKRAINE	0.771	good	100					0.916	n/a	0.659	
ShW_UA_3	0.771	good	100					0.916	n/a	0.659	
ShW_UA_5	0.771	good	100					0.916	n/a	0.659	
ShW_UA_1	0.771	good	100					0.916	n/a	0.659	
ShW_UA_7	0.771	good	100					0.916	n/a	0.659	
ROMANIA	0.759	good	86.8					0.766	0.435	0.799	
BLK_RO_RG_MT01	0.759	good	86.8					0.766	0.435	0.799	

SAU	NEAT value	Status class	Confidence	Phytoplankton	Zooplankton	Benthic macroinvertebrates	Water column Nutrients	Sediments Contaminants	Biota Contaminants	Water column Contaminants	Mesozooplankton
BULGARIA	0.434	moderate	99.5					n/a	0.434	n/a	
BLK-BG-AA-Shelf-South	0.434	moderate	99.5					n/a	0.434	n/a	
TURKEY	0.935	high	100					0.860	1.000	0.969	
TR-KARD1	0.935	high	100					0.860	1.000	0.969	
D9 MSFD											
Black Sea - ANEMONE JOINT CRUISE	0.880	high	100						0.880		
UKRAINE	n/a	n/a	n/a						n/a		
ShW_UA_3	n/a	n/a	n/a						n/a		
ShW_UA_5	n/a	n/a	n/a						n/a		
ShW_UA_1	n/a	n/a	n/a						n/a		
ShW_UA_7	n/a	n/a	n/a						n/a		
ROMANIA	0.840	high	100						0.840		
BLK_RO_RG_MT01	0.840	high	100						0.840		
BULGARIA	0.875	high	100						0.875		
BLK-BG-AA-Shelf-South	0.875	high	100						0.875		
TURKEY	0.941	high	100						0.941		
TR-KARD1	0.941	high	100						0.941		

6 Conclusions and future outlook

The Black sea's ecosystem has no borders. While some of the pressures are attributable to each riparian country, their effects are transboundary. That's why the joint efforts establishment and strengthening of sustainable networks are critical. ANEMONE Joint Cruise was a cooperation platform between regional partners, capable of providing a real contribution in addressing priorities of common concern related to marine environment monitoring and protection. This joint marine monitoring initiative was aimed at the exchange of best practices and use of harmonized new methodologies, with the final goals of filling the knowledge gaps and improving the availability of cross-border compatible environmental monitoring data and information within the Black Sea Basin among scientists, the general public and relevant stakeholders.

The cruise was organized to apply the Black Sea Monitoring and Assessment Guideline (BSMAG) findings and advice. BSMAG represents the first comprehensive regional recommendation on the implementation of a harmonized methodological framework for the monitoring and assessment of the Black Sea environmental status. BSMAG was developed in line with the European legal requirements laid down in the Martine Strategy Framework Directive that aims at implementing a precautionary and holistic ecosystem-based approach for managing European marine waters. BSMAG advised a common framework for regional-level environmental status assessment of pelagic habitats, benthic habitats biodiversity and seabed integrity, non-commercial fish, marine mammals, eutrophication, contaminants in the marine environment and seafood, and marine litter according to the most recent criteria and methodological standards of Commission Decision (EU) 2017/848.

The cruise was designed to assess the status of biodiversity (D1,4 and 6), eutrophication (D5), contamination in water, sediments, and biota (D8 and D9) and marine litter (D10). The data and their assessment have limitations and one of the most recommended actions was the use of dedicated monitoring surveys for different purposes (e.g. mammals).

This holistic assessment shows that the pelagic habitats of the Black Sea are still not in a healthy state. Major pressures on the Black Sea – eutrophication and hazardous substances were all at higher than sustainable levels during the ANEMONE joint cruise. These pressures might be also the ones causing the most widespread impacts. The ecosystem is affected by these pressures and is potentially sensitive to them, directly or indirectly.

6.1 Key priorities for the Black Sea's ecosystem protection

6.1.1 Climate change

Warming of the atmosphere in response to climate change may increase the tendency for atmospheric transport of certain substances (nutrients and contaminants), more rain and floods can result in higher run-off from land and increased storminess may lead to additional remobilization of contaminants from marine sediments. Change in seawater temperature and other possible biological impacts of climate change add to the stress on organisms and coupled with pollution effects may make marine organisms more vulnerable to chemical contamination (Morton, D., 2016). An improved understanding of these processes may lead to the need for a regular review of assessment criteria.

6.1.2 Eutrophication

Although the effects of eutrophication are reduced compared with the 90s, the introduction of nutrients from the upstream watershed is a significant issue in the studied area, mainly in the N-NW and S Black Sea. Thus, the development of the Maximum Allowable Inputs at the regional level is recommended as one of the critical indicators for reducing the impact of the nutrients. In other words, the identification of tipping points consists of the critical nutrient loading thresholds beyond which the whole system is changing into an alternative steady state. Coupled atmosphere-river-coastal sea models need to be developed at the regional scale for the estimate of critical nutrient loads from terrestrial sources, concerning coastal retention, and chemical and biological target indicators. Another priority is the research on natural background nutrient enrichment (e.g. import by upwelling; import from pristine/good status rivers) for determination of pristine state and separation of natural productive status from anthropogenic impacted eutrophic status.

6.1.3 Pollution

New data on a wide range of contaminants in seawater, sediments and biota from the NW, W and SW Black Sea were obtained following ANEMONE Joint Cruise, 2019, thus contributing to further integrated assessments of the Black Sea state of the environment. Generally, the concentrations of the contaminants were at a non-sustainable level showing a need for the increased reduction from human activities. Levels of pollution effects on the ecosystem components, having regard to the selected biological processes and taxonomic groups where a cause/effect relationship is established should be monitored. Implementing biological effects techniques used in environmental health assessment, like assays for specific inhibition of enzymes, induction of proteins, pollutant metabolites, DNA microarrays, immunotoxicity, physiological responses and pathology, is needed. Introducing an ecotoxicology monitoring program will allow the integration of chemical and biological effects measurements. A combination of biological effects and chemical measurements will provide an improved assessment due to the ability to address effects that are potentially caused by a wide range of contaminants as well as those that are more clearly linked to specific compounds or groups of compounds (Vethaak et al., 2017). Little is known on the relationship between the mechanisms of entry of pollutants (riverine, atmospheric, land-based and sea-based sources) into marine waters and their availability and potential effects on organisms and ecosystems. Research is needed on long time series that relate pollutant exposure and cycling to effects on organisms and ecosystem functioning (HELCOM, 2010). Data for better quantification of contaminants fluxes and inputs into the marine environment and their sea/air and water/sediments interfaces exchanges is lacking. Monitoring programmes would need to be designed to allow tracing back chemicals from the environment via their pathways to the sources to allow the appropriate development of programmes of measures to achieve good environmental status and assess the progress being made. Monitoring programme should allow the combination of the data covering waterborne and atmospheric inputs, environmental concentrations, and biological effects of hazardous substances.
The transfer of contaminants through the food chain needs to be better understood, and also the possibility of additive, synergistic and antagonistic effects. The toxic effects of chemical contaminants on marine organisms are dependent on bioavailability and persistence, the ability of organisms to accumulate and metabolize contaminants, their interference with specific metabolic or ecological processes. Little is known about contaminant uptake in the first trophic levels (plankton), and how different biogeochemical statuses of marine ecosystems favour the bioaccumulation and cycling of contaminants (Chouvelon et al., 2019).

6.1.4 Assessment Tools

Data from ANEMONE Joint cruise was used for the different assessment using indicators – Shannon 95, Menhinick, Shannon Weaver, M_AMBI (n), TUBI, TRIX and tools – BEAST, CHASE, NEAT. Most of them need further development of classification systems at the regional level. Thus, the integrated tool's results might be used as governance performance indicators - evaluating the success of policies developed to effectively manage the coastal and marine environment.
The capability of the Black Sea’s riparian countries and its catchment to adjust to environmentally sustainable living is a major factor at all stages of authority. Opportunities for the Black Sea region are seen in research, knowledge, and education, forming a basis for further ecological understanding, technical and social innovation, and evidence-based policies. The knowledge sharing, cooperation and interaction among scientists, organizations and initiatives around the Black Sea, contribute to sustainable human activities and achieving a healthy Black Sea ecosystem.

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ANNEX Species list identified during the Joint Cruise

List of phytoplankton taxa

Species / Group	UA	RO	BG	TR
Bacillariophyceae				
Amphora ovalis A. Schmidt in Schmidt et al., 1875			+	
Asterionella frauenfeldii Grunow, 1863			+	
Bacillaria sp. J.F. Gmelin, 1788	+			
Cerataulina bergonii Ostenfeld, 1903	+	+	+	
Ceratoneis fasciola Ehrenberg, 1839				+
Chaetoceros aequatorialis Yendo, 1905			+	
Chaetoceros affinis Lauder, 1864			+	+
Chaetoceros compressus Cleve, 1894				+
Chaetoceros curvisetus Hustedt in Schmidt, 1920		+		+
Chaetoceros decipiens Cleve, 1873			+	+
Chaetoceros laciniosus F.Schütt, 1895	+			
Chaetoceros lorenzianus Van Breemen, 1905			+	
Chaetoceros peruvianus Gran, 1908		+		
Chaetoceros rigidus Ostenfeld, 1902		+		
Chaetoceros similis Cleve, 1896				+
Chaetoceros simplex Ostenfeld, 1902				+
Chaetoceros socialis H.S.Lauder, 1864			+	
Chaetoceros sp. C.G. Ehrenberg, 1844	+			
Chaetoceros spores			+	
Chaetoceros wighamii Grunow in Van Heurck, 1882			+	
Coscinodiscus angustelineatus Schmidt in Schmidt et al., 1878				+
Coscinodiscus centralis A. Schulze, 1879				+
Coscinodiscus granii Gough, 1905			+	+
Coscinodiscus perforatus Cleve & Möller, 1878				+
Coscinodiscus sol C.G.Wallich, 1860			+	
Coscinodiscus sp. C.G. Ehrenberg, 1839			+	
Cyclotella caspia Grunow, 1878		+	+	
Cyclotella meneghiniana Kützing, 1844		+		
Cyclotella planctonica Brunnthaler, 1901	+			
Cyclotella sp. (F.T. Kützing) A. de Brébisson, 1838				+
Cylindrotheca closterium (Ehrenberg) Reimann & J.C.Lewin, 1964				+
Ditylum brightwellii (T.West) Grunow, 1885			+	+
Halamphora hyalina (Kützing) Rimet & R. Jahn in Rimet et al., 2018	+			
Lennoxia faveolata H.A.Thomsen & K.R.Buck, 1993		+	+	
Leptocylindrus danicus Schutt, 1900				+
Leptocylindrus minimus Gran, 1915	+			
Navicula sp. J.B.M. Bory de Saint-Vincent, 1822			+	
Nitzschia closterium Eulenstein, 1868		+		
Nitzschia longissima (Brébisson) Ralfs, 1861			+	
Nitzschia tenuirostris Manguin in Bourrelly & Manguin, 1952			+	
Nitzschia sp. A.H. Hassall, 1845				+
Paralia sulcata (Ehrenberg) Cleve, 1873	+		+	
Pleurosigma elongatum Auerswald in litt. ed sched. Rabenhorst, 1863		+	+	+
Proboscia alata (Brightwell) Sundström, 1986		+	+	+
Pseudo-nitzschia calliantha Lundholm, Moestrup & Hasle, 2003			+	
Pseudo-nitzschia delicatissima (Cleve) Heiden, 1928	+	+	+	+
Pseudo-nitzschia pungens (Grunow ex Cleve) G.R.Hasle, 1993			+	
Pseudo-nitzschia seriata (Cleve) H.Peragallo, 1899	+		+	
Pseudosolenia calcar-avis (Schultze) B.G.Sundström, 1986	+	+	+	+
Rhizosolenia fragilissima f. fragilissima Bergon, 1903				+
Rhizosolenia styliformis T.Brightwell, 1858				+
Sphenella parvula Kützing, 1844	+			
Stephanodiscus hantzschii Grunow, 1880	+			
Syndendrium diadema Ehrenberg, 1854			+	
Synedra nitzschioides f. nitzschioides Grunow, 1862	+	+	+	+
Synedra sp. C.G. Ehrenberg, 1830	+			
Synedra ulna var. subcontracta Østrup in Héribaud et al., 1920				+

Species / Group	UA	RO	BG	TR
Thalassiosira baltica (Grunow) Ostenfeld, 1901	+			
Thalassiosira eccentrica (Ehrenberg) Cleve, 1904			+	+
Thalassiosira minima Mertz, 1966			+	
Thalassiosira nordenskioldii Cleve emend Berg, 1952			+	
Thalassiosira rotula Meunier, 1910			+	
Thalassiosira sp. P.T. Cleve, 1873 emend. Hasle, 1973	+		+	
Thalassiosira subsalina Proshkina-Lavrenko, 1955		+		
Trachyneis sp. P.T. Cleve, 1894	+			
Dinophyceae				
Akashiwo sanguinea (K.Hirasaka) Gert Hansen & Moestrup, 2000		+	+	
Alexandrium minutum Halim, 1960			+	
Alexandrium sp. Halim, 1960		+	+	
Amphidinium acutissimum Schiller, 1933			+	
Amphidinium carterae Hulburt, 1957			+	
Amphidinium crassum Lohmann, 1908		+	+	
Amphidinium curvatum Schiller, 1928			+	
Amphidinium extensum Wulff, 1919		+		
Amphidinium flagellans Schiller			+	
Amphidinium longum Lohmann, 1908			+	
Amphidinium sp. Claperède & Lachmann, 1859		+	+	
Amphidinium turbo Kofoid & Swezy, 1921			+	
Amphidoma languida Tillmann, Salas & Elbrachter, 2012			+	
Archaeperidinium minutum (Kofoid) Jørgensen, 1912			+	
Aureodinium pigmentosum Dodge, 1967			+	
Azadinium sp. Elbrächter & Tillmann, 2009			+	
Azadinium spinosum Elbrächter & Tillmann, 2009			+	
Biecheleria cincta (Siano, Montresor & Zingone) Siano, 2012			+	
Cochlodinium archimedes (Pouchet) Lemmermann, 1899		+		
Cochlodinium pupa Lebour, 1925			+	
Cochlodinium sp. Schütt, 1896	+			
Dinophyceae Fritsch, 1927	+			
Dinophysis acuminata Claparède & Lachmann, 1859	+		+	
Dinophysis acuta Ehrenberg, 1839	+	+	+	+
Dinophysis caudata Saville-Kent, 1881	+	+	+	+
Dinophysis fortii Pavillard, 1924				+
Dinophysis hastata F.Stein, 1883		+		
Dinophysis odiosa (Pavillard) Tai & Skogsberg, 1934			+	
Dinophysis sacculus F.Stein, 1883	+	+	+	+
Diplopsalis lenticula Bergh, 1881	+		+	+
Diplopsalis sp. Bergh, 1881	+			
Diplopsalopsis orbicularis (Paulsen) Meunier, 1910	+			
Ensiculifera sp. Balech ex K.Matsuoka, S.Kobayashi & G.Gains, 1990			+	
Glenodiniopsis uliginosa (A.J.Schilling) Woloszynska, 1928			+	
Glenodinium paululum Lindemann, 1928	+	+		
Glenodinium pilula (Ostenfeld) Schiller, 1935	+	+	+	
Glenodinium pulvisculum (Ehrenberg) Stein, 1883			+	
Glenodinium sp. Ehrenberg, 1836	+		+	+
Goniodoma sphaericum Murray & Whitting, 1899			+	
Gonyaulax ceratocoroides Kofoid, 1910		+	+	+
Gonyaulax minima Matzenauer, 1933	+		+	
Gonyaulax monacantha Pavillard, 1916				+
Gonyaulax polygramma F.Stein, 1883			+	+
Gonyaulax sp. Diesing, 1866			+	
Gonyaulax verior Sournia, 1973			+	
Gymnodinium agiliforme Schiller, 1928		+		
Gymnodinium albulum Er.Lindemann, 1928			+	
Gymnodinium aureolum (E.M.Hulburt) Gert Hansen, 2000			+	
Gymnodinium catenatum H.W.Graham, 1943			+	
Gymnodinium fuscum (Ehrenberg) F.Stein, 1878	+		+	
Gymnodinium hamulus Kofoid & Swezy, 1921			+	
Gymnodinium lachmannii Kent, 1881			+	
Gymnodinium lacustre J.Schiller, 1933			+	

Species / Group	UA	RO	BG	TR
Gymnodinium lantzschii Utermöhl, 1925			+	
Gymnodinium latum Skuja, 1948			+	
Gymnodinium najadeum J.Schiller, 1928	+	+	+	
Gymnodinium nanum Schiller, 1928			+	
Gymnodinium opressum Conrad, 1926			+	
Gymnodinium pulchrum J.Schiller, 1928			+	
Gymnodinium punctatum Pouchet, 1887			+	
Gymnodinium rubrum Koifoid & Swezy, 1921			+	
Gymnodinium sp. F. Stein, 1878	+	+	+	
Gymnodinium uberrimum (G.J.Allman) Kofoid & Swezy, 1921			+	
Gymnodinium verruculosum P.H.Campbell, 1973			+	
Gymnodinium wulffii J.Schiller, 1933	+	+	+	
Gyrodinium cochlea Lebour, 1925			+	
Gyrodinium contortum (Schütt) Kofoid & Swezy, 1921	+			
Gyrodinium cornutum (Pouchet) Kofoid & Swezy, 1921	+			
Gyrodinium dominans Hulbert, 1957			+	
Gyrodinium flagellare Schiller, 1928			+	
Gyrodinium fusiforme Kofoid & Swezy, 1921	+	+	+	+
Gyrodinium helveticum (Penard) Y.Takano & T.Horiguchi, 2004		+	+	
Gyrodinium lachryma (Meunier) Kofoid & Swezy, 1921	+			+
Gyrodinium ovum (Schütt) Kofoid & Swezy, 1921			+	
Gyrodinium pingue (Schütt) Kofoid & Swezy, 1921	+		+	
Gyrodinium sp. Kofoid & Swezy, 1921			+	
Gyrodinium spirale (Bergh) Kofoid & Swezy, 1921			+	
Herdmania litoralis J.D.Dodge, 1981		+	+	
Heterocapsa lanceolata Iwataki & Fukuyo, 2002			+	
Heterocapsa minima A.J.Pomroy, 1989			+	
Heterocapsa niei (Loeblich III) Morrill & Loeblich III, 1981			+	
Heterocapsa rotundata (Lohmann) Gert Hansen, 1995		+	+	+
Heterocapsa sp. Stein, 1883			+	
Karenia sp. G.Hansen & Moestrup, 2000	+			
Karlodinium veneficum (D.Ballantine) J.Larsen, 2000			+	
Katodinium glaucum (Lebour) Loeblich III, 1965	+		+	
Kryptoperidinium foliaceum (F.Stein) Lindemann, 1924			+	
Kryptoperidinium triquetrum (Ehrenberg) U.Tillmann, M. Gottschling, M.Elbrächter, W.-H.Kusber & M.Hoppenrath, 2019	+	+		+
Lepidodinium chlorophorum (M.Elbrächter & E.Schnepf) Gert Hansen, Botes & Salas, 2007			+	
Lessardia elongata Saldarriaga & F.J.R.Taylor, 2003	+	+	+	
Lingulodinium polyedra (F.Stein) J.D.Dodge, 1989	+		+	+
Margalefidinium citron (Kofoid & Swezy) F.Gómez, Richlen & D.M.Anderson, 2017		+		
Mesoporos perforatus (Gran) Lillick, 1937		+		
Nematodinium armatum (Dogiel) Kofoid & Swezy, 1921			+	
Nematodinium sp. Kofoid & Swezy, 1921			+	
Neoceratium sp. F.Gómez, D.Moreira & P.López-Garcia, 2010	+			
Oblea rotunda (Lebour) Balech ex Sournia, 1973		+	+	
Oxyrrhis marina Dujardin, 1841			+	
Pentapharsodinium dalei Indelicato & Loeblich III, 1986			+	
Peridiniella danica (Paulsen) Y.B.Okolodkov & J.D.Dodge, 1995			+	
Peridiniella globosa (P.A.Dangeard) Okolodkov, 2006			+	
Peridinium cysts		+	+	
Peridinium minusculum J.Pavillard		+		
Peridinium sp. Ehrenberg, 1830			+	
Peridinium vegetative stages		+		
Phalacroma rotundatum (Claparéde & Lachmann) Kofoid & J.R.Michener, 1911	+	+	+	+
Polykrikos kofoidii Chatton, 1914		+	+	
Polykrikos schwartzii Bütschli, 1873	+			
Preperidinium meunieri (Pavillard) Elbrächter, 1993		+		+
Pronoctiluca pelagica Fabre-Domergue, 1889			+	+
Pronoctiluca spinifera (Lohmann) Schiller, 1932			+	
Prorocentrum compressum (Bailey) T.H.Abé ex J.D.Dodge, 1975	+	+	+	+
Prorocentrum cordatum (Ostenfeld) J.D.Dodge, 1975	+	+	+	+

Species / Group	UA	RO	BG	TR
Prorocentrum maximum (Gourret) Schiller, 1937	+			
Prorocentrum micans Ehrenberg, 1834	+	+	+	+
Prorocentrum obtusum Ostenfeld, 1908	+			
Prorocentrum ponticus Krachmalny & Terenko, 2002	+			
Prorocentrum scutellum Schröder, 1900		+		
Prosoaulax lacustris (F.Stein) Calado & Moestrup, 2005			+	
Protoceratium reticulatum (Claparède & Lachmann) Bütschli, 1885		+	+	
Protodinium simplex Lohmann, 1908		+	+	
Protoperidinium bipes (Paulsen, 1904) Balech, 1974	+		+	+
Protoperidinium breve (Paulsen, 1908) Balech, 1974		+	+	
Protoperidinium brevipes (Paulsen, 1908) Balech, 1974	+		+	+
Protoperidinium bulla (Meunier, 1910) Balech, 1974		+		
Protoperidinium claudicans (Paulsen, 1907) Balech, 1974				+
Protoperidinium conicum (Gran) Balech, 1974				+
Protoperidinium curtipes (Jørgensen, 1912) Balech, 1974				+
Protoperidinium depressum (Bailey, 1854) Balech, 1974	+	+	+	+
Protoperidinium divergens (Ehrenberg) Balech, 1974	+	+	+	+
Protoperidinium granii (Ostenfeld) Balech, 1974	+	+		
Protoperidinium knipowitschii (Usachev, 1927) Balech, 1974	+			
Protoperidinium leonis (Pavillard, 1916) Balech, 1974				+
Protoperidinium oblongum (Aurivillius) Parke & Dodge, 1976				+
Protoperidinium pallidum (Ostenfeld, 1899) Balech, 1973			+	+
Protoperidinium pellucidum Bergh, 1881			+	+
Protoperidinium pentagonum (Gran, 1902) Balech, 1974			+	
Protoperidinium sinaicum (Matzenauer, 1933) Balech, 1974			+	
Protoperidinium sp. Bergh, 1881	+			
Protoperidinium steinii (Jørgensen, 1899) Balech, 1974	+	+	+	+
Protoperidinium subinerme (Paulsen) Loeblich III, 1969			+	
Scaphodinium mirabile Margalef, 1963		+		
Scrippsiella acuminata (Ehrenberg) Kretschmann, Elbrächter, Zinssmeister, S.Soehner, Kirsch, Kusber & Gottschling, 2015	+	+	+	+
Scrippsiella spinifera G.Honsell & M.Cabrini, 1991			+	
Speroidium fungiforme (Anisimova) Moestrup & Calado, 2018	+		+	
Torodinium robustum Kofoid & Swezy, 1921		+	+	+
Torodinium teredo (Pouchet) Kofoid & Swezy, 1921			+	
Triadinium polyedricum (Pouchet) Dodge, 1981			+	
Tripos furca (Ehrenberg) F.Gómez, 2013	+	+	+	+
Tripos fusus (Ehrenberg) F.Gómez, 2013	+	+	+	+
Tripos muelleri Bory de Saint-Vincent, 1826	+	+	+	+
Chlorophyceae				
Chlamydomonas sp. Ehrenberg, 1833			+	+
Chlorogonium sp. Ehrenberg, 1836			+	
Chlorophyceae sp. Wille, 1884		+	+	
Desmodesmus communis (E.Hegewald) E.Hegewald, 2000				
Kirchneriella lunaris (Kirchner) Möbius, 1894	+			
Monoraphidium contortum (Thuret) Komárková-Legnerová, 1969	+		+	
Monoraphidium sp. *Komárková-Legnerová, 1969*			+	
Raphidocelis danubiana (Hindák) Marvan, Komárek & Comas, 1984	+			
Scenedesmus quadricauda var. ellipticus West & G.S.West, 1895			+	
Schroederia setigera (Schröder) Lemmermann, 1898		+		
Schroederia sp. Lemmermann, 1898		+		
Schroederia spiralis (Printz) Korshikov, 1953		+		
Tetraëdron trigonum (Nägeli) Hansgirg, 1888		+		
Tetrastrum staurogeniiforme (Schröder) Lemmermann, 1900	+			
Willea crucifera (Wolle) D.M.John, M.J.Wynne & P.M.Tsarenko, 2014			+	
Chlorodendrophyceae				
Pachysphaera sp. Ostenfeld, 1899		+		
Tetraselmis inconspicua Butcher, 1959	+			
Tetraselmis sp. F.Stein, 1878	+			
Cryptophyceae				
Chroomonas sp. Hansgirg, 1885		+	+	+
Cryptomonas sp. Ehrenberg, 1831		+		

Species / Group	UA	RO	BG	TR
Cryptophyceae sp. Fritsch, 1927	+		+	
Hemiselmis sp. Parke, 1949			+	
Hillea fusiformis (J.Schiller) J.Schiller, 1925	+	+	+	+
Hillea marina Butcher, 1952	+			
Hillea sp. Schiller, 1925	+			
Komma caudata (L.Geitler) D.R.A.Hill, 1991		+		
Leucocryptos marina (Braarud) Butcher, 1967			+	
Plagioselmis prolonga Butcher ex G.Novarino, I.A.N.Lucas & S.Morrall, 1994	+			
Plagioselmis sp. Butcher ex G.Novarino, I.A.N.Lucas & S.Morrall, 1994			+	
Rhodomonas marina (P.A.Dangeard) Lemmermann, 1899			+	
Microflagellates	+	+	+	
Teleaulax sp. Hill, 1991			+	
Telonema sp. Griessmann, 1913			+	
Cyanophyceae				
Aphanothece sp. C.Nägeli, 1849			+	
Chroococcus sp. Nägeli, 1849			+	
Cyanophyceae sp. Stanier ex Cavalier-Smith, 2002	+	+		
Glaucospira laxissima (G.S.West) Simic, Komárek & Dordevic, 2014	+			
Jaaginema sp. Anagnostidis & Komárek, 1988	+			
Monoraphidium contortum (Thuret) Komárková-Legnerová, 1969	+			
Phormidium hormoides Setchell & N.L.Gardner, 1918		+		
Phormidium sp. Kützing ex Gomont, 1892			+	
Romeria sp. Koczwara, 1932			+	
Euglenoidea				
Euglena acusformis J.Schiller, 1925			+	
Eutreptia globulifera Goor, 1925			+	
Eutreptia lanowii Steuer, 1904	+	+	+	
Eutreptia sp. Perty, 1852	+		+	
Eutreptiella sp. A.da Cunha, 1914			+	
Lepocinclis acus (O.F.Müller) B.Marin & Melkonian, 2003		+		
Phacus sp. Dujardin, 1841			+	
Prymnesiophyceae				
Acanthoica acanthifera Lohmann ex Lohmann, 1913	+			
Acanthoica coronata Lohmann, 1903	+			
Acanthoica quattrospina Lohmann, 1903	+	+		
Acanthoica sp. Lohmann, 1903			+	
Braarudosphaera bigelowii (Gran & Braarud) Deflandre, 1947			+	
Calyptrosphaera oblonga Lohmann, 1902			+	
Calyptrosphaera sp. Lohmann, 1902	+			
Chrysochromulina sp. Lackey, 1939			+	
Coccolithus sp. E.H.L.Schwarz, 1894	+		+	
Coronosphaera mediterranea (Lohmann) Gaarder, 1977			+	
Coronosphaera binodata (Kamptner) Gaarder, 1977	+			
Emiliania huxleyi (Lohmann) W.W.Hay & H.P.Mohler, 1967	+	+	+	+
Holococcolithophora sphaeroidea (Schiller) J.W.Jordan, L.Cros & J.R.Young, 2005			+	
Prymnesiophyceae sp. Hibberd	+			
Syracosphaera sp. Lohmann, 1902	+			
Chrysophyceae				
Ochromonas sp. Vysotskii [Wissotsky], 1887			+	
Ollicola vangoorii (W.Conrad) Vørs, 1992	+			
Dictyochophyceae				
Apedinella radians (Lohmann) P.H.Campbell, 1973		+	+	
Octactis octonaria (Ehrenberg) Hovasse, 1946	+		+	+
Octactis speculum (Ehrenberg) F.H.Chang, J.M.Grieve & J.E.Sutherland, 2017			+	+
Ebriophyceae				
Ebria tripartita (J.Schumann) Lemmermann, 1899		+		+
Hermesinum adriaticum O.Zacharias, 1906			+	
Trebouxiophyceae				
Actinastrum hantzschii Lagerheim, 1882			+	
Crucigenia tetrapedia (Kirchner) Kuntze, 1898		+		
Micractinium pusillum Fresenius, 1858		+		
Trochiscia sp. Kützing, 1834			+	+

Species / Group	UA	RO	BG	TR
Haptophyta				
Alisphaera ordinata (Kamptner) Heimdal, 1973	+			
Nephroselmidophyceae				
Nephroselmis astigmatica Inouye & Pienaar, 1984			+	
Nephroselmis pyriformis (N.Carter) Ettl, 1982			+	
Pavlovophyceae				
Diacronema lutheri (Droop) Bendif & Véron, 2011			+	
Pyramimonadophyceae				
Polyblepharides amylifera (Conrad) H.Ettl, 1982			+	
Pseudoscourfieldia marina (J.Throndsen) Manton, 1975			+	
Pyramimonas sp. Schmarda, 1849			+	
Raphidophyceae				
Heterosigma akashiwo (Y.Hada) Y.Hada ex Y.Hara & M.Chihara, 1987			+	
Synurophyceae				
Synura sp. *Ehrenberg, 1834*			+	
Ulvophyceae				
Binuclearia lauterbornii (Schmidle) Proschkina-Lavrenko, 1966			+	

List of zooplankton taxa

Species / Group	UA	RO	BG	TR
Oligotrichea				
Tintinnopsis campanula Ehrenberg, 1840			+	
Tintinnopsis cylindrica Daday, 1887			+	
Tintinnopsis minuta Wailes, 1925		+		
Metacylis mediterranea Mereschkowsky, 1880 Jörgensen, 1924		+		
Favella ehrenbergii Claparède & Lachmann, 1858 Jörgensen, 1924		+	+	
Amphorellopsis acuta Schmidt, 1902		+	+	+
Eutintinnus lusus-undae Entz, 1885			+	+
Eutintinnus tubulosus Ostenfeld, 1899, Kofoid & Campbell, 1939		+	+	+
Salpingella decurtata Jörgensen, 1924		+	+	+
Rhizodomus tagatzi Strelkow & Wirketis, 1950			+	+
Appendicularia				
Oikopleura (Vexillaria) dioica Fol, 1872	+	+	+	+
Bivalvia				
Bivalvia Linnaeus, 1758	+	+	+	+
Branchiopoda				
Evadne spinifera P.E.Müller, 1867	+	+	+	+
Penilia avirostris Dana, 1849	+	+	+	+
Pseudevadne tergestina Claus, 1877	+	+	+	+
Pleopis polyphemoides Leuckart, 1859	+	+	+	+
Podonevadne trigona G.O. Sars, 1897	+			
Podon leuckartii G.O. Sars, 1862			+	
Clitellata				
Oligochaeta Grube, 1850	+			
Dinophyceae				
Noctiluca scintillans Macartney Kofoid & Swezy, 1921	+	+	+	+
Gastropoda				
Gastropoda Cuvier, 1795	+	+	+	+
Hexanauplia				
Acartia (Acartiura) clausi Giesbrecht, 1889	+	+	+	+
Pseudocalanus elongatus Boeck, 1865	+	+	+	+
Oithona similis Claus, 1866	+	+	+	+
Centropages ponticus Karavaev, 1895	+	+	+	+
Paracalanus parvus parvus Claus, 1863	+	+	+	+
Harpacticoida Sars M., 1903	+	+		
Cyclopoida Burmeister, 1834	+	+		
Calanus euxinus Hulsemann, 1991	+	+	+	+
Oithona davisae Ferrari F.D. & Orsi, 1984	+	+	+	+
Balanus Costa, 1778	+	+	+	
Acartia (Acanthacartia) tonsa Dana, 1849	+		+	

Species / Group	UA	RO	BG	TR
*Acartia sp.*Dana, 1846			+	
Pontella mediterranea Claus, 1863			+	+
Calanus euxinus Hulsemann, 1991	+	+	+	+
Copepoda Milne Edwards, 1840	+			+
Cirripedia Burmeister, 1834				+
Centropages spinosus Krichagin, 1873	+			
Calanipeda aquaedulcis Krichagin, 1873	+			
Malacostraca				
Decapoda Latreille, 1802		+	+	+
Isopoda Latreille, 1817			+	
Polychaeta				
Polychaeta Grube, 1850	+	+	+	+
Spionidae Grube, 1850	+			
Neanthes Kinberg, 1865	+			
Sagittoidea				
Parasagitta setosa J. Müller, 1847	+	+	+	+
Phoronida				
Phoronis Wright, 1856			+	
Phoronida Hatschek, 1888				+
Bryozoa				
Bryozoa			+	+
Platyhelminthes				
Platyhelminthes sp. Minot, 1876			+	
Nemertea				
Nemertea sp.			+	
Actinopterygii				
Syngnathidae Bonaparte, 1831			+	
Hydrozoa				
Sarsia tubulosa M. Sars, 1835			+	
Leptocardii				
Branchiostoma lanceolatum Pallas, 1774			+	
Nuda				
Beroe ovata Bruguière, 1789	+	+	+	+
Scyphozoa				
Aurelia aurita Linnaeus, 1758	+	+	+	+
Tentaculata				
Pleurobrachia pileus O. F. Müller, 1776	+	+	+	+
Mnemiopsis leidyi A. Agassiz, 1865		+	+	+
Pisces				
Pisces (ova, larvae)	+		+	+

List of ichthyoplankton taxa

Species / Group	RO	BG	TR
Actinopterygii			
Merlangius merlangus Linnaeus, 1758	+	+	+
Mullus barbatus ponticus Essipov, 1927		+	+
Sprattus sprattus Linnaeus, 1758	+	+	

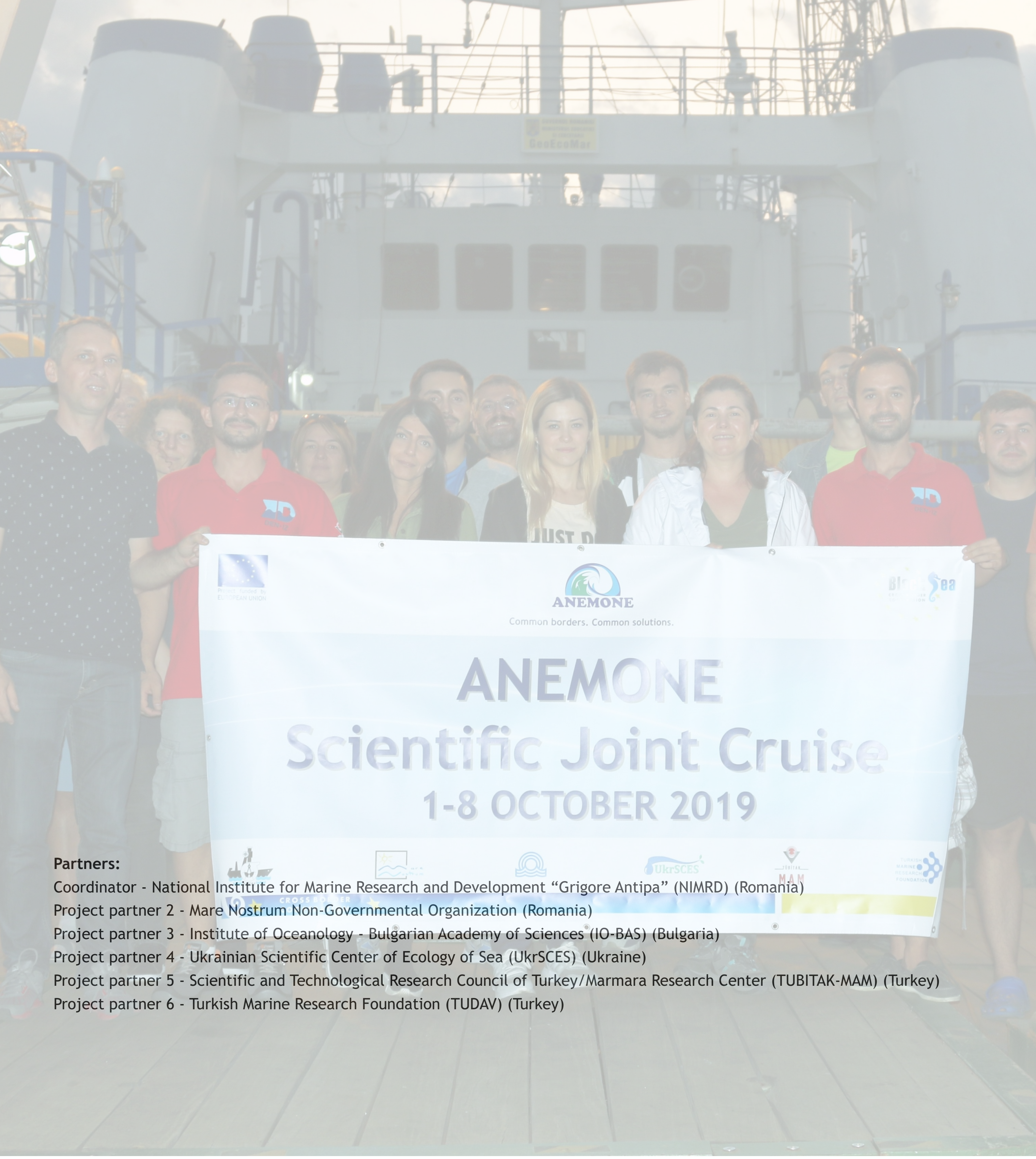
List of zoobenthos taxa

Species / Group	UA	RO	BG	TR
Demospongiae				
Suberites carnosus Johnston, 1842		+	+	
Anthozoa				
Anthozoa sp. Ehrenberg, 1834		+	+	+
Actinia sp.	+			
Actinia equina Linnaeus, 1758	+			
Diadumene lineata Verrill, 1869			+	
Pachycerianthus solitarius Rapp, 1829		+	+	+
Sagartiogeton undatus Müller, 1778		+	+	+
Nemertea				
Leucocephalonemertes aurantiaca Grube, 1855				

Species / Group	UA	RO	BG	TR
	+			
Nemertea Schultze, 1851	+	+	+	+
Gastropoda				
Calyptraea chinensis Linnaeus, 1758	+			+
Doto coronata Gmelin, 1791	+			
Eulimidae sp. Philippi, 1853			+	
Philine cf. quadripartita Ascanius, 1772			+	
Retusa truncatula Bruguière, 1792		+		+
Retusa umbilicata Montagu, 1803			+	+
Tritia neritea (Linnaeus, 1758)				+
Trophon sp. Montfort, 1810		+		+
Trophonopsis breviata Jeffreys, 1882				+
Bivalvia				
Abra alba W.Wood, 1802		+	+	+
Abra nitida O. F. Müller, 1776			+	+
Abra prismatica Montagu, 1808			+	
Abra spp.			+	+
Acanthocardia paucicostata G.B. Sowerby II, 1934		+	+	+
Anadara kagoshimensis Tokunaga, 1906	+			
Chamelea gallina Linnaeus, 1758	+		+	+
Flexopecten glaber ponticus Bucquoy, Dautzenberg & Dollfus, 1889	+			
Gonilia calliglypta Dall, 1903			+	
Gouldia minima Montagu, 1803	+	+	+	
Kurtiella bidentata Montagu, 1803			+	+
Modiolula phaseolina Philippi, 1844		+	+	+
Moerella donacina Linnaeus, 1758	+			
Mytilus galloprovincialis Lamarck, 1819	+	+	+	+
Papillicardium papillosum Poli, 1791		+	+	+
Parvicardium exiguum Gmelin, 1791	+			
Parvicardium simile Milaschewitsch,1909		+	+	+
Pitar rudis Poli, 1795		+	+	+
Polititapes aureus Gmelin, 1791	+	+	+	
Spisula subtruncata da Costa, 1778		+	+	+
Clittelata				
Oligochaeta Grube, 1850		+	+	+
Polychaeta				
Amphicorina armandi Claparede, 1864			+	
Amphitritides gracilis Grube, 1860	+			
Aonides paucibranchiata Southern, 1914	+	+	+	+
Aricidea (*Strelzovia*) *claudiae* Laubier, 1967			+	+
Capitella capitata Fabricius, 1780	+	+	+	+
Capitella minima Langerhans, 1881		+	+	
Chone sp. Krøyer, 1856		+		
Cossura soyeri Laubier, 1964			+	
Dipolydora quadrilobata Jacobi, 1883	+	+	+	
Ditrupa arietina O. F. Müller, 1776	+			
Euchone limnicola Reish, 1959		+	+	+
Eumida sanguinea Oersted, 1843		+	+	+
Eunereis longissima Johnston, 1840				+
Exogone naidina Örsted, 1845		+	+	+
Harmothoe imbricata Linnaeus, 1767	+			
Harmothoe impar Johnston, 1839			+	+
Harmothoe reticulata Claparede, 1870	+	+	+	+
Harmothoe sp. Kinberg, 1856		+	+	+
Hediste diversicolor O. F. Muller,1776	+		+	+
Heteromastus filiformis Claparede, 1864	+	+	+	+
Lagis koreni Malmgren, 1866	+			
Lindrilus flavocapitatus Uljanin, 1877		+	+	
Melinna palmata Grube, 1870		+	+	+
Micronephthys longicornis Perejaslavtseva, 1891		+	+	+
Mysta picta Quatrefages, 1866	+			
Neanthes sp. Kinberg, 1865				+

Species / Group	UA	RO	BG	TR
Nephtys hombergii Savigny in Lamarck, 1818	+	+	+	+
Nephtys hystricis McIntosh, 1900		+	+	+
Nephtys sp. Cuvier, 1817			+	+
Nereis zonata Malmgren, 1867	+			
Notomastus latericeus Sars, 1851				+
Notomastus profundus Eisig, 1887		+		
Oriopsis armandi Claparède, 1864			+	+
Pholoe inornata Johnston, 1839	+			
Phyllodoce maculata Linnaeus, 1767	+			
Phyllodoce mucosa Örsted, 1843		+	+	+
Polycirrus jubatus Bobretzky, 1868		+	+	
Polycirrus sp. Grube, 1850		+	+	
Polynoe scolopendrina Savigny, 1822	+			
Prionospio cirrifera Wirén, 1883	+	+	+	+
Prionospio maciolekae Dagli & Çinar, 2011		+	+	+
Pterocirrus macroceros Grube, 1860		+	+	
Sphaerosyllis bulbosa Southern, 1914		+	+	
Sphaerosyllis hystrix Claparède, 1863		+	+	+
Sphaerosyllis taylori Perkins, 1981				+
Spio decoratus Bobretzky, 1870			+	
Spio filicornis O. F. Muller, 1776			+	
Syllides longocirratus Örsted, 1845		+	+	+
Syllis sp. Lamarck, 1818				+
Terebellides stroemii Sars, 1835	+	+	+	+
Arachnida				
Thalassarachna basteri Johnston, 1836		+		
Pycnogonida				
Callipallene phantoma Dohrn, 1881		+		
Pantopoda sp. Gerstaecker, 1863		+	+	
Thecostraca				
Amphibalanus improvisus Darwin, 1854	+		+	
Malacostraca				
Ampelisca diadema Costa, 1853	+	+	+	+
Ampelisca pseudosarsi Bellan-Santini & Kaim-Malka, 1977			+	+
Ampelisca pseudospinimana Bellan-Santini & Kaim-Malka, 1977		+	+	+
Ampelisca sarsi Chevreux, 1888		+	+	+
Ampelisca sp. Krøyer, 1842		+	+	
Amphipoda sp. Latreille, 1816		+	+	+
Apherusa bispinosa Bate, 1857	+	+	+	
Apherusa chiereghinii Giordani- Soika, 1949		+		+
Apseudopsis acutifrons Sars, 1882		+		
Apseudopsis latreillii Milne Edwards, 1828			+	+
Apseudopsis ostroumovi Bacescu & Carause, 1947	+	+	+	+
Athanas nitescens Leach, 1813	+			+
Brachynotus sexdentatus Risso, 1827 in Risso, 1826-1827	+			
Caprella acanthifera Leach, 1814	+	+	+	+
Caprella sp. Lamarck, 1801			+	
Crangon crangon Linnaeus, 1758	+			
Cumella (*Cumella*) *pygmaea* G.O. Sars, 1865				+
Deflexilodes gibbosus Chevreux, 1888		+		
Dexamine spinosa Montagu, 1813	+	+		
Diogenes pugilator Roux, 1829				+
Eudorella truncatula Bate, 1856		+	+	+
Gammarellus carinatus Rathke, 1843			+	
Gammaropsis palmata Stebbing & Robertson, 1891				+
Hyale sp.	+			
Iphinoe elisae Bacescu, 1950		+	+	+
Iphinoe serrata Norman, 1867			+	+
Iphinoe sp. Bate, 1856			+	
Iphinoe tenella Sars, 1878			+	+
Iphinoe trispinosa Goodsir, 1843				+
Isopoda sp. Latreille, 1817		+		

Species / Group	UA	RO	BG	TR
Liocarcinus navigator Herbst, 1794	+		+	
Medicorophium runcicorne Della Valle, 1893			+	+
Megaluropus agilis Hoeck, 1889		+		
Megamphopus cornutus Norman, 1869				+
Microdeutopus algicola Della Valle, 1893	+			
Microdeutopus damnoniensis Bate, 1856		+	+	
Microdeutopus gryllotalpa Costa, 1853	+	+	+	+
Microdeutopus versiculatus Spence Bate, 1857		+	+	+
Nototropis guttatus Costa, 1851		+		
Nototropis massiliensis Bellan-Santini, 1975		+	+	
Orchomene grimaldii Chevreux, 1890		+		
Orchomene humilis Costa, 1853	+	+		+
Orchomene sp. Boeck, 1871			+	
Perioculodes longimanus Spence Bate &Westwood, 1868	+		+	+
Phtisica marina Slabber, 1749	+	+	+	+
Pisidia bluteli Risso, 1816	+			
Pseudoprotella phasma Montagu, 1804		+	+	
Stenosoma capito Ratche, 1837		+		
Synchelidium haplocheles Grube, 1864		+	+	
Synchelidium maculatum Stebbing, 1906			+	+
Upogebia pusilla (Petagna, 1792)				+
Ophiuroidea				
Amphiura stepanovi Dijakonov, 1956	+	+	+	+
Ophiuroidea sp. Müller & Troschel, 1840		+	+	+
Holothuroidea				
Holothuroidea sp. Blainville, 1834		+		+
Holothuroidea sp. Burmeister, 1837			+	
Holothuroidea sp.			+	
Leptosynapta inhaerens O.F. Muller,1776		+		+
Oestergrenia digitata Montagu, 1815	+			
Stereoderma kirchsbergii (Heller, 1868) Panning, 1949			+	
Phoronida				
Phoronis sp. Wright, 1856		+	+	+
Ascidiacea				
Ascidiella aspersa Müller, 1776	+		+	
Ciona intestinalis Linnaeus, 1767		+		
Eugyra adriatica Drasche, 1884		+	+	+
Molgula appendiculata Heller, 1877		+	+	+
Tunicata sp. Lamarck, 1816		+	+	+



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