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Project: **INNOVATIVE INSTRUMENTS FOR ENVIRONMENTAL ANALYSIS IN NORTH WESTERN BLACK SEA BASIN (BLACK SEA e-EYE)**
Cod proiect: **MIS-ETC No 1475**

GA1: Monitoring physical-chemical and biological parameters of surface aquatic systems in North-Western Black Sea Basin

Research initiative 2

Elaboration of sampling and analysis methods for parameters and indicators (Danube delta)

Danube Delta National Institute for research and Development (DDNITL Partner 1)

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Introduction

One of the basic problems of environmental water analysis is that generally it must be carried out with selected portions (i.e., samples) of the water of interest, and the quality of this water must then be inferred from that of the samples. If the quality is essentially constant in time and space, this inference would present no problem. However, such constancy is rare if ever observed in the real world; in most circumstances virtually all waters show both spatial and temporal variations in quality. It follows that the timing and choice of location for taking water samples must be chosen with great care. Also, since an increase in the number of sampling locations and sampling occasions increases the cost of the measurement program, it is important to attempt to define the minimal number of sampling positions and occasions needed to provide the desired information. The whole process of analyzing a material consists of several steps: sampling, sample storage, sample preparation, measurement, evaluation of results, comparison with standards or threshold values, and assessment of results (Nollet 2007).

2. Elaboration of sampling and analysis methods for parameters and indicators

Areas/sites and sampling frequency

Danube River – Danube Delta – Black Sea is a very complex system, with water flow, together with its pollutants, from upstream to Black Sea discharge. Danube Delta is an important ecosystem, that receive and same time naturally and partially cleans discharging water into Black Sea, having a buffer role against pollutants.

To understand and study this complicated process it was considered to monitor and analyze historical data regarding water physical-chemical and biological parameters on Danube River at the delta entrance with splitting point of Tulcea arm in between and to those three branches discharge into Black Sea adding four large lakes inside the delta.

The sampling points were established in 9 points from Danube Delta Biosphere Reserve (Ceatal Chilia, Ceatal Sf. Gheorghe, Periprava, Sulina, Sf. Gheorghe, Fortuna Lake, Isac Lake, Merhei Lake, Rosu Lake). (**Figure 2.1**)

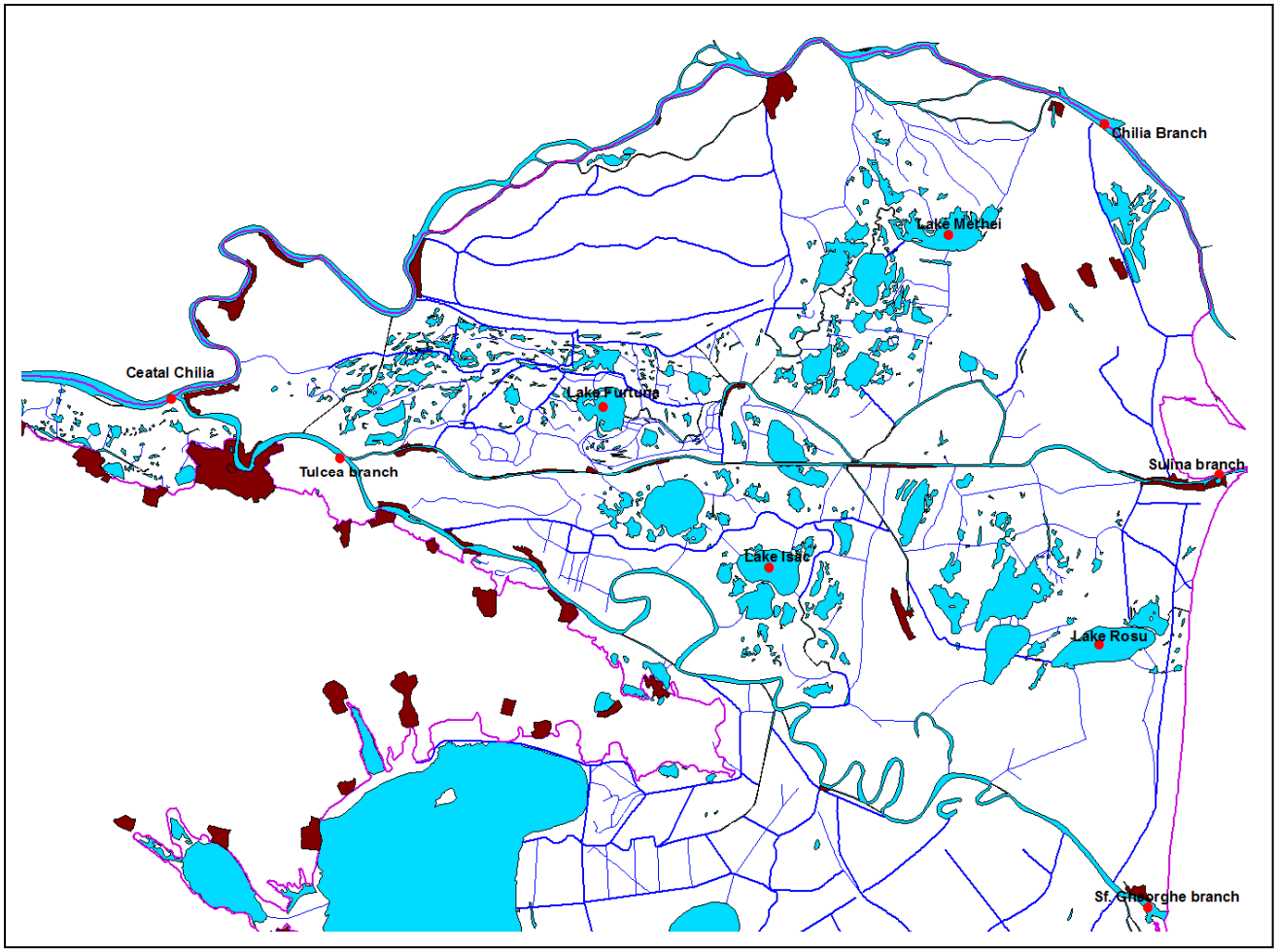

Figure 2.1 Sampling points from Danube Delta Biosphere Reserve

The coordinates (GPS latitude and longitude) of 9 sampling points and the frequency of chemistry, phytoplankton, zooplankton, macroinvertebrates, macrophytes and fish sampling are presented in **Table 2.1**.

Table 2.1 Sampling points of surface aquatic systems in North-Western Black Sea Basin, coordinates and sampling frequency

No	Waterbody code	Monitoring water bodies	GPS N latitude	GPS E longitude	Chemistry	Phytoplankton	Zooplankton	Macroinvertebrates	Macrophytes	Fish
1		Danube river - Ceatal Chilia	45.228785°	28.734599°	6 sampling expedition x 1 station	6 sampling expedition x 5 mixing samples	6 sampling expedition x 3 stations	0	0	0
2		Tulcea branch - Ceatal Sf. Gheorghe	45.188082°	28.887228°	6 sampling expedition x 1 stations	6 sampling expedition x 5 mixing samples	6 sampling expedition x 3 stations	0	0	0
3		Chilia Branch - Periprava	45.389181°	29.591428°	6 sampling expedition x 1 station	6 sampling expedition x 5 mixing samples	6 sampling expedition x 3 stations	0	0	0
4		Sulina branch - Sulina	45.161426°	29.684776°	6 sampling expedition x 1 stations	6 sampling expedition x 5 mixing samples	6 sampling expedition x 3 stations	0	0	0
5		Sf. Gheorghe branch - Sf. Gheoreghe	44.885216°	29.607264°	6 sampling expedition x 1 station	6 sampling expedition x 5 mixing samples	6 sampling expedition x 3 stations	0	0	0
6		Danube Delta - Lake Furtuna	45.216682°	29.127284°	6 sampling expedition x 1 station	6 sampling expedition x 5 mixing samples	6 sampling expedition x 5 stations	3 sampling expedition x 3 stations	3 sampling expedition x 6 transects	3 sampling expedition x 12 stations with nordic gillnets + 3 stations with electrofishing
7		Danube Delta - Lake Isac	45.110258°	29.273903°	6 sampling expedition x 1 station	6 sampling expedition x 5 mixing samples	6 sampling expedition x 5 stations	3 sampling expedition x 3 stations	3 sampling expedition x 6 transects	3 sampling expedition x 12 stations with nordic gillnets + 3 stations with electrofishing
8		Danube Delta - Lake Merhei	45.321178°	29.445967°	6 sampling expedition x 1 station	6 sampling expedition x 5 mixing samples	6 sampling expedition x 5 stations	3 sampling expedition x 3 stations	3 sampling expedition x 6 transects	3 sampling expedition x 12 stations with nordic gillnets + 3 stations with electrofishing
9		Danube Delta - Lake Rosu	45.054600°	29.571263°	6 sampling expedition x 1 station	6 sampling expedition x 5 mixing samples	6 sampling expedition x 5 stations	3 sampling expedition x 3 stations	3 sampling expedition x 6 transects	3 sampling expedition x 12 stations with nordic gillnets + 3 stations with electrofishing

All the physical-chemical and biological parameters necessary to be analyzed to achieve the project objectives are presented in the **Table 2.2**.

Table 2.2 Physical-chemical and biological parameters of surface aquatic systems in North-Western Black Sea Basin

Physical-chemical	Phytoplankton	Zooplankton	Macroinvertebrates	Macrophytes	Fish	Indicators for chemical and/or ecological status possible to be investigated	Relationships possible be be investigated
Water temperature (^oC)	Richness	Richness	Richness	Richness	Richness	Water Quality Index (WQI) for Chemistry	Investigation of correlation between determined present and historical pollution and the quality of marine costal ecosystems.
Secchi disk transprency (m)	Abundance	Abundance	Abundance	Abundance	Abundance	Ecological risk factors (heavy metals in sediments) for Chemistry	heavy metals (surface water)-heavy metals (tissue of fish)
Water depth (m)	(Biomass)	(Biomass)	(Biomass)	(Biomass)	Biomass	Saprobic Index (Pantle Buck Method) for Phytoplancton, Zooplancton and Macroinvertebrates	nutrients (total N and/or P) - chlorophyll-a
pH						Diversity – Shannon Wienner for Phytoplancton, Zooplancton, Macroinvertebrates, Macrophites and Fish	transparency - aquatic vegetation
Dissolved oxygen						WFD indicators for chemistry and biota	log cchlorophill-a - log Seechy disk transparency
Biochemical oxygen demand						Trophic State Index (TSI) Carlson et al 1996	vegetation - fish compozition
Chemical Oxygen Demand						Secchi disk transparency/Depth	
Ammonium							
Nitrite							
Nitrate							
Total nitrogen							
Phosphate							
Total phosphorus							
Chlorophyll “a”							
Filterable residue dried at 105^0C							
Chloride							
Sulphate							
Calcium							
Magnesium							
Sodium							
Total chromium							
Copper							
Zinc							
Arsenic							
Lead							
Cadmium							
Total iron							
Mercury							
Total manganese							
Nickel							
Anionic detergent active							

2.1. Physical-chemical indicators

In this chapter are presented the sampling and the analysis methods for physical-chemical quality elements from all selected sampling points.

2.1.1. Sampling methods

2.1.1.1. General physical-chemical quality elements (thermal, salinity, oxygenation)

Chemistry parameters are sampled using a fast speed boat by a one day delta round trip reaching all sampling points.
For the analysis of general physical-chemical quality elements (**thermal, salinity**) the samples are transported to the laboratory in completely full, tightly stoppered bottles with no preservatives added.
The water samples should be stored in a cool, dark place to 1-5°C, for analysis in the laboratory.

The method for determination of **dissolved oxygen** is titration method (the Winkler method). This method involves the chemical fixation of the oxygen in a water sample collected in an air-tight bottle. Fixation is carried out in the field and the analysis, by titration, is carried out in the laboratory.
Samples taken for analysis by titration must be taken with great care to ensure no air bubbles are trapped in the bottle, which must be filled to overflowing and stoppered. The necessary reagents must be added for oxygen fixation immediately the sample is taken and the bottles must be protected from sunlight until the determination is carried out, which should be as soon as possible. Regardless of the analytical method, the water temperature must be measured at the time of sampling. For dissolved oxygen determination a preservation with 1 ml $MnSO_4$ + 2 ml alkali reagent (NaOH or KOH +KI or NaI in 50 ml water +NaN_3) and storage for analysis in the laboratory is needed.

Water samples collected for **Biochemical oxygen demand** (**BOD)** measurement must not contain any added preservatives and must be stored in glass bottles. Ideally the sample should be tested immediately since any form of storage at room temperature can cause changes in the BOD (increase or decrease depending on the character of the sample) by as much as 40 per cent. Storage should be at 5° C and only when absolutely necessary.

Samples for **Chemical Oxygen Demand (COD-Cr)** analysis are collected in bottles which do not release organic substances into the water, such as glass-stoppered glass bottles. Ideally samples should be analyzed immediately, or if unpolluted, within 24 hours provided they are stored cold. If analysis cannot be carried out immediately, the samples should be preserved with sulphuric

acid. For prolonged storage samples should be deep frozen. Unfiltered samples containing settleable solids should be homogenised prior to sub-sampling. The standard method for measurement of COD is oxidation of the sample with potassium dichromate in a sulphuric acid solution (although other oxidants can be used which may have different oxidation characteristics) followed by a titration. It is extremely important that the same method is followed each time during a series of measurements so that the results are comparable.

2.1.1.2. Nutrients

All water samples for **nutrients** analysis are preserved with 1 ml chloroform for 500 ml water, store at 4^0 C and analyzed.
For the analysis of organic nitrogen (mg/L) the samples were preserved on the field with 1 mL H_2SO_4 concentrate for 100 mL sample, store at 4^0 C and analyzed in the laboratory.
Prior to analysis, water samples are filtered through 0.45 μm pore-size membranes.

Samples taken for **chlorophyll "a"** analysis in the laboratory are collected in polythene bottles. The water sample is immediately filtered after collection through a glass filter of 4.5 cm diameter. The volume of the sample shall be 50 mL – 2 L depending on trophic conditions and the amount of suspended solids. The filter is folded with the exposed side inside. Samples are stored in a cool dark place for up to 8 hours. However, once filtered through a glass fibre filter, the filter can be stored frozen for a short period prior to analysis.

2.1.1.3. Metals

Samples for metals analysis are usually pre-treated by acidification prior to transportation to the laboratory to suppress hydrolysis, sorption and other processes which affect concentration. However, such preservation techniques destroy the equilibrium of the different forms of the metals, and can be used only for determination of total concentrations.
For determination of dissolved metals, it is recommended that the samples are filtered through 0.45 μm pore diameter membrane filters (using ultra-clean equipment in a laminar flow hood). The filtered sample should be acidified for preservation. Removal of the particulate matter by filtration prevents dissolution or desorption of trace metals from the particulate phase to the dissolved phase within the sample. A very high degree of cleanliness in sample handling at all stages of collection and analysis is necessary (such as, use of ultra-pure acids to clean glassware or PTFE (polytetrafluoroethene) utensils, use of a laminar flow hood for sample manipulation and special laboratories with air filtration and purification systems) to avoid contamination and incorrect results.

2.1.2. Analysis methods

All indicators (general physical-chemical quality indicators, nutrients and heavy metals) were analyzed using European Standards adapted to Romanian legislation.

2.1.2.1. General physical-chemical quality elements (thermal, oxygenation, salinity)

Temperature (°C) - Temperature is immediately measured after sampling using Multi parameter instrument pH/conductivity WTW 340i.

Transparency (cm) – is an in-situ measurement using Secchi disc.
Transparency is easily measured in the field. It is determined by lowering a circular disc, Secchi disc, on a calibrated cable into the water until it just disappears. The depth at which it disappears, and just reappears, is recorded as the depth of transparency. A Secchi disc is usually 20-30 cm in diameter (although the result is not affected by the disc diameter), and colored white or with black and white sectors.

pH - is determined *in situ* using Multi parameter instrument pH/conductivity WTW 340i. As pH is temperature dependent, the water temperature must also be measured in order to determine accurately the pH.
If field measurement is not possible, samples are transported to the laboratory in completely full, tightly stoppered bottles with no preservatives added.
The analysis standard used for pH determination is SR ISO 10523/2009 – Water quality. Determination of pH.
The basic principle of electrometric pH measurement is determination of the activity of the hydrogen ions by potentiometric measurement using a standard hydrogen electrode and a reference electrode.

Electrical conductivity mS/cm - is usually measured *in situ* using Multi parameter instrument pH/conductivity WTW 340i.
If field measurement is not possible, Conductivity is measured in laboratory according with SR EN 27888/1997 (ISO 7888) Water quality. Determination of electrical conductivity.

Chloride mg/L
In the laboratory, chloride concentrations are determined according with SR ISO 9297/ 2001. Water quality. Determination of chloride. Silver nitrate titration with chromate indicator.
In a neutral or slightly alkaline solution, potassium chromate can indicate the end point of the silver nitrate titration of chloride. Silver chloride is precipitated quantitatively before red silver chromate is formed.

Total hardness mg-eq/L
The water samples are stored in a cool place to 2-5°C, for analysis in the laboratory.

Determination of total hardness is made using EDTA Titrimetric Method according with STAS 3026-62. Titrimetric determination of total hardness.
Ethylenediaminetetraacetic acid and its sodium salts (EDTA) form a chelated soluble complex when added to a solution of certain metal cations. If a small amount of a dye such as Eriochrome Black T is added to an aqueous solution containing calcium and magnesium ions at a pH of 10 ± 0.1, the solution becomes wine red. If EDTA is added as a titrant, the calcium and magnesium will be complexed, and when all of magnesium and calcium has been complexed the solution turns from wire red to blue, marking the end point of the titration. Magnesium ions must be present to yield a satisfactory end point. To insure this, a small amount of complexometrically neutral magnesium salt of EDTA is added to the buffer; this automatically introduces sufficient magnesium and obviates the need for a blank correction.
The sharpness of the end point increases with increasing pH. However, the pH cannot be increased indefinitely because of the danger of precipitating calcium carbonate, $CaCO_3$, or magnesium hydroxide, $Mg(OH)_2$, and because the dye changes color at high pH values. The specified pH of 10 ± 0.1 is a satisfactory compromise. A limit of 5 min is set for the duration of the titration to minimize the tendency toward $CaCO_3$ precipitation.

Sulphates, mg/L
The concentration of sulphates is determined by spectrometric method. Sulphate ions are precipitated as barium sulphate in acidic medium with barium chloride. The absorption of light by this precipitated suspension is measured spectrophotometrically at 400 nm.

Suspended solids, mg/L **-** are determined using the filtration through glass fibre filters according with SR EN 872-2005. Water quality. Determination of suspended solids. Method by filtration through glass fibre filters.
A well-mixed sample is filtered through a weighed standard glass fibre filters and the residue retained on the filter is dried to a constant weigh at 103 to 105°C. The increase in weight of the filter represents the total suspended solids. If the suspended material clogs the filter and prolongs filtration, the difference between the total dissolved solids may provide an estimate of the total suspended solids.

Filterable residuum, mg/L **-** are determined using STAS 9187-84 – Surface, underground and waste waters. Residuum determination.
A well-mixed sample is evaporated in a weighed dish and dried to constant weight in an oven at 103 to 105°C. The increase in weight over that of the empty dish represents the total solids.

Anionic surfactants, mg/L **-** are determined according with SR EN 903 / 2003. Water quality. Determination of anionic surfactants by measurement of the methylene blue index MBAS.
Methylene blue active substances (MBAS) bring about the transfer of methylene blue, a cationic dye, from an aqueous solution into an immiscible organic liquid upon equilibration. This occurs through ion pair formation by the

MBAS anion and the methylene blue cation. The intensity of the resulting blue color in the organic phase is a measure of MBAS. Anionic surfactants are among the most prominent of many substances, natural and syntethetic, showing methylene blue activity.

Dissolved oxygen, mg O_2/L - is determined using Automatic Titrator 702 SM Titrino according with SR EN 25813 ISO 5813 June 2000, Water quality. Determination of dissolved oxygen content. Iodometric method.
The iodometric test is the most precise and reliable titrimetric procedure for DO analysis. It is based on the addition of divalent manganese solution, followed by strong alkali, to the sample in a glass-stoppered bottle. DO rapidly oxidizes an equivalent amount of the dispersed divalent manganous hydroxide precipitate to hydroxide of higher valency states. In the presence of iodide ions in an acidic solution, the oxidized manganese reverts to the divalente state, with the liberation of iodine equivalent to the original DO content. The iodine is then titrated with a standard solution of thiosulfate. The titration end point can be detected visually, with a starch indicator.

Biochemical Oxygen Demand (BOD_5) mg O_2/L
Incubation at 20^0C for a defined period of time, 5 days in the dark, completely filled and tightly sealed bottles.
Determination of BOD_5 using Automatic Titrator 702 SM Titrino according with SR EN 1899-2/2002. Water quality. Determination of biochemical oxygen demand after n days (BOD_n). Method for undiluted samples.
The method consists of filling with sample, to overflowing, an airtight bottle of the specified size and incubating it at the specified temperature for 5 days. Dissolved oxygen is measured initially and after incubation, and the BOD is computed from the difference between initial and final DO. Because of the initial DO is determined immediately after the dilution is made, all oxygen uptake, including that occurring during the first 15 min, is included in the BOD measurement.

Chemical Oxygen Demand (COD-Cr)
The Chemical Oxygen Demand (COD-Cr) concentrations are determined according with SR ISO 6060/ 1996. Water quality. Determination of the chemical oxygen demand.
The water samples were preserved by acidification to pH ≤ 2 using concentrate H_2SO_4.
Most type of organic matter is oxidized by a boiling mixture of chromic and sulfuric acid. A sample is refluxed in a strongly acid solution with a known excess of potassium dichromate ($K_2Cr_2O_7$). After digestion, the remaining unreduced $K_2Cr_2O_7$ is titrated with ferrous ammonium sulfate to determine the amount of $K_2Cr_2O_7$ consumed and the oxidizable organic matter is calculated in terms of oxygen equivalent.

2.1.2.2. Nutrients

Ammonium (N -NH_4^+), mg N/L

The ammonium concentration, $N\text{-}NH_4^+$ mg/L was determined at 655 nm by measuring the absorption of the blue compound formed by the reaction of ammonium ion with salicylate and hypochlorite ions in the presence of sodium nitroprusside, according with SR ISO 7150-1. Water quality. Determination of ammonium. Part 1. Manual spectrometric method at UVVIS Lambda 10 Perkin Elmer Spectrometer.
For ammonium it was made the flow chart and the calibration curve. The calibration curve was made using the ammonium chloride, Scharlau, reagent grade, ACS, ISO. The calibration curve is linear and was made in five points. The flow chart was made using the ammonium standard solution traceable to SRM from NIST NH_4Cl in H_2O 1000 mg/L NH_4^+ CertiPUR. Using the Excel interface, for each calibration curve, was calculated, the equation and the R^2 coefficient. The coefficient R^2, for the calibration curve has the value 0.9999 that represents a very good correlation between the absorbance and the standard concentrations.

Nitrite ($N\text{-}NO_2^-$), mg N/L

Nitrite ($N\text{-}NO_2^-$) is determined through formation o reddish purple azo dye produced at pH 2.0 to 2.5 by coupling diazotized sulfanilamide with N-(1-naphthyl)-ethylendiamine dihydrochloride, according with SR EN 26667/ ISO 6777/2002. Determination of nitrite. Molecular absorption spectrometric method, using the UVVIS Lambda 10 Perkin Elmer Spectrometer at 540 nm. The analysis was made on filtered water.
For nitrite it was made the flow chart and the calibration curve. The calibration curve was made using the sodium nitrite, GR for analysis ACS, Reag. Ph Eur. The calibration curve is linear and was made in five points. The flow chart was made using the nitrite standard solution traceable to SRM from NIST $NaNO_2$ in H_2O, 1000 mg/L NO_2^- CertiPUR. Using the Excel interface, for each calibration curve, was calculated, the equation and the R^2 coefficient. The coefficient R^2, for the calibration curve has the value 0.9998 that represents a very good correlation between the absorbance and the standard concentrations.

Nitrate ($N\text{-}NO_3^-$), mg N/L

The nitrate, expressed as $N\text{-}NO_3^-$, was determined according with SR ISO 7890-3:2000 – Water quality. Determination of nitrate. Part 3. Spectrometric method using sulfosalicylic acid, by spectrometric measurement of yellow compound absorbance formed by reaction of sulfosalicylic acid (formed by addition of sodium salicylate in the sample and sulfuric acid) with nitrate followed by treatment with alkaline solution, at UVVIS Lambda 10 Perkin Elmer Spectrometer (415 nm).
For nitrate it was made the flow chart and the calibration curve. The calibration curve was made using the potassium nitrate, Scharlau, GR for analysis ACS, Reag. Ph Eur. The calibration curve is linear and was made in five points. The flow chart was made using the nitrate standard solution traceable to SRM from NIST $NaNO_3$ in H_2O, 1000 mg/L NO_3^- CertiPUR. Using the Excel interface, for each calibration curve, was calculated, the equation and the R^2 coefficient. The coefficient R^2, for the calibration curve

has the value 0.9999 that represents a very good correlation between the absorbance and the standard concentrations.

Organic nitrogen, mg/L

For organic nitrogen, the samples were analyzed according with SR ISO 5663-1984. Water quality. Determination of Kjeldahl nitrogen. Method after mineralization with selenium. The samples were mineralization to form ammonium sulfate, from which ammonia is liberated. The next step is the conversion of nitrogen compounds responding to the test of ammonium sulfate by mineralization of the sample with sulfuric acid, containing a high concentration of potassium sulfate to raise the point o the mixture, in the presence of selenium which acts as a catalyst. The third step is the determination of ammonium ion in the mineralizate by spectrometry on UVVIS Lambda 10 Perkin Elmer Spectrometer, at 655 nm.

For organic nitrogen it was made the flow chart and the calibration curve. The calibration curve was made using the ammonium chloride, Scharlau, reagent grade, ACS, ISO. The calibration curve is linear and was made in five points. The flow chart was made using the ammonium standard solution traceable to SRM from NIST NH_4Cl in H_2O 1000 mg/L NH_4^+ CertiPUR. Using the Excel interface, for each calibration curve, was calculated, the equation and the R^2 coefficient. The coefficient R^2, for the calibration curve has the value 0.9999 that represents a very good correlation between the absorbance and the standard concentrations.

Ortho-phosphate ($P\text{-}PO_4^{3-}$), mg/L

Filtration through a 0.45 µm –pore – diameter separates dissolved phosphorus from suspended forms of phosphorus.

The ortho-phosphate ($P\text{-}PO_4^{3-}$) expressed as mg/L, was determined according with SR EN 6878/2005. Water quality. Determination of phosphorus. Ammonium molybdate spectrometric method, using UVVIS Lambda 10 Perkin Elmer Spectrometer at 880 nm.

Ammonium molybdate and potassium antimony tartrate react in acid medium with orthophosphate to form a heteropoly acid – phosphomolybdic acid – that is reduced to intensely colored molybdenum blue by ascorbic acid.

For ortho-phosphate was made the flow chart and the calibration curve. The calibration curve was made using the potassium dihydrogen phosphate, Scharlau, reagent grade, ACS, ISO. The calibration curve is linear and was made in five points. The flow chart was made using the phosphate standard solution traceable to SRM from NIST KH_2PO_4 in H_2O 1000 mg/L PO_4^{-3} CertiPUR. Using the Excel interface, for each calibration curve, was calculated, the equation and the R^2 coefficient. The coefficient R^2 for the calibration curve has the value 0.9999 that represents a very good correlation between the absorbance and the standard concentrations.

Total phosphorus, mg/L

For total phosphorus, the samples were treated on unfiltered water.

The ortho-phosphate ($P\text{-}PO_4^{3-}$) expressed as mg/L, was determined according with SR EN 6878/2005. Water quality. Determination of phosphorus.

Ammonium molybdate spectrometric method, using UVVIS Lambda 10 Perkin Elmer Spectrometer at 880 nm.
Ammonium molybdate and potassium antimonyl tartrate react in acid medium with orthophosphate to form a heteropoly acid – phosphomolybdic acid – that is reduced to intensely colored molybdenum blue by ascorbic acid.

Chlorophyll "a", µg/L
The sample is collected with a volumetric sampler.
The concentrations of chlorophyll "a", was determined according with SR ISO 10260/ 1996. Water quality. Measurement of biochemical parameters – Spectrometric determination of the chlorophyll "a" concentration.
Ethanol is heated to 75 ^{0}C, poured over the samples, grinded and extracted for 5 min, cooled under running water and further extracted over-night in a refrigerator in the dark. The extract is then clarified by filtration into a calibrated flask of 10 ml capacity and analyzed using UVVIS Lambda 10 Perkin Elmer Spectrometer at 665 and 750 nm using ethanol as blank. Results are reported as µg/L.

2.1.2.3. Metals

The heavy metals concentrations (Cr, Cu, Zn, As, Pb, Cd, Fe, Mn, Ni, Na, K) are determined by Inductively Coupled Plasma with Mass Spectrometer Perkin Elmer Elan DRCe according to SR EN ISO 17294-2/ 2005. Water quality. Application of inductively coupled plasma mass spectrometry (ICP MS). Part 2. Determination of 62 elements.
Surface water samples were filtered through 0.45 µm pore-size membranes on-board, acidified to pH<2 with nitric acid and stored in a plastic bottle.
The ICP MS method measures ions produced by a radio-frequency inductively coupled plasma. Analyte species originating in a liquid are nebulized and the resulting aerosols are transported by argon gas into the plasma torch. The ions produced are entrained in the plasma gas and introduced, by means of an interface into a mass spectrometer. The ions produced in the plasma are sorted according to their mass-to-charge ratios and quantified with a channel electron multiplier.
The calibration curves were made using the Perkin Elmer Pure Plus Atomic Spectroscopy Standard, certified reference material 10 µg/ml, Multi-element ICP-MS calibration STD.3, matrix 5% HNO_3. The calibration curves are linear and made in five points. Using the Excel interface, for each calibration curves, were calculated, the equations and the R^2 coefficients.
The coefficient R^2, for the calibration curves has the values between 0.9994 - 0.9997, that represent a very good correlation between the intensity and the standard concentrations.

Mercury
The mercury concentration is determined by Cold Vapor Atomic Absorption (CVAA) Spectrometer FIMS 400 PerkinElmer according to SR EN 1783/ February 2003. Water quality. Determination of mercury.

Surface water samples were filtered through 0.45 µm pore-size membranes on-board, acidified to pH<2 with nitric acid and stored in a plastic bottle.
The CVAA procedure is a physical method based on the absorption of radiation at 253.7 nm by mercury vapor. The analyst may use a Flow Injection Mercury Analysis System (FIAS) in conjunction with an atomic absorption spectrometer or a stand-alone Flow Injection Mercury System (FIMS). Organic mercury compounds are oxidized and the mercury is reduced to the elemental state and aerated from solution in a closed system. The mercury vapor passes through a cell positioned in the light path of an atomic absorption spectrometer. Alternatively, the stand-alone mercury analyzer can be used which contains a light source and detector specific for mercury and does not require the use of a separate atomic absorption spectrometer. Absorbance (peak height) is measured as a function of mercury concentration and recorded in the usual manner.
The calibration curves were made using the Perkin Elmer Pure Plus Atomic Spectroscopy Standard, certified reference material 10 µg/ml Mercury in 5% HNO_3.
The calibration curves are linear and made in five points. Using the Excel interface, for each calibration curves, were calculated, the equations and the R^2 coefficients. The coefficient R^2, for the calibration curves has the values between 0.9994 - 0.9997, that represent a very good correlation between the intensity and the standard concentrations.

2.2. Phytoplankton

Water quality affects the abundance, species composition, stability, productivity, and physiological condition of indigenous populations of aquatic organisms. Therefore, the nature and health of the aquatic communities is an expression of the quality of the water. Biological methods used for assessing water quality include the collection, counting, and identification of aquatic organisms; biomass measurements; measurements of metabolic activity rates; measurements of toxicity, bioconcentration, and bioaccumulation of pollutants; and processing and interpretation of biological data. Information from these types of measurements may serve one or more purposes.

The term ‘’plankton” refers to those microscopic aquatic forms having little or no resistance to currents and living free-floating and suspended in open or pelagic waters. The phytoplankton occurs as unicellular, colonial, or filamentous forms. Phytoplankton have been long used as indicators of water quality. Some species flourish in highly eutrophic waters while others are very sensitive to organic and/or chemical wastes. Some species develop noxious blooms, sometimes creating offensive tastes and odors or anoxic or toxic conditions resulting in animal deaths or human illness.

Because of their short life cycles, plankters respond quickly to environmental changes, and hence their standing crop and species composition are more likely to indicate the quality of the water mass in which they are found. They

strongly influence certain non-biological aspects of water quality, and in a very practical sense, they are a part of water quality.

The frequency and location of sampling is dictated by the purpose of the study. Locate sampling stations as near as possible to those selected for chemical and bacteriological sampling to insure maximum correlation of findings. Establish a sufficient number of stations in as many locations as necessary to define adequately the kinds and quantities of plankton in the waters studied. The physical nature of the water will influence greatly the selection of sampling stations. Sampling frequency depends on the intent of the study as well as the range of seasonal fluctuations, the immediate meteorological conditions, adequacy of equipment, and availability of personnel. Select a sampling frequency at some interval shorter than community turnover time is recommended. This requires consideration of life-cycle, length, competition, predation, flushing and current displacement. Frequent plankton sampling is desirable because of normal temporal variability and migratory character of the plankton community.

Test procedures using algae are valuable for determining the primary productivity of waterbodies. The procedures that follow measure the response of certain algae to the nutritional status of water or measure the response of algae to the materials that interfere with their normal metabolism. Because water may vary greatly in time and point of collection, must be establish sampling programs to obtain representative and comparable data (*1989-Standard methods for the examination of water and wastewater. American Public Health Association, Washington, U.S.A).

There are many biological methods used for assessing, processing and interpretation of biological data, information from these types of measurements being able to serve for different purposes Török (2003-2004)
Phytoplankton samples were prelevated from locations on Danube branches.

The samples were collected in plastic containers of 1 liter and preserved with 5 ml Lugol solution. Prelevation point as well as site characteristics of area are noted in samples notebook for more information about weather, water colour and other parameters that can indicate water quality.
Samples may be taken from a established depth or from more samples from different depths in case of deep waters. In case on a sample from more depths is necessary to mix collected samples to obtain one sample from sampling point (Török, 2006).

The organisms contained in water samples must be concentrated in the laboratory before analysis. Sedimentation is the preferred method of concentration because it is nonselective and nondestructive, although many of the picoplankton, the smaller nanoplankton, and actively swimming flagellates may not settle completely. The volume concentrated varied inversely with the abundance of organisms and is related to sample turbidity. It may be as small

as 1 ml for use with an inverted microscope or as large as 1 l for general phytoplankton enumeration.

For microscopic analysis were followed few steps:

a. sedimentation for 7 days
b. siphoning until 100 ml
c. sharing sample in two test tubes, one of 40 ml (for diatoms determination) and 15 ml for quantitative and qualitative analyses of the sample.

Under microscopic analyses was identified of phytoplankton species, elaborated species list and calculate number of individuals per liter from every sample.

Some phytoplankton species are unicellular while others are multicellular. To enumerate plankton use a counting cell or chamber that limits the volume and area for ready calculation of population densities. To identify organisms use standard bench references. Do not count dead cells or broken diatom frustules. Magnification is important in phytoplankton identification and enumeration. The greatest disadvantage associated with the cell is that objectives providing high magnification cannot be used. Use either a standard or an inverted compound microscope for algal identification. Equip standard or inverted compound microscope with a mechanical stage capable of moving all parts of counting cell past the objectives lens. Use objectives to provide adequate working distance for the counting chamber. Magnification requirements vary with the plankton fraction being investigated, the type of microscope, counting chamber used, and optics. (*1989-Standard methods for the examination of water and wastewater. American Public Health Association, Washington, U.S.A)

For “in situ” phytoplankton biomass determination was used bbe FluoroProbe, which can quickly assess the concentration of chlorophyll “a” in water weight. This achieve analyse by emitting a beam of light of different intensities for each group of algae. This device, can difference primary groups of algae from mixed populations and can determinate quantity of organic matter find in decomposition until a depth of 100 m.

Based on relative intensity of fluorescence light of four light waves are differenced next taxonomic groups of algae: 470nm LED for green algae; 610nm LED for blue-green algae; 525nm LED for diatoms; 570nm LED for cryptophyceae) and other fluorescing matter (for example, yellow substances) to enhance the accuracy of the measurements (Török 2009).

2.3 Zooplankton

Zooplankton is mainly applied as species-indicators of saprobic or trophic state of the system. For a long time, the use of zooplankton community

characteristics as a tool in trophic condition has been considered of rather low information value. Now, are more valuable as indicators than has generally been realized.

The frequency and location of zooplankton sampling is dictated by the purpose of the study. Locate sampling stations as near as possible to those selected for phytoplankton, benthic organisms and physical-chemical sampling.
Surface water sample was collected from the lakes in five stations per lake and three stations per Danube branch stations.

Zooplankters are collected by filtering 30 liters of water from the surface of the water body through plankton net (55 μm mesh size) and was fixed immediately with absolute ethanol, into plastic container (Clesceri et al. 1989, Wetzel and Likens 1991).
The systematic identification of zooplankton was made by using standard keys of principals taxonomic groups Rotifera, Copepoda and Cladocera (Margalef 1953, Voigt 1956-1957, Rudescu 1960, Dussart and Gras 1966, Dussart 1967, Dussart 1969, Pontin 1978, Negrea 1983, Dumont et al. 1984, Clesceri et al. 1989). The quantitative analysis of planktonic organisms was carried out using Sedgwick Rafter's American Public Health Association (1989) plankton counting chamber.

Characteristics of zooplankton community recommended for using in monitoring as indices of eutrophication process:

-The number of dominant species;
-Number of individuals (no ind./L);
-Biomass (mg/l) w.w.- zooplankton data is expressed as wet weigh.

The diversity index of Shannon and Weaver (1948) is used to assess the equilibrium of the zooplankton community.

The degree of organic pollution was determined using the saprobic index of Pantle and Buck (1955). Five levels of organic pollution (standard methods 161/2006) which have been realized from the analysis of the saprobity scale for water contamination is used (**Table 2.3.1**.)

Table 2.3.1. Saprobity scale for water pollution.

Biological status class u/m	Term	Colour	Saprobic index Romanian (standard 161/2006)
I	high	blue	1.8
II	good	green	2.3
III	moderate	yellow	2.7
IV	poor	orange	3.2
V	bad	red	>3.2

blue	very good; natural view without any significant restrictions
green	good; overall view natural with only smaller signs of anthropogenic changes
yellow	moderate; partly changed, but still near natural view
orange	poor; strongly changed
red	unnatural; completely changed

2.4 Macroinvertebrates

It is considered most important to recognize that no one sampling method will provide enough data to reflect the actual biological community which exists in the area sampled. The use of the kick-net method used to obtain samples of the macro-invertebrate communities cannot be used to obtain samples in deep slow flowing rivers or lakes and different sampling methods have to be designed and used for this purpose.

Biological data for any given aquatic habitat does not of itself define that habitat; many other factors must be taken into account before even a basic definition of the habitat can be obtained. Among the more important are the hydrological and hydro-morphological variables which include flow rate, turbulence, and catchments characteristics. Physical-chemical factors are also important in particular, temperature, pH (acidification status), oxygenation, salinity and presence of pollutants. Other biological components of the habitat must also be considered such as macrophytes, fish and algae communities.

Standardised methods of macroinvertebrate sampling

The use of macroinvertebrates (defined as invertebrates of size greater than 0.5 mm) as indicators of surface water quality have been used extensively in last decades. This concept has however been shown in practice to be unreliable and is no longer used in river management being replaced by biological quality of surface waters, which encompasses not only macroinvertebrates but many other parameters shaping water quality.

In general the standardization of biological methods of sampling involves a definition of procedure, data interpretation and presentation, this later being referred to as biological classification.

Quantitative sampling with Ekmann dredge (ISO 10870:2012) is useful only for sampling mud, silt, muck, and sludge in water with little current. It is difficult to use when rocky or sandy bottoms or moderate macrophyte growth are present because small pebbles or grit or macrophyte stems prevent proper jaw closure. The grab weighs approximately 3.2 kg. The box-like part holding the sample has spring-operated jaws on the bottom that must be cocked manually. At the top of the grab are two hinged overlapping lids that are held open partially during descent by water passing through the sample compartment. These lids are held shut by water pressure when the sampler is being retrieved.

When sampling qualitatively, organisms are searched in as many different habitats as possible. *Dip, kick nets* are the most versatile collecting devices for shallow, flowing water, and are useful also for shoreline collecting in lakes. When combined with a standardized kicking technique, these nets are

appropriate for quantitatively sampling macroinvertebrates. A three minute kick is made in the important habitats of the aquatic ecosystem.

Sample preservation, containers, handling, and storage

After a representative sample has been collected, animals, vegetation, and substrate are preserved for picking, sorting, and analysis (e.g., taxonomic, statistical). The organisms from each sample are placed into a separate jar or vial and covered with a preservative such as 70 percent (%) ethyl alcohol, 40% isopropyl alcohol, or neutral formalin. Alcohol is less irritating to use than formalin therefore it is recommended to use.

Laboratory Processing

The preserving fluid may be decanted off and the sample rinsed with water to prevent exposure of preservative fumes to the processor. The sample content is emptied into a 500 micrometer sieve and rinse with water to remove the fixative or preservative. The rinsate is collected and disposed of properly. The content of the sieve is emptied onto a white plastic tray. Samples collected may contain a mixture of mud, sand, and debris, in addition to the desired organisms. The organisms are removed from the unwanted material and separate them into similar taxonomic groupings (e.g., Order; Family) for identification and enumeration. It is recommended that the processor use illuminated dissecting lamps to aid in this task.

A number of three points are sampled per lake. The index period (preferred period for sampling) is March-September.

2.5 Macrophytes

Method: macrophytes assessment method
General information: *South East Europe, Danube Delta Biosphere Reserve, Romania, Relevant intercalibration lake types.*
Category: Lakes (Fortuna, Isac, Merhei, Roşu)
Biological Quality Element (BQE): Macrophytes
Detected pressure(s):
Increased nutrient concentrations (eutrophication), Pollution by organic matter
Pressure-impact-relationship: No, pressure-impact relationship has not been tested.
Comments:
The macrophytes assessment method, which is primarily based on dominating indicator taxa, has been complemented with Water Framework Directive (2000/60/CEE) macrophytes assessment method, adding two more parameters: species composition and depth limit of submerged plants. Macrophytes are the indicators that can be used as monitoring species because they are sensitive to human disturbance (Short & Wyllie-Echeverria, 1996). As macrophytes are disturbance sensitive their presence, health and

abundance are likely to indicate a water body's quality status; they can be considered at good or high status if there is no evidence of degradation or loss of species from localities where they were previously found in the water body. Importantly, despite much recent research effort, the ideal environmental parameters for supporting macrophytes are not entirely understood, so that absence of species from areas apparently suited to its growth are not always explicable (Krause-Jensen *et al.*, 2003). Absence therefore, does not necessarily suggest a catastrophic loss of species, unless a historic bed was previously recorded and is no longer present.
Loss of macrophytes abundance occurs in many wetland environments (Short & Wyllie- Echeverria, 1996), often from natural causes such as high energy storms. Anthropogenic hydrodynamic stress from dredging and other activities can effects macrophyte beds due to increased suspended sediment in the water column (blocking light) or excess sedimentation (causing smothering). Macrophytes can also be sensitive to nutrient enrichment and in some areas, the species number decrease logarithmically and percentage loss of habitat increases logarithmically as nitrogen loading rates increase (Hauxwell *et al.*, 2003). However, macrophytes species can recover if conditions improve.

Nutrient enrichment may also lead to excessive growth of opportunistic epiphytic algal species, or blooming species that limit macrophyte development.

Data acquisition - Field sampling/surveying (**Figure 2.5.1 b**)
Sampling/Survey guidelines: Transect method (**Figure 2.5.1 a**)

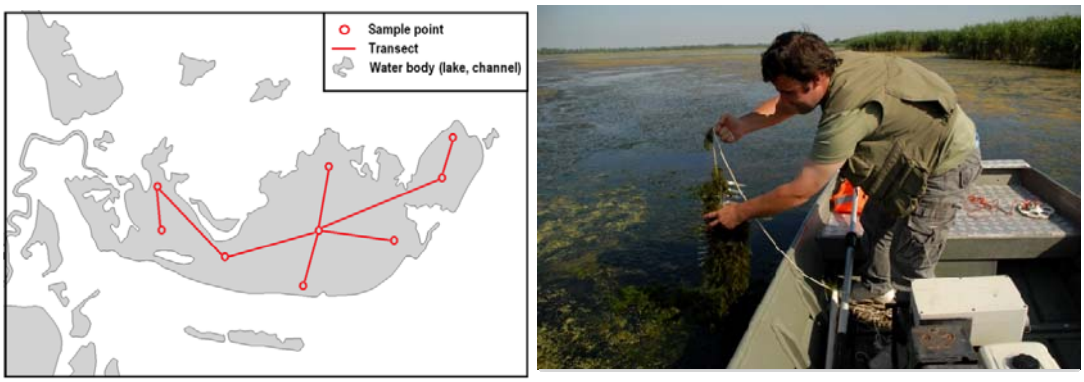

Figure 2.5.1 Example – Merheiul Mare Lake
a) Transects and sampling (stations) points; **b) Grapnel/rake macrophytes investigation**

Short description:
Passing the surface of the whole lake by boat, relative abundance of the macrophytes species of all belts (emergent, floating-leaved etc.) and all taxonomical groups are estimated for the lake in the 7 point scale (1=<1%;2=1-5%;3=5-15%;4=15-25%;5=25=50%;6=50-75%;7=75-100%)

(Kohler,1978). Using the plant hook with marked rope (or stock), the zonation and depth limits of macrophytes are determined on transects.

The frequency of transects depends on the character of the lake; they have been made after 100-500 m (**Table 2.5.1**.).

- In small lakes (< 5 ha), relevé registration of occurring macrophytes in one transect will be made on 100-200 m long sections. The transect is selected as well as possible after superficial inspection of the whole lake. It should be representative of the whole lake. A single relevé includes that part of the lake that can be readily seen from the bank and can be waded or can be sampled using a grapnel.
- In medium lakes, one transect will be made on 100-300 m long and it should be representative of the whole lake. In large lakes (e.g. Merhei), one transect will be made on 100 – 500 m long.

The relevés include both submerged and floating macrophytes and helophytes, which are rooted in the water for most of the year.

Table 2.5.1: Lakes selected for macrophytes monitoring within "Black Sea e-Eye" project

Crt. No.	Lake name	Lake type	Monitoring programme		
			surveillance	operational	investigative
1	Furtuna	2		x	x
2	Isac	1	x		
3	Merheiu Mare	1		x	
4	Rosu	1		x	x

*data according to the shape file of trans-boundary vegetation map of Danube Delta Biosphere Reserve

Method to select the sampling/survey site or area: Expert knowledge
Sampling/survey device: grapnel, rake, long-roped rake
Sampled/surveyed habitat:
Sampled habitat: All available habitats per site (Multi-habitat)
Sampling/survey month(s): June – August
Sampling should be completed from June through to August. For lakes type 3 the sampling can be carried out in September also, but no later than first decade if the temperature allows it. Monitoring is not recommended outside of this period due to seasonal variations that could affect the classification outcome and possibly lead to misclassification (**Table 2.5.2.).** Sampling should be carried out during spring low tides in order to expose the maximum area of intertidal and full bed extent.

Table 2.5.2: Sampling protocols

Surveillance Monitoring / Operational Monitoring	
Characteristics	**Macrophytes**
Measured parameters that indicate ecological status	Diversity and species abundance
Species presence in lakes	Abundant, rare in lakes

Sampling methods	Ortho- photo images and/or perpendicular sampling section on the lake shore
Natura 2000 habitats	3150, 3160
Frequency for monitoring according to WFD	once in one year every 3 years
Frequency for lake seasonality	twice in one year every year
Sampling Month	June/August
Quantity of sampling	3-10 profiles / lake with 2-3 locations in four points for each profile it should be sufficient for most of the lakes
Sampling difficulty degree	Variable, specialized sampling equipment
Field observations/laboratory	Field measures sustained by aero – photo data, profile sampling, species identification
Sampling quality/data comparison	The reference values are in accordance typical values indicators and species diversity from undisturbed significantly habitats by anthropogenic activities
Current use of biotic indicators/ ranking score	*Trophic Ranking Score* (TRS), species with small values of TRS are present especially in poor nutrient waters, and vice versa are associated with eutrophic waters, the level of diversity; The presence of functional species groups (sensitive, tolerant, indifferent); trophic macrophyte index (TMI)

Number of sampling/survey occasions (in time) to classify site or area:
One occasion per sampling season
Sample processing - **Level of taxonomical identification:**
Level: Family, Genus, Species/species groups
Specification of level of determination:
Taxa should be identified at least to genus or family level if it is not possible to identify species.
In Annex V (1.2) of the WFD, normative definitions describe the aspects of the macrophytes community that must be included in the ecological status assessment of a water body. The abundance macrophytes tool is a multimetric index composed of three individual components referred to as metrics, these are:

- Presence of characteristic taxa and indicator species;
- Total aquatic flora species number;
- Taxonomic composition (macrophytes communities and species diversity)
- The individual metrics are considered separately and have equal weighting in the final multimetric calculation.

It is not possible for a single metric to be used in isolation to derive a robust WFD classification for a water body; all metrics must be used to assess ecological status.

The macrophytes tool operates over an Ecological Quality Ratio (EQR) range from zero (major disturbance) to one (reference/minimally disturbed).
Abundance of certain groups of macrophytes species (cover such as *Isoetids, Eloids, Nymphaeids, Lemnids, Characeae*) determined by key species shoot density and spatial extent - expressed as loss/gain in percentage;
To calculate the tool, the percentage cover change in shoot density and bed extent, along with changes in taxonomic composition, are required. Taxonomic composition is based upon the stability of species richness and limited to a maximum of five species. The percentage shoot density metric has been developed to classify data using both a single sampling event and a rolling 3 year mean.

2.6 Fish

Area
General information: *South East Europe, Danube Delta Biosphere Reserve, Romania, Relevant intercalibration lake types.*
Category: Lakes
Biological Quality Element (BQE): Fish

Fish are not distributed randomly in the lakes but according to the fish species ontogeny, therefore fish species are distributed at various depths or in different horizontally areas of the lake. Even over a year the distribution is not constant but is influenced by temperature and seasonal variation. To cover this non-uniform sampling is necessary randomized and where necessary must be done a stratified sampling (not applicable in Danube Delta, for this lake-complexes not exceeding 3 meters depths, so according to "Water analysis", 2002 such a stratified sampling is required in lakes with a depth greater than 3 meters).

Two complementary methods of sampling will be use: electrical fishing will perform with SAMUS or DEKA 7000 W electrofisher machine, during 10 minutes per site, 3 sites per lake and multi meshes gillnet fishing with Northern gillnets (30 m length x 1.8 or 1.5 m high each). The Northern gillnets have 12 randomly joined panels, 2,5 meters length each panel, with multiples meshes: (4)6, 6, 8, 10, 12, 16, 20, 24, 30, 35, 45, 55 millimeters (most effective methods after Năvodaru (2008), *DIN EN 147:2005 Water quality, **CEN/TC 230, „Water analysis” 2002).

Electric fishing will carry out during the day time, in border zone with shallow water, with rich vegetation, while gillnet fishing during the night (12 hour), in the open deep water of lake, with less vegetation. Gillnets sampling period is 12 hours, 18-20 p.m. hours recommended for installation in the evening and 6-8 a.m. in the morning for removal.
The catch per unit effort (CPUE) will standardize to: individuals and/or biomass per 1-hour electric fishing and individuals and/or biomass per 100 m² gillnets / night for gillnet fishing.

The fish species have been identified using the recognized determination keys after Bănărescu (1964), and taxonomic name by recent revision (Kottelat 1997, Kottelat & Freyhof 2007, Nelson 2006, Oţel 2007 and Froese & Pauly 2014, www.fishbase.org 2014).

The relative abundance or dominance (D) was calculated as proportion of species to the total catch (Mühlenberg 1993 cited by Şindrilariu et.al. 2002): Di = ni/N ·100 (%), where, Di = dominance of species i, ni = individuals of the species i, and N = total number of individuals (**Table 2.6.1**).

Table 2.6.1 Dominance (relative abundance) classification

Relative Dominance/Abundance	
(%)	Classification
<1	sporadic
1 (2^0) - <2	subrecedent
2 (2^1) - <4	recedent
4 (2^2) - <8	subdominant
8 (2^3) - 16	dominant
>16 (2^4)	eudominant

Table 2.6.2: Lakes selected for fish monitoring

Crt. No.	Lake name	Lake type	Monitoring programme			
			Total	electric	gillnets	questionnaire
1	Furtuna	2	3 sampling expedition x 12 stations with northern gillnets + 3 stations with electrical fishing	X	X	X
2	Isac	1	3 sampling expedition x 12 stations with northern gillnets + 3 stations with electrical fishing	X	X	X
3	Merhei	1	3 sampling expedition x 12 stations with northern gillnets + 3 stations with electrical fishing	X	X	X
4	Roşu	1	3 sampling expedition x 12 stations with northern gillnets + 3 stations with electrical fishing	X	X	X

Biometric measurements used fish measurements accuracy of 1 millimeter for length and weight using electronic scales precision 1 gram.

Ecological diversity study is very important in modern ecology, but also very complex, with several issues (Odum, 1975 Gomoiu & Skolka, 2001 Sârbu & Benedek, 2004). Regarding α diversity, in addition to species richness, heterogeneity, etc. more other is equitability. The main index used in the analysis of biodiversity is Shannon-Wiener index:

Method to select the sampling/survey site or area: Expert knowledge
Sampling/survey device: electrofisher machine and gillnets
Sampled/surveyed habitat:

Sampled habitat: All available habitats per site (Multi-habitat)
Sampling/survey month(s): March – November

Table 2.6.3: Sampling protocols

Surveillance Monitoring / Operational Monitoring	
Fish	
Sampling Month	March/November
Frequency for monitoring according to WFD	once in one year every 3 years

Number of sampling/survey occasions (in time) to classify site or area:
One occasion per sampling season
Sample processing - **Level of taxonomical identification:**
Level: Family, Genus, Species/species groups
Specification of level of determination:
Taxa should be identified at least to genus or family level if it is not possible to identify species.

Table 2.6.4: Description of the characteristics of fish at each WFD status class in accordance with the normative definitions (WFD Annex V)
Definitions for high ecological status, good and moderate in lakes (WFD Annex V)

Element	High	Good	Moderate
Fish fauna	Species composition and abundance correspond totally or nearly totally undisturbed conditions. Are present all specific sensitive fish species. Age structure of fish communities show little sign of anthropogenic disturbance and does not indicate a disturbance in reproduction or development of particular species.	There are slight changes in species composition and abundance of the type specific communities attributable to the impact on physical-chemical quality elements or Hydromorphological. Age structure of fish communities show signs of disturbance attributable to anthropogenic impacts on physico-chemical quality elements or Hydromorphological and, in some cases, is an indicator of disturbances in reproduction or development of certain species to the extent that some age classes may be missing.	Composition and abundance of fish species differ moderately from the type-specific due to human impact on the physico-chemical quality elements or hydromorphological. Age structure of fish communities shows major signs of disturbance, which are attributed to anthropogenic impacts on physico-chemical quality elements or Hydromorphological so that a moderate proportion of the type specific species are absent or are less abundant.

Conclusions

In the second research initiative, sampling methods (preservation, transport and storage) and analysis methods (analysis standards and methods principles) for each indicator selected (including sampling sites and sampling frequency) were performed.

Plankton and macroinvertebrates methods used for assessing water quality include the collection, counting, and identification of organisms, biomass measurements and processing and interpretation data. To define “good ecological status” the biological communities were classified following Romanian Order 161/2006.

The key parameter for other biological components of the lake, the macrophytes have an important role in lake metabolism. However, their monitoring is not frequently used for ecological quality assessment.

In lakes of Danube Delta Biosphere Reserve – fish sampling methods are gillnets fishing and electrical fishing, while analysis methods for fish parameters and indicators are fish species composition (richness species), dominance (abundance) and biomass in CPUE (capture per units effort) in accordance with tested system - ECOFRAME for implementation of the Water Frame Directive.

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