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Environmental Monitoring in the Black sea

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Black Sea Monitoring Guidelines

Macroplankton (Gelatinous plankton)

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CONTENTS

1 Introduction

Pelagic gelatinous zooplankton organisms where adults have size over 10 mm according to Omori and Ikeda (1984) have been included into the Black Sea macrozooplankton, although some of the species included in this manual have benthic stage in their ontogenic development and the representatives of Hydromedusae often have smaller size. The main components among them comprise representative of Coelenterata (Scyphomedusae, Hydromedusae) and Ctenophora. There are two species of Scyphomedusae (*Rhizostoma pulmo* (Macri, 1778; *Aurelia aurita* (L., 1758); three species of ctenophores, (one native species *Pleuroblachia pileus* (O.F. Müller, 1776) and two invasive *Mnemiopsis leidyi* (A. Agassiz 1865) and *Beroe ovata* sensu Mayer 1912, one more (*Bolinopsis vitrea* L. Agassiz , 1860) Mediterranean species was recorded in two locations of the Black Sea but its naturalization in the Black Sea is not definitely known (Ozturk, et al., 2001); 12 species of Hydromedusae (two of them are alien species *Blackfordia virginica* Mayer, 1910 and *Bougainvillia megas* (Kinne,1956) in the Black Sea) (Annex 1).

Gelatinous plankton plays important role in the functioning of the marine ecosystems and in the cases of excessive proliferation its role is negative. Since 1980, native gelatinous species have considerably increased their population size and distribution areas in the Black Sea. They often created blooms during past decades under the influence of different anthropogenic factors and climate change, the first among which were man-made eutrophication and increasing surface water temperature.

As it was mentioned above, two invasive ctenophores were introduced in the Black Sea, first the *Mnemiopsis leidyi* in 1982 that greatly affected the Black Sea ecosystem (Vinogradov et al.,1989) and ten years later its predator *Beroe ovata* (Konsulov and Kamburska, 1997). Both species were released with ballast waters into the Black Sea (Ghabooli et al, 2011). These two invaders are playing now a role of drivers of entire pelagic ecosystem functioning, both bottom-up and top-down (Shiganova et al., 2014). In addition, these invasive ctenophores *Mnemiopsis leidyi* and *Beroe ovata* spread further in the adjacent seas. First *Mnemiopsis leidyi* dispersed in the Sea of Azov via Kerch Strait (Studenikina et al., 1991), then it was brought with ballast waters to the Caspian Sea (Ivanov et al., 2000) and spread south, to the Sea of Marmara (Shiganova, 1993) and to the eastern Mediterranean Sea (Shiganova et al., 2001a), Adriatic Sea (Shiganova & Malej, 2009), Levantine Sea (Galil et al., 2009; Fuentes et al., 2010), Italian (Boero et al., 2009) and Spanish waters (Fuentes et al., 2010). Both currents and shipping are probable ways of *Mnemiopsis* transport within the Mediterranean Sea (Ghabooli at al., 2014).

Beroe ovata followed *M.leidyi* - first it spread from the Black Sea to the Sea of Azov (Shiganova et al., 2001b), the Sea of Marmara (Tarkan et al., 2000) and further to the eastern and western Mediterranean (Shiganova et al. 2007; Shiganova and Malej, 2009).

2 Purposes of macrozooplankton (gelatinous plankton) monitoring

The main goal of gelatinous plankton monitoring is to determine species composition, pattern of distribution, biomass, abundance and, using obtained data, to assess the impact of gelatinous plankton species, both native and non-native, on the ecosystem functioning.

The objectives of gelatinous plankton monitoring are as follows:

- Identification of species composition, their abundance, biomass and spatial distribution;
- Early registration of new non-native gelatinous macroplankton species introduction in the region;
- Study of seasonal, annual, interannual and long-term variability in macrozooplankton abundance, biomass and species composition;

To achieve comparability of the data collected during monitoring programs in the different Black Sea littoral states the standard methodology for macrozooplankton sampling and processing should be created. Therefore information on the sampling and processing methods, assessment of abundance and biomass of gelatinous species has been provided in the Manual. Other methods and equipment can be used as well, but the extended inter-calibration with the suggested standard technique is strongly recommended.

3 Sampling

3.1 Equipment

3.1.1 The nets and their devices

Sampling of gelatinous macroplankton must be performed using the plankton net with 500 μm or minimal 300 μm mesh size. As a rule, Georgia, Russia and Ukraine use a Bogorov Rass (BR) net (upper ring diameter 113 mm, opening 1 m^{-2}, lower diameter 140 mm, 500 μm mesh size) for gelatinous plankton sampling in the Black Sea or its smaller size modification (0.2 m^2 opening, 500 μm mesh size). SIO RAS uses a smaller size modification of BR net (0.2 m^2 opening, 500 μm mesh size). The Hensen Nets were provided to the representatives of all the Black Sea countries by NATO TU Black Sea project [1] in the 1990s. The advantage of this net is that it is equipped with the best large size collector where all individuals are collected in good conditions (Fig.1). Specially constructed collector can be used for BR net to obtain animals also in good conditions (Fig.2). Ichthyoplankton net (IN), which is a smaller modification of the same BR net (upper ring diameter 80 mm, lower - 113 mm), can be used for sampling from small vessels. For vertical distribution study net should be equipped with closing device.

The Hensen net (d=0.7 m, opening 0.38 m^{-2}, 300 μm mesh size (Fig.1) or WP 3 nets with mesh size 300 or 500μm are the best option for gelatinous plankton.

Fig.1 Sampling with Hensen Net

[1] NATO TU Black Sea project (1993-1997), which united all the Black Sea countries under the leadership of Middle East Technical University, Institute of Marine Sciences, supported by NATO Science Commitee

Fig.2 Handmade collector for Bogorov Rass Net

Ovae and larvae of ctenophores should be collected with a net of mesh size 180-200 μm (Juday net or WP-2 net) and preserved in 2-4 % buffered formaldehyde.

The gelatinous zooplankton can be sampled also with "Bongo" net, opening diameters d=2x0.60 m, mesh size of 500/300 μ, and cod-ends. The net should be provided with flow-meter. Oblique tows at a towing speed of around 2.5 knots (1.25m/sec) are recommended with the Bongo system. This entails a constant towing speed until the set depth is achieved and then recovery, again at a set winch speed. Any pause at the surface, bottom or at any other point during the haul will cause an over-estimation of plankton abundance at that depth. Thus, a smooth, continuous pay-out and recovery winch speed is essential for representative sampling. After the tow, the catch should be gently washed into the cod-end.

It is recommended to equip a net with a flowmeter assembled at ¼ of the diameter of the ring (UNESCO, 1968). The flowmeter must be calibrated for assessment of filtration ratio before the sampling process. If there is no flowmeter the length of the wire is used to calculate the volume of water filtered.

Ovae and small larvae <5 mm of ctenophores, ephyrae and planulae of medusae should be collected using Juday net (see mesozooplankton manual).

3.1.2 Sampling site and sampling depth

In general, ***Mnemiopsis leidyi*** and ***Beroe ovata*** share vertically the same layer – the water column from the surface down to the upper boundary of the thermocline, which is easily identified in the water temperature vertical profiles measured with CTD probe (Kideys and Romanova 2001, Mutlu et al., 2009, Shiganova et al, 2001a, Shiganova et al, 2003; Finenko et al, 2003). *Pleurobrachia pileus* and in some cases *Aurelia aurita* can occur deeper.

Prior to sampling, CTD probe should be used to measure vertical stratification of the sea water characteristics (temperature, salinity and density – see Fig.3) at each station, which should be included into routine protocols and used to obtain data on bottom depth, boundary of anoxic layer, depth of thermocline and Cold Intermediate Layer (CIL – layer, where temperature drops down to 8 °C and below). The boundary of anoxic layer is determined as function of the depth of sigma-

theta=16.2 varying between 60 m (the centre of the main gyres) and down to 220 m in the areas of downwelling in Rim Current[2].

Sampling of ctenophores, the first vertical haul should be taken from the upper boundary of the thermocline to the surface. It is better to take the second vertical haul using a closing device from the boundary of anoxic layer (sigma-theta=16.2) to the lower boundary of the thermocline. In case when closing device is not available or the weather is rough, the second haul has to be performed from the upper boundary of the anoxic layer (the depth of sigma-theta=16.2) to the surface.

In the cold seasons, when there is no thermocline, the general haul from the anoxic layer to the surface and standard hauls of 25-0m and 50-0m should be performed because *M.leidyi* and particularly *P.pileus* and *Aurelia aurita* may occur deeper, especially in winter when cooling of upper layer is strong. No hauls shorter than 5 meters should be made.

For fractionated hauls, the following intervals can be performed:

1. from upper layer of thermocline to the surface;
2. from lower boundary of thermocline to upper boundary of thermocline;
3. from lower boundary of Cold Intermediate Layer (CIL) to upper boundary of CIL;
4. from upper boundary of anoxic layer to lower boundary of CIL.

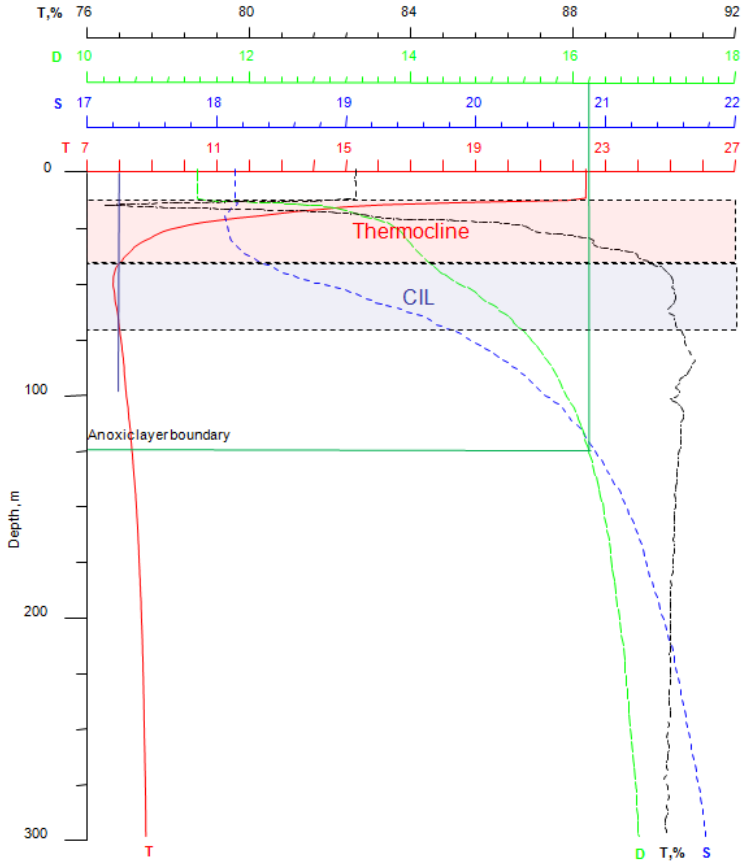

Fig. 3. Vertical profiles of water temperature (T° C), salinity (S‰), relative potential density (sigma-theta, T%) and transparency (D, m)

[2] Rim Current –the main circular cyclonic current of the Black Sea

3.2 Sampling procedure and sub-samples calculation

Sampling is performed by vertical hauls from a research vessel or other type of ship using a winch at a speed of about 0.5 m/s. The wire angle is measured and a correction for the wire-out is re-calculated on-the-fly using the following equation:

$$Z1 = Z / \cos(\theta),$$

where:

Z1 - length of wire-out,

Z - sampling depth,

Θ - wire angle in degrees.

If wire angle exceeds 40º, the sample should be discarded.

After each sampling the net should be always washed with gentle water flow and all remained organisms should be transferred from the collector into container.

3.3 Sampling frequency

To obtain the most representative results samplings should be performed monthly or better. if possible, every two weeks around a year or at least frequency should be increased from early March to late November. Each station should be sampled at least twice at the same station for more accurate results.

4 Preservation

Since the fixation of gelatinous species is very problematic, identification, measurements of size, counting and weighing of these organisms should be provided in vivo immediately after sampling.

Ovae and small larvae should be preserved with 2 percent of formaldehyde.

5 Taxonomical identification

Taxonomical identification should be based on the Annex II and the descriptions of the Black Sea macrozooplankton species (Annex 1); for hydromedusae identification guidance from special taxonomic publications (Baullion et al, 2006) should be used.

Biological monitoring should pay particular attention to indicator species and their abundance, which comprises most of gelatinous species - that helps to determine trends in environmental status of a basin. Special attention should also be given to taxonomic identification of non-indigenous species (non-native, alien, exotic species), those in most cases are recent arrivals to the Black Sea ecosystem. They are also often used as indicators of disturbed ecosystems.

6 Calculation of gelatinous plankton abundance and biomass

6.1 Calculation of M.leidyi and B.ovata abundance and biomass

The total number of ctenophores or/and jellyfish should be calculated in the sample or in the case of very high numbers of individuals a sub-sample should be taken (definite part of the sample) and then numbers for total sample calculated. After that the total abundance and biomass for the area (i.e. per m^2) and/or water volume (per m^3) is assessed taking the sampling depth into account.

Quantitative characteristics of species include their abundance and biomass calculated per square meter under the sampled water column and per cubic meter of filtrated water. The total number

N_{total} (the number of individuals in the sample) of *M. leidyi* and *B. ovata* in the sample is used to calculate the abundance.

Abundance ind/m^2 ($Ab_{sp/m2}$) is calculated using the following equation:

$$Ab_{sp/m2} = \frac{N_{total}}{S_{Net\_mouth}}$$, where S_{Net_mouth} is the square of the net mouth calculated as follows:

S_{Net_mouth} = 3.14 * *Net_Diameter*^{2} / 4

Abundance ind/m^3 is calculated by dividing the numbers (N_{total} per volume (V) of filtrated water).

$$Ab_{sp/m3} = \frac{N_{total}}{V_{fw}}$$

The volume of filtrated water V_{fw} should be estimated with flowmeter or by calculating the total filtrated water as follows:

$V_{fw} = S_{Net\_mouth} * Z1$, where *Z1* is the length [m] of wire-out.

In case the flowmeter and wire angle information are not available, the $Ab_{sp/m3}$ can be estimated with the following formula:

$$Ab_{sp/m3} = \frac{Ab_{sp/m2}}{D_l - D_u},$$

where D_l and D_u are the lower and upper sampling depths correspondingly.

The formulas for the biomass are similar:

$$Bm_{sp/m2} = \frac{W_{total}}{S_{Net\_mouth}}$$

$$Bm_{sp/m3} = \frac{Bm_{sp/m2}}{D_l - D_u}$$

Some investigators use coefficient for the filtering efficiency of the net. If a correction factor is applied, this should be stated in the method description.

The sampling procedures should be described and the obtained data on abundance and biomass at every station should be recorded using metadata and data format templates provided in Annex 1. If gelatinous species are not found in a sample, respective data should be recorded as 0 (zero).

Calculation of abundance for the Bongo Net is described further.

A: Rotor constants of flow-meter:

Standard speed rotor constant = 26.873

B: Distance (in meters):

$$Distance = \frac{Counts\,Difference\ X\ Rotor\,Constant}{999999}$$

Counts Difference: the difference between the indications of the flow-meter stopwatch before and after sampling.

C: Speed (cm/sec):

$$Speed\ cm/sec = \frac{Distance\ X\ 100}{time\,sec}$$

D: Volume of water (m^3):

$$Volume = \frac{3{,}14\ x\ Net\,diameter^2}{4}\ (x)\ Distance$$

Volume of the filtered water should be multiplied by 2, as the net has two rings.

E: Abundance ($ind.m^{-3}$):

$N(ind.m^{-3}) = N_{sample}/V_{filtrated\ water}$

$N(ind.m^{-2}) = N(ind.m^{-3})*h;$ where h - horizon depth measured by depth-meter

6.2 Calculation of ctenophores (M.leidyi, B.ovata and Pleurobrachia pileus) length, weight, length/weight ratio and biomass.

Ctenophores ***M. leidyi*** and ***B. ovata***, obtained by hauling at a station, should be immediately separated from other organisms with 2 mm mesh sieve and the sieve should be rinsed. Total number of individuals and total wet weight of *M. leidyi* and *B. ovata* should be determined to estimate abundance and biomass (Shiganova et al., 2000; Finenko et al., 2001, Mutlu, 1999).

If there are less than 100 individuals in a sample, all individuals should be measured, otherwise a sub-sampling can be performed (1/2, 1/3, 1/4 etc. of total), then recalculation for the entire sample should be done. Measurement has to be done as follows: individuals are sorted out to the size groups with ruler; in each group the number of individuals is counted and group is weighed. The total number N_{total} and wet weight W_{total} should be computed as respective sums through all size groups.

Recommended size groups for ***M.leidyi***:

<2 mm hatched larvae

3-5 mm larvae

6-10 mm cydippid larvae

11-20 mm juvenile individuals

21-30 mm juvenile individuals

31-40 mm stage of beginning maturity

41-50 mm adult individuals

51-60 mm adult individuals

>60 mm adult individuals

We suggest to measure length as total length with lobes of ***M.leidyi***. Individuals of ***M.leidyi*** should be measured by ruler with millimetre scale and small larvae with binocular microscope. The individuals have to be put in a Petri dish or other transparent dish; they should be suspended in water. This procedure allows for more accurate length measurement.

Under many circumstances, measurements on board a ship of the wet weight by any balance could not be precise, therefore calculation of weight is recommended to be done by using the length-weight (L-W) or volume-weight (V-W) equations.

To determine wet weight of ctenophores the biovolume (V in ml) is usually used, which is roughly equivalent to wet weight (WW in g). For biomass calculation in field studies linear regression with average length for size class live individuals is used, which usually equals to 5 mm for ***Mnemiopsis leidyi*** and ***Beroe ovata.***

Equations for estimation of wet weight or biovolume of ***Mnemiopsis leidyi*** and ***Beroe ovata*** were obtained by several scientists in different areas of the Black Sea. Some equations were derived using measurements of the total length of Mnemiopsis, other were based on length without lobes (oral-aboral length) (Table 1).

Equations for estimation of wet weight for native ctenophore *Pleurobrachia pileus* are also included into the Table 1.

Table 1. Equations for calculating Wet Weight (WW) or biomass of Ctenophore species in the Black Sea

Organisms	Wet Weight WW (Biomass)	Reference
Mnemiopsis leidyi WW (mg) (L<45mm) (total length)	$3{,}100 \cdot L^{2{,}22}$	Vinogradov et al., 2000, northeastern including central part
Mnemiopsis leidyi WW (mg) (L≥45mm) (total length)	$3{,}800 \cdot L^{2{,}22}$	Vinogradov et al., 2000, northeastern including central part
Mnemiopsis leidyi (WW (g), L (2-160 mm) (total length)	$W = 0043 \cdot L^{1.896}$ $R^2=0.944$ (n=300, p< 0.01)	Shiganova et al., 2000, 2001c; 2004 northeastern Black Sea (spring, summer, autumn)
Mnemiopsis leidyi WW (g), L (mm) (total length)	$WW=0{,}0339L^{1{,}39}$	Kamburska, 2004, western Black Sea, summer
Mnemiopsis leidyi WW (g), L (mm) (oral-aboral length)	$WW=0{,}0928\ L^{2.231}$	Mutlu, 1999, Southern Black Sea
Mnemiopsis leidyi WW (mg), L (5-70 mm) (oral-aboral length)	$1.31\ L^{2.49}$	Finenko et al., 2003; western Black Sea Anninsky, 1994
Mnemiopsis leidyi WW (mg), L (2-10 mm) (oral-aboral length)	$1.074\ L^{2.74}$	Finenko et al., 2003; western Black Sea
Mnemiopsis leidyi larvae (L 0.5-2 mm)	-	Anninsky et al., 2007; western Black Sea
Beroe ovata (8<L<162 mm)	$WW=0{,}0036L^{2{,}02}$ (R^2 =0,9353, n=40, p < 0.01)	Shiganova et al., 2001; northeastern Black Sea
Beroe ovata (L 10-120 mm)	$0.85\ L^{2.47}$	Finenko et al., 2003; Anninsky et al., 2005
Beroe ovata larvae (L 0,5-6 mm)	-	Anninsky et al., 2007; western Black Sea
Pleurobrachia pileus	$WW=0.682\ L^{2.52}$	Mutlu, 1994; Anninsky, 1994
Pleurobrachia pileus (L 3-25 mm)	$WW=0.250 \cdot L^3$	Vinogradov &Shushkina, 1987

Wet Weight (WW) (biomass) could be estimated using the given equations and the researchers could make their choice, however they should keep in mind for which area and season the equation was derived and which range of length was included into assessment. A special equation should be used for larvae of *M.leidyi* and *B.ovata* (Annensky et al., 2007).

6.3 Measurements of length, weight of jellyfish *Aurelia aurita*

Recommended intervals for jellyfish ***Aurelia aurita*** grouping is 1 cm. In each size group jellyfishes are counted and their diameters measured. The jellyfish *A. aurita* can be fixed in 2% buffered formaldehyde.

To determine wet weight of ***Aurelia aurita*** the biovolume (V ml) as displacement volume is also usually used. To calculate biomass in field investigations linear regression is used with average length for size class live individuals, which usually equals to 1 cm – for ***Aurelia aurita***, then wet weight is estimated using general linear function.

6.4 Measurements of length, weight of jellyfish *Rhyzostoma pulmo*

Jellyfish *Rhyzostoma pulmo* should be taken carefully from the net, length and diameter of each animal should be measured and then weighted on balance.

Table 2. Equations for calculating the WW (biomass, biovolume) Cnidaria*

Organisms	WW (Biomass) , mg	Reference
Cnidaria, Hydrozoa (meduza stage)	$**0.140 \cdot L^3$	Vinogradov & Shushkina, 1987
Cnidaria, Scyphozoa		
Aurelia aurita (D* 5- 235 mm)	$WW=0.058D^{1.9037}$ ($R^2=0.93$)	Shiganova (unpublished)
Aurelia aurita (D* 2-247 mm)	$WW=\_0.051\ D^{2.994}$	Anninsky, 2009

*D -diameter of bell

Because of varied morphology of small gelatinous organisms the best way to measure their body mass is the technique proposed by Eiji (1987). Organisms should be gently compressed between two glass plates at a known distance (0.2 – 1 mm) thus taking easily measurable form of a disk with the density about 1 mg/ mkl.

In this case length and wet weight will be estimated more accurately (Larson, 1985; Schneider, 1988; Bamstedt, 1990; 1994; Hirst & Lucas, 1998; Olesen, 2004; etc.). The jellyfish diameter is measured as a distance between ropalia at the moment of maximal relaxation of the specimen.

Biomass should be calculated as a sum of wet weight of all species and finally total biomass estimated as a total biomass in sample and further should be estimated per square meter or cubic meter.

7 Sampling information note.

After taking a sample the information on sampling should be uniformed and noted in accordance with agreement between littoral states (or participants of a particular program). The following information should be recorded in a way shown in the example below:

N	Acronym	Name	Example
1	RV	Name of R/V and cruise number	30 RV Akademik
2	Station	Station number	5
3	Depth	Depth (m)	38
4	Year	Year	2009
5	Month	Month	7
6	Day	Day	1
7	Time	Time of sampling	17:30
8	Ndec	Coordinate of station: Latitude (Degree)	45.6593
9	Edec	Coordinate of station: Longitude (Degree)	31.6113
10	Net	Type of the plankton net	Juday 0.1 m^2
11	Mesh	Mesh size (µm)	150
12	Layer	Depth range of net haul (m)	0-25
13	Angle	Angle of wire (Grad)	30^0
14	Wind	Wind speed (m/s)	10
15	Filtrated volume (FV)	Volume of water filtered by the net estimated as: wire length multiplied by mouth area (m^3)	2.5
16	Flowmeter	Volume of water filtered by the net estimated based on flowmeter reading (m^3)	2.0
17	Volume	Volume of sample (ml)	150
18	Taxon 1 SS	Total volume of aliquots which were taken for counting under binocular microscope and for calculation of abundance of each individual taxon (ml)	7
19	Taxon 1 K	Coefficient K = Total volume (N17) / aliquot volume (N18)	21,43
20	Taxon 1 N	Number of taxa enumerated in aliquots (ind.)	65
21	Taxon 1 Ind	Number of taxa in the whole sample = K (N19) * N (N20)	1393
22	Taxon 1 Ab	Abundance of individuals per cubic meter ind. (N21) / FV (N15) (ind/m^3)	557*
23	Taxon 1 B	Biomass = Ind/m^3 (N22) * sum of individual weights of taxa in cubic m (mg/m^3)	XXX.XX**
…	Taxon NN		
…	Group 1 C	Total concentration of certain taxonomic group (ind./m^3)	XXXX
…	Group 1 B	Total biomass of certain taxonomic group (mg/m^3)	XXX.XX
	Total C	Total concentration of macrozooplankton (ind./m^3)	XXXX
	Total C	Total biomass of macrozooplankton (mg/m^3)	XXX.XX

* Ind./m^3 can be less than 1 in case of few specimens in the sample, less in number than filtrated volume. More than 10 ind./m^3 should be rounded to whole number.

** For biomass calculation additional columns should be added to the data set:

- Average length of each zooplankton taxon.

- Individual weight of each taxon in terms of wet weight, dry weight or organic carbon.

Further work with summarizing metadata and data in tables as metadata table and dataset format are presented in ANEX 4.

8 Quality control

Throughout a year, gelatinous plankton monitoring results are highly variable. Therefore accurate quality control procedures should be provided for all organizations participating in monitoring. Quality control procedures need to be applied for the whole process of sampling site/depth selection, sampling and sub-sampling procedures, sample processing (identification) and reporting. Quality control procedures need to be unified strictly by all the monitoring organizations/laboratories (external verification QC). In addition a joint sampling should be provided with comparison of sampling methods and catchability of nets, sample processing, calculation of abundance and biomass.

8.1 Use of standardized equipment

All organizations/laboratories preferably should use standardized Black Sea zooplankton sample collection/processing equipment, consisting of:

1. Bogorov_Rass Net or other modifications with mesh size 300-500 μm
2. Juday net for hydromedusae, ovae and larvae of ctenophores, ephyrae and planulae medusa (diameter of net mouth 36 cm, mesh size 150 -180 μm).
3. Winch
4. Flowmeter.
5. Closing deviser for vertical distribution study
6. Stempel-pipette.
7. Bogorov's chamber for small size items examination and calculation
8. Graduated cylinder for displacement volume of animal determination
9. Binocular microscope.

8.2 Standard sampling methodology

Sampling methodology should be agreed between the participants of monitoring and should be provided with standard methods and equipment. High filtration capacity of the mesh should be maintained by washing the net with detergent after sampling. "Bad" samples (containing large amount of phytoplankton or jelly-fish) should be discarded and sampling repeated.

8.3 Sample storage and processing (identification and counting).

Samples of large gelatinous animals as a rule cannot be stored. Identification and counting should be done with live individuals immediately after sampling. Only their ovae and larvae <2 mm can be preserved by 2% buffered formaldehyde or 2% Lugole solutions. Ctenophore *Pleurobrachia pileus* and all species of the Black Sea Hydromedusae may be preserved as well.

8.4 Inter-laboratory proficiency testing. Reporting and data storage procedures

Identical procedures should be adopted for the laboratories involved in monitoring. These needs to be agreed on and the format developed. For processing of samples to have more precise biomass determination it is strictly recommended to measure length of examined species, if possible weight and their developmental stages. Sampling notes reporting on macrozooplankton is not yet formalised at national, regional or global level, so concrete recommendations on that can be given in each concrete case.

8.5 Staff training

Scientists working with samples analyses should participate in training courses (as funding allows). The results of the internal quality control schemes (re-analysis of at least 3-5-10% of the samples by colleagues) and inter-laboratory proficiency tests should be used.

8.6 Data control (data checking)

Data control is based on the regulation of quality control (QC). Considering the steps of the whole procedure it could be possible to assess the errors on each stage in *percent*. The assessment could not be done automatically but only manually.

Stages of macrozooplankton studying procedure: **S** - sampling, **C** - counting, **T** - data management, **P** - data presentation

Mesh size of the net (passing, 10-30% up to 100%) **S**

Mesh size of the net (clogging, 20-30% up to 100%) **S**

Quality of formalin (dissolving, 10% up to 30-40%) **S**

Subsampling device (under-overestimation, 5-10% up to 30%) **C**

Number of counted specimens (under-overestimation, 20-40%, up to 60%) **C**

Abundance and biomass calculation (0% up to 100%) **T**

Checking with the List of the Black Sea species (Flag) and guides **T**

Comparison of abundance and biomass values with literature (Flag) **T**

Typing errors (0% up to 5%) **T**

Metadata and data presentation units (0% up to 100%) **P**

Database format, including column titles (0% up to 100%) **P**

References

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ANNEX 1 Taxonomic composition of the most important groups of gelatinous meso- and macrozooplankton and their distribution in the national waters of the Black Sea countries*

№	Taxa	Bulgaria	Georgia	Romania	Russia	Turkey	Ukraine
	Phylum CNIDARIA (COELENTERATA)						
	Class HYDROZOA						
	Subclass HYDROIDOLINA						
	Order ANTHOATHECATA						
	Family CORYMORPHIDAE Allman, 1872						
	Corymorpha nutans M. Sars,1835	+					+
	Family CORYNIDAE Johnston, 1836						
	Sarsia tubulosa (M. Sars, 1835)	+	+	+	+		+
	Family CLADONEMATIDAE Gegenbaur, 1857						
	Cladonema radiatum Dugardin, 1843						+
	Eleutheria dichotoma Quatrefages, 1842						+
	Family HYDRACTINIIDAE L. Aggasiz, 1862						
	Podocoryna carnea M. Sars, 1846 Syn.: *Hydractinia carnea* (M. Sars, 1846)						+
	Family MOERISIIDAE Poche, 1914						
	Odessia maeotica (Ostroumoff, 1896) Syn.: *Moerisia maeotica* (Ostroumov, 1896)	+	+				+
	Family RATHKEIDAE Russell, 1953						
	Rathkea octopunctata (M. Sars, 1835)	+					+
	Family TUBULARIIDAE Goldfuss, 1818						
	Hybocodon prolifer Agassiz, 1860 Syn.: *Tubularia prolifer* (L.Agassiz, 1862)						+
	Order LEPTOTHECATA						
	Family BLACKFORDIIDAE Bouillon, 1984						
	Blackfordia virginica Mayer, 1910 (alien)	+					+
	Family CAMPANULARIIDAE Johnston, 1836						
	Clytia hemisphaerica (Linnaeus, 1767) *Syn.:Campanularia johnstoni* (Alder, 1856)						+
	Obelia longissima (Pallas, 1766)	+|					+
	Отряд LEPTOLİDA						
	Family BOUGAİNVİLLİDAE						
	Bougainvillia megas (Kinne,1956)(alien)	+		+			+
	Class SCYPHOZOA						
	Subclass DYSCOMEDUSAE						
	Order RHIZOSTOMEAE						

№	Taxa	Bulgaria	Georgia	Romania	Russia	Turkey	Ukraine
	Family RHIZOSTOMATIDAE Cuvier, 1799						
	Rhizostoma pulmo (Macri, 1778)	+	+	+	+	+	+
	Order SEMAEOSTOMEAE						
	Family ULMARIDAE						
	Aurelia aurita (L., 1758)	+	+	+	+	+	+
	Phylum CTENOPHORA						
	Class NUDA						
	Order BEROIDA						
	Family BEROIDAE Eschscholtz, 1829						
	Genus BEROE Gronov, 1760						
	Beroe ovata (sensu) Mayer 1912 (alien) Syn.:*Beroe ovata* Chamisso and Eysenhardt, 1821	+	+	+	+	+	+
	Class TENTACULATA						
	Subclass CYCLOCOELA						
	Order LOBATA						
	Family BOLINOPSIDAE Bigelow, 1912						
	Genus BOLINOPSIS L. Agassiz, 1860						
	Bolinopsis vitrea (L. Agassiz, 1860) (alien)	+				+	
	Genus MNEMIOPSIS L. Agassiz , 1860						
	Mnemiopsis leidyi (A.Agassiz) 1865 (Alien) Syn.: *Mnemiopsis gardeni* L. Agassiz,1860; *M. mccradyi* Mayer,1900	+	+	+	+	+	+
	Subclass TYPHLOCOELA						
	Order CYDIPPIDA						
	Family PLEUROBRACHIIDAE Chun, 1880						
	Genus PLEUROBRACHIA Fleming, 1822						
	Pleurobrachia pileus (O.F. Müller, 1776) **Syn.: *Pleurobrachia rhodopis* Chun,1879	+	+	+	+	+	+

* Taxonomic status of above mentioned representatives of gelatinous meso- and macrozooplankton is given according to the World Register of Marine Species (WoRMS) http://www.marinespecies.org/index.php.

**According to (Zaika, 2012)

ANNEX 2 Identification of the Black Sea gelatinous organisms

TYPE COELENTERATA

Class Scyphozoa in the Black Sea:

Fam. Ulmaridae

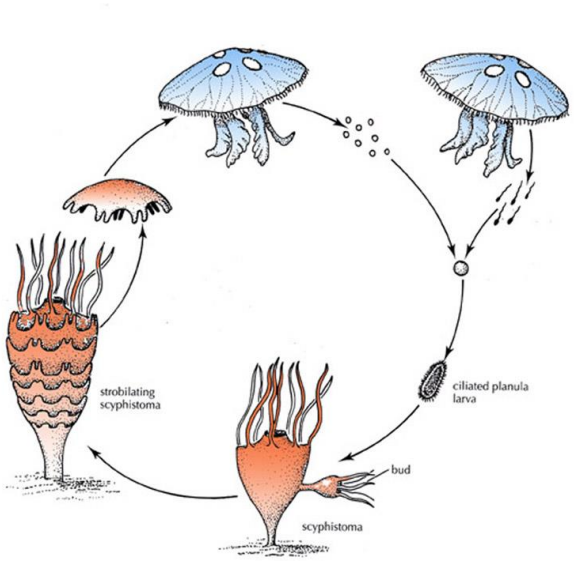
***Aurelia aurita* (L) (Fig. 1).**

A. aurita is one of the most abundant species among native gelatinous species (Fig.1).

The medusa *Aurelia aurita* or common sea jellyfish can be easily identified by its four horseshoe-shaped gonads. Its gelatinous body resembles a flat umbrella. The edges of the umbrella are decorated by numerous short coreless tentacles and eight marginal corpuscles (ropalia). Ropalia represent sensitive organs of the medusa; they control its position in the water and the rhythm of the umbrella contractions. It has four thickened arms, each with a central furrow rimmed by more convolute lips. The mouth is located in the middle of the lower side of the body; it leads to the throat where the intestine begins. The undigested remains are removed via the mouth. Sexual glands are located near the stomach or the radial channels.

Fig.1. Adult medusa stage of *Aurelia aurita* (photo Tihomir Makovec)

Scyphozoa has complex type of reproduction with alternation sexual and asexual reproduction (Metagenesis). Each stage (medusa, planula, scyphistoma, ephyra) has specific morphology and the way of living.



Larva stage *planula* develops in specific lobes of mouth of female and then lives in water about one week, then sinks on the bottom.

Scyphistoma develops from the planula, which is similar to polyp with 16-32 feelers. They usually occur in shallow areas. This stage can live 3 and more years, depending on trophic conditions and temperature. Scyphistoma can gemmate and strobile ephyrae. Ephyrae appear in spring and late autumn in the Black Sea.

Fig.2. Life cycle of *Aurelia aurita* (http://jellieszone.com/scyphomedusae.html)

Distribution.

Both spatial and vertical distributions of medusas are extremely irregular. The spatial in homogeneity in the medusa distribution is caused by their transport by currents and manifests

itself in the form of accumulations observed as individual patches or bands, sometimes extended along the shore or, in open regions, along the direction of the wind. The sizes of these kinds of accumulations may be rather significant.

Usually, the bulk of the animals are concentrated in the subsurface layers of the sea or at depths of 30–50 m, where up to 90% of individuals may occur; meanwhile, accumulations may also be encountered in the layer 70–80 m. In the near-shore zone, the number of medusas that prefer dwelling in the near-bottom layers in the warm periods is greater than that in cold ones.

Fam. Rhizostomidae

Gen. Rhizostoma Cuvier

***Rhizostoma pulmo* (Macri)** is a rather usual species in the Black Sea; it dwells mainly in the near-shore regions of the Black Sea and sometimes penetrates with currents to its open part. Usually, isolated individuals are encountered; meanwhile, it may also feature outbursts of the abundance as it was observed in the northwestern part of the sea in the 1960s–early 1970s.

Fig. 3 *Rhyzostoma pulmo* (Macri,1778) (photo Tihomir Makovec)

TYPE CTENOPHORA

Order Cydippidea

Fam.Pleurobrachiidae

***Pleurobrachia pileus* O. Muller (syn. *P.rodopis* Chun)**

Pleurobrachia refers to the most primitive order of ctenophores Cydippidea and up to the 1980s, *Pleurobrachia pileus* was the only species of Ctenophora in the Black Sea. They are small ctenophores; in the Black Sea, their length ranges from 5 to 25 mm. The bodies of ctenophores are transparent and have oval or spherical shapes. At the distal end of the body, the slit-like mouth is located. Usually, it is closed. When capturing the prey, the mouth of Pleurobrachia widely opens. *Pleurobrachia pileus*, similarly to all the ctenophores, has 8 rows of swimming combs that commence at a distance from the aboral pole.

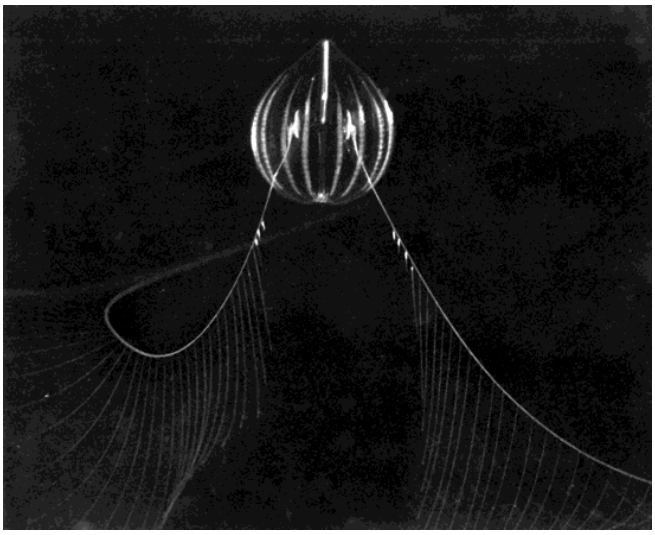


Fig.4 Hunting Pleurobrachia pileus

The length of the swimming combs in the meridional direction equals two-thirds of the total body length. *P.pileus* moves due to the operation of the swimming combs having its oral part ahead. The aboral end hosts the aboral organ. Near it, on either side, two tentacular bulbs are located into which the ctenophore can retract its tentacles. *P.pileus* can swim with its tentacles completely hidden. When the tentacles are outside, they may stretch to reach a length 20 times as great as the length of the animal proper. Extended tentacles feature a row of numerous long lateral filaments each. At the surface, sticky cells or colloblasts are located; they help the animal to catch its prey. *P.pileus* is capable of rather long-term hanging with its oral end up (feeding position); in so doing, its tentacles are extended downward and on the sides to form a sort of catching net. *Pleurobrachia pileus* is zooplanktivorous comb-jelly, inhabits mainly the intermediate layer of the Black Sea.

Invasive Ctenophores in the Black Sea.

Mnemiopsis leidyi

TAXONOMY AND BRIEF DESCRIPTION

Mnemiopsis leidyi (A.Agassiz) 1865 (Fig.1)

Phylum Ctenophora Esch

Class Tentaculata Chun

Order Lobata Esch

Fam. Mnemiidae Ech.

Genus Mnemiopsis L.Agassiz , 1860

Mnemiopsis leidyi (A.Agassiz) 1865

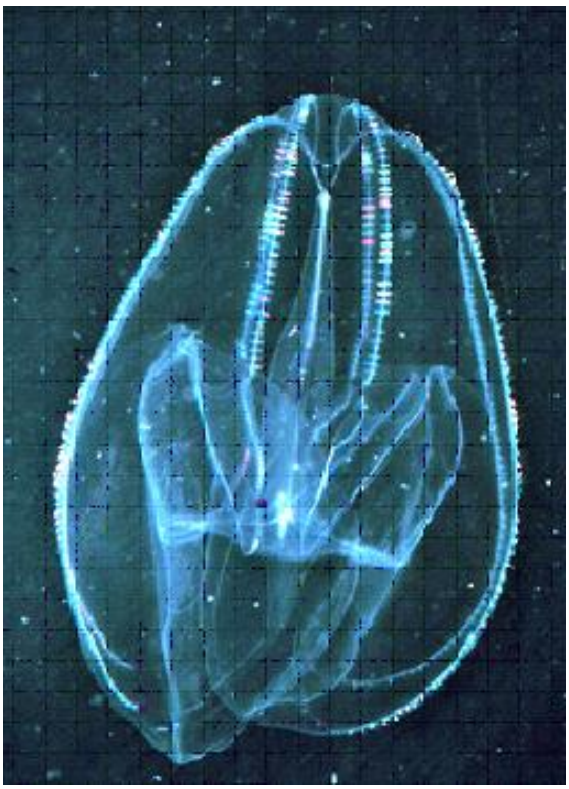
Synonyms: *Mnemiopsis gardeni* L. Agassiz,1860; *M. mccradyi* Mayer,1900.

Mnemiopsis leidyi is characterized by the presence of two large lobes referred to as lateral or oral lobes. The oral lobes are derivatives of the ctenophore body (spherosome). Four smaller lobes – auricules are situated under the two principal oral lobes. Closing down with one another by their distal edges, they completely envelop the mouth area of the animal (Agassiz, 1860; Seravin, 1994). The morphology of this ctenophore in the Black Sea appears to be rather variable (adult from 40 to maximal 180 mm) depending on the environment conditions and prey availability.

Fig. 1. *Mnemiopsis leidyi* from the Black Sea (photo T.Shiganova).

***Bolinopsis vitrea* (L.Agassiz, 1860)**

In 2007 and 2010 another ctenophore *Bolinopsis vitrea* (L.Agassiz, 1860**)**, which occurs in the Mediterranean Sea was recorded in the southern and north-western Black Sea (Ozturk, Michneva and Shiganova, 2011). This species is morphologically very similar to *Mnemiopsis leidyi* and herewith we provide information on features that are characteristic for *Bolinopsis vitrea* and allow distinguishing in between the two mentioned species.



Representative of *B. vitrea* can be easily distinguished morphologically from *M .leidyi* (Fig.2) knowing the following similarities and differences. Both species have an oval body with considerable lateral compression, and two oral lobes are derivatives of the ctenophore body (spherosome). Four smaller lobes –auricles– are situated under the principal two oral lobes. The main difference in between *Bolinopsis vitrea* and *Mnemiopsis leidyi* is in the position of the oral lobes. In *M. leidyi*, the oral lobes originate near the level of infundibulum, whereas in *B. vitrea* they originate approximately half-way between the mouth and the infundibulum (Fig. 2). In addition, *M. leidyi* has papillae on the body, while *B. vitrea* does not (Shiganova and Maley, 2009).

Fig. 2. *Bolinopsis vitrea* (photo of Tihomir Makovec)

***Beroe ovata* sensu Mayer 1912**

Phylum Ctenophora Eschscholtz, 1829

Order Beroida Eschscholtz, 1829

genus *Beroe* Browne, 1756

Beroe ovata (sensu) Mayer 1912

Beroe ovata is another species of Ctenophora, non-native for the Black Sea. The body of this species is mitten-shaped, wider at the oral end and not tapered at the aboral end. The lateral compression of the body is remarkable, being no less than three-fold in the paragastric plane, with a length to width ratio (*l/w*) 1.1–1.2 (Fig.3) (Mayer, 1912).

Fig. 3. *Beroe ovata* from the Black Sea (photo T. Shiganova)

Younger individuals are wider both in the oral and aboral parts of the body. Meanwhile, under the influence of environment conditions, the body shape of the ctenophore is variable to a certain extent; for example, the aboral end may be significantly stretched. Usually, ctenophores feature a pink coloration; the largest individuals are more intensively colored with a brownish tint. The size of large adult individuals in the Black Sea ranges from 81 to a maximum of 162 mm at the average value of 40–70 mm.

Individuals of *Beroe ovata* have a widely opening mouth, which provides the animal with a possibility of sucking preys without hunting tenticulars as the ctenophore *Mnemiopsis leidyi* does. The mouth leads to a vast stomodeum, which actually represents the stomach of the animal.

The ctenophore body looks bag-shaped, due to the vast stomodeum, which occupies 4/5 of the animal body width and extends up to the flattened wall of the aboral end of the body, where a relatively small infundibulum is located. If one cuts out the lips of the mouth, it can be seen under binoculars that, at a certain distance from the lip end of the mouth, the inner surface of the frontal edge of the stomodeum is covered with large ciliate structures (macrocilie). Macrocilie rim the frontal part of the stomodeum (behind the corners of the mouth) in the form of a ring; they are used as teeth that can help the animal to bite off parts of large preys if it cannot swallow it whole. After swallowing the food, the ends of macrocilie in the mouth area join together closing the mouth for the time when the prey is in the stomodeum (Shiganova et al., 2004).

ANNEX 3 References for gelatinous species identifications

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ANNEX 4 Examples of data reporting / Dataset description format

Dataset description format (example)

Dataset: Bilim, 1991 June

Research vessel: Bilim

Organization: IMS METU

Project_Name: NATO TU-Black Sea and TUBITAK

Data: *Mnemiopsis leidyi* biomass and abundance

Were other gelatinous species measured or not: Yes (*Beroe ovata, Aurelia aurita, Pleurobrachia pileus*)

Number of samples: 68

Area of sampling: Turkish exclusive economic zone

Net type: Hensen net.

Mesh size: 300um.

Net mouth opening: 0.7m.

Sampling, analysis and processing methods: Hauls from 0-100 offshore stations and from 0-bottom at shallow stations (<200 m, 2 m above the bottom to be the last depth sampled). Hauling speed: 1.5m/s. Samples were sorted out and enumerated without prior preserving them and weighed onboard with balance or using method of displacement volume. Sampling volume was estimated by multiplying the mouth area (the diameter of the opening of the net) with the wire length.

Data originator: Dr. Erhan Mutlu.

Data provider: Dr. Erhan Mutlu.

Quality control: Dr. Erhan Mutlu.

Remarks and comments:

Metadata format (example)

Cruise	Station	Date	Time	Latitude	Longitude	Station sea depth	Upper sampling depth	Lower sampling depth	Depth of sigma-theta=16.2	Depth of CIL' upper limit	Comment
Bilim, 1995 March	N00K12	19.03.1995	19:30	43	28.2	100	0	40			
Bilim, 1995 March	M30K13	18.03.1995	21:45	42.5	28.216667	100	0	85			
Bilim, 1995 March	M11K15	18.03.1995	19:00	42.18333	28.25	100	0	92			
Bilim, 1995 March	L56K17	18.03.1995	17:15	41.93333	28.283333	100	0	60			
Bilim, 1995 March	N00K30	19.03.1995	17:40	43	28.5	100	0	90			
Bilim, 1995 March	L59K35	18.03.1995	15:20	41.98333	28.583333	100	0	95			
Bilim, 1995 March	M30K42	19.03.1995	00:55	42.5	28.7	100	0	190			
Bilim, 1995 March	M40L15	19.03.1995	07:00	42.66667	29.25	100	0	150			

Note1: Please indicate in comments any important information such as weather conditions or sea state or details of sampling

Data format (example)

Cruise	Station	Date	Time	Latitude	Longitude	Station sea depth	Upper sampling depth	Lower sampling depth	ML* biomass (mg-m3)	ML abundance (ind-m3)	BO** biomass (mg-m3)	BO abundance (ind-m3)	Sigma 16.2 depth (optional)	Wire angle (optional)	Comment
Bilim, 1995 March	N00K12	19.03.1995	19:30	43	28.2	100	0	40	0.13	0.03	1	1			
Bilim, 1995 March	M30K13	18.03.1995	21:45	42.5	28.216667	100	0	85	0.76	0.4	1	1			
Bilim, 1995 March	M11K15	18.03.1995	19:00	42.18333	28.25	100	0	92	2.17	0.88	1	1			
Bilim, 1995 March	L56K17	18.03.1995	17:15	41.93333	28.283333	100	0	60	1.33	0.92	1	1			
Bilim, 1995 March	N00K30	19.03.1995	17:40	43	28.5	100	0	90	1.5	0.4	1	1			
Bilim, 1995 March	M40L15	19.03.1995	07:00	42.66667	29.25	100	0	150	0.8	0.15	1	1			

* ML-*Mnemiopsis leidyi*
** BO –*Beroe ovata*

Notes:
1) Inclusion of data on other gelatinous is encouraged.
2) Please indicate in comments any important information such as weather conditions or sea state or details of sampling.