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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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1. Introduction

Native and established non-native gelatinous zooplankton species have been recognised as the Black Sea macrozooplankton, although some of these species pass benthic stage in their onthogenic development. Most of pelagic gelatinous zooplankton organisms at adult stage have size over 10 mm and, therefore, included in macrozooplankton, according to Omori and Ikeda (1984). However, some of Hydromedusae species often have smaller size (less than 10 mm). Their main representatives include **Coelenterata** (**Scyphomedusae, Hydromedusae**) and **Ctenophora**. There are two native species of **Scyphomedusae:** ***Rhizostoma pulmo*** (**Macri, 1778**) and ***Aurelia aurita*** (**L., 1758**) in the Black Sea. However, recent genetic analyses showed that the Black Sea ***Aurelia aurita*** belongs to clade Borealis (Ramsak et al., 2012) and should be referred as ***Aurelia sp.*** until the final global molecular analyses will be done.

There are three species of Ctenophora occur in the Black Sea now, one native species ***Pleuroblachia pileus*** **(O.F. Müller, 1776)** and two non-native ***Mnemiopsis leidyi*** **A. Agassiz 1865** and ***Beroe ovata*** **Bruguière, 1789**. According to our genetic analyses both species were released with ballast waters into the Black Sea from the vicinity of the Gulf of Mexico or Caribbean areas. (Ghabooli, Shiganova et al., 2011., 2013; Reusch et al., 2010; Bayha et al., 2015; Johansson, Shiganova et al., 2018).

As to their origin, native gelatinous species are the cold-water ones. Among them are ctenophore ***Pleurobrachia pileus***, scyphomedusa ***Aurelia aurita*** and ***Rhisostoma pulmo.*** In addition, pyrophyte alga ***Noctiluca scintillans*** **(Macartney) Kofoid & Swezy, 1921** can be attributed to them. Two warm-water invasive ctenophores ***Mnemiopsis leidyi*** and ***Beroe ovata*** arrived and established in the upper warm layer of the sea (Fig.1).

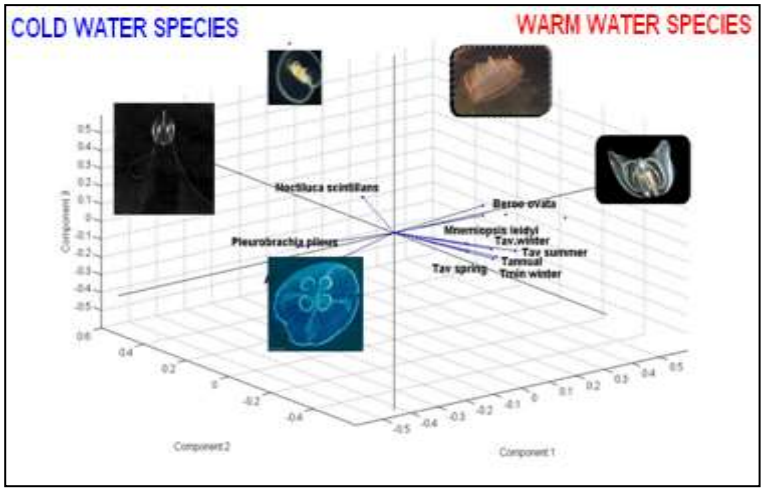

Fig. 1. Distribution of the native and non-native gelatinous species in the Black Sea according to seasonal, annual and minimal winter SST (based on main component analyses of field data).

In addition, 12 species of Hydromedusae occur in the Black Sea, including two non-native species: ***Blackfordia virginica*** **Mayer, 1910** and ***Calyptospadix cerulea Clarke, 1882*** **(syn.*Bougainvillia megas*** **(Kinne,1956)** (ANNEX 1; 2).

Gelatinous plankton plays important role in the functioning of the marine ecosystems and in the cases of excessive proliferation, its role is harmful. Since 1980, native population and distribution areas in the Black Sea of gelatinous species ***Aurelia aurita*** have considerably increased. Their medusae often generated blooms during last decades due to the influence of various anthropogenic factors and climate change. The main of them were man-made eutrophication and increasing of water temperature. Prior to 1988, ***A. aurita*** was the dominant gelatinous predator in the near-surface horizons with a plentiful supply of zooplankton. But since 1988 a mass outbreak of harmful invader ***Mnemiopsis leidyi*** occurred in the Black Sea, resulting in a rapid decrease in abundance of warm-water zooplankton species and ***A. aurita*** population, as a competitor, was also diminished (Shiganova, 2009).

There were any of ***M. leidyi*** predators and it established under optimal temperatures and food concentration in the Black Sea, reaching high abundances. Its basic food is zooplankton, fish eggs and small larvae while its larvae feeding on microzooplankton (Sullivan & Gifford, 2004; 2007). As a

result, in following years cascading effects were observed at the most levels of the ecosystem. Bottom-up effects, including collapse of planktivorous fish populations, drop of large pelagic fish and dolphins stocks, followed. Top-down effects included decreasing of zooplankton species diversity and stocks (maximum annual zooplankton biomass declined to ca. 0.5 mg C m^{-3}, which was almost two orders of magnitude lower than during the previous period) and increasing of phytoplankton biomass, released from zooplankton pressure. In addition, bacterioplankton increased growing due to the high production of mucus, released by ***M.leidyi*** and its degradation fragment, heterotrophic flagellates and ciliates increase follow, which fed on the overgrown biomass of bacteria (Shiganova et al., 2004, 2019a). By the late 1980s, the pelagic ecosystem had become dominated by gelatinous plankton, where ***M.leidyi*** comprised the major part of biomass (Shiganova et al., 2003; Finenko et al., 2003).

Ten years later its predator ***Beroe ovata*** (Konsulov and Kamburska, 1998; Shiganova et al., 2000; Seravin et al., 2002) was introduced in the Black Sea with ballast waters. ***B. ovata*** is a known predator of zooplanktivorous ctenophores, mostly of ***M. leidyi*** in Northern and Southern American waters (Bayha et al., 2004; Mianzan, 1999). In the upper layer of the Black Sea ***B. ovata*** feeds on ***M. leidyi***. However, it may feeds on the native ctenophore ***Pleurobrachia pileus***, which generally lives in deeper offshore waters and is not available for ***B.ovata***, occurring in surface layer during active period of its life (Shiganova et al., 2001b). After the arrival of ***B. ovata***, the Black Sea ecosystem began to recover progressively (Shiganova et al., 2014; 2018). A supporting factor that favored the recovery of the ecosystem was a decrease of eutrophication, resulted from reduced anthropogenic nutrient inputs (Cociasu et al., 2008). That was accompanied by decrease of total phytoplankton biomass and harmful algae blooms. The combination of these factors in the late 1990s led to a general recovery of the Black Sea ecosystem (Oguz and Velikova 2010; Shiganova et al., 2014). Nevertheless, these two invaders are still stressors of entire pelagic ecosystem functioning, both bottom-up and top-down (Shiganova et al., 2014).

Since the interannual abundance of the ***M. leidyi*** depends on variability of surface water temperature and food concentration, i.e. edible micro- and mesozooplankton, the level of control by ***B.ovata*** after its arrival varies (Shiganova et al., 2014; 2018).

These invasive ctenophores ***M. leidyi*** and ***B. ovata*** spread further in the adjacent seas. First, ***M leidyi*** was dispersed in the Sea of Azov via Kerch Strait (Studenikina et al., 1991), where it can live only during warm seasons. Then it was introduced with ballast waters into the Caspian Sea (Ivanov et al., 2000). Also, it spread southward, to the Sea of Marmara (Shiganova, 1993) and to the eastern Mediterranean Seas (Shiganova et al., 2001b): Levantine Sea (Galil et al., 2009; Fuentes et al., 2010), central and western Mediterranean Sea: the Adriatic Sea (Shiganova & Malej, 2009), Italian (Boero et al., 2009) and Spanish waters (Fuentes et al., 2010). Both sea currents and shipping are probable vectors of *M.leidyi* transport within the Mediterranean Sea (Ghabooli at al., 2013).

B. ovata followed ***M.leidyi*** invasion pathway. Initially it spread from the Black Sea into the Sea of Azov (Shiganova et al., 2001a), the Sea of Marmara (Tarkan et al., 2000) and further to the eastern Mediterranean (Shiganova et al., 2007; Shiganova and Malej, 2009; Mamish et al., 2020; Badreddine et al.,2020).

2. Purposes of macrozooplankton (gelatinous plankton) monitoring

The main goal of gelatinous plankton monitoring is to determine species composition, patterns of its distribution, biomass, abundance, and, using obtained data along with the other parameters, to identify their role in trophic webs and to assess their impact on the ecosystem functioning.

The objectives of gelatinous plankton monitoring:

- 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, , interannual and long-term variability in macrozooplankton abundance, biomass and species composition;
- Study impact on the ecosystem state.

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 introduced. 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 attached devices

Sampling of gelatinous macroplankton should be performed using the plankton net with 500 μm or minimal 300 μm mesh size. Usually, 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, 300 or 500 μm mesh size). SIO RAS team 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 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.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.2) or WP 3 nets with mesh size 300 or 500μm are the best option for gelatinous plankton.

Fig.2 Sampling with Hensen Net (Shiganova T. and Skirta A., SIO RAS)

[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

Ovae and small larvae <5 mm of ctenophores, ephyrae and planulae of medusae, all size individuals ***Pleurobrachia pileus*** and pelagic stages of all Hydromedusae should be collected using Juday net (maximal 200 μm mesh size, opening mouth 0.1 m) (see mesozooplankton manual).

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.

3.1.2. Sampling sites and 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,b, Shiganova et al, 2003; Finenko et al, 2003). ***Pleurobrachia pileus*** and ***Aurelia aurita*** occurs in deeper waters (Vinogradov et al., 1992).

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 the Rim Current[2].

While sampling the 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 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*** 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 should be used:

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.

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

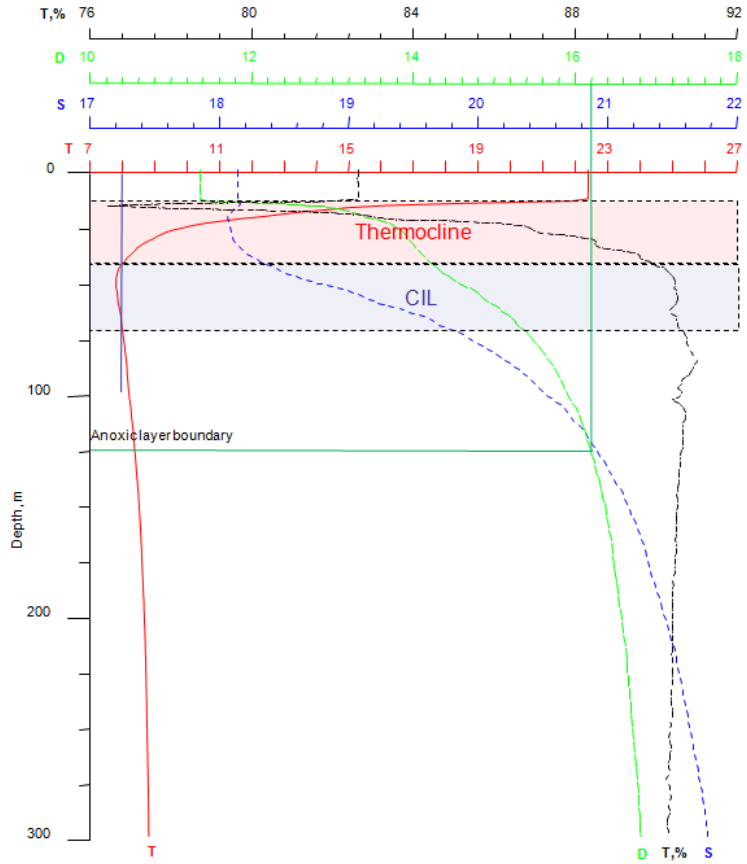

Fig. 3. Vertical profiles of water temperature (T° C), salinity (S‰), relative potential density (sigma-theta, σ_θ) and transparency (D, m)

3.2. Sampling procedure and sub-sampling method

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 washed with gentle water flow and all remained organisms should be transferred from the collector into container with the sample.

3.3. Sampling frequency

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

4. Samples 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 of ctenophores, ephyrae, planulae and all pelagic stages of Hydromedusae species should be preserved with 2% formaldehyde.

5. Taxonomical identification

For taxonomical identification the ANNEX I and II should be used, where list of the Black Sea macrozooplankton species and their descriptions and illustrations are presented, in addition ANNEX 3 is given with a list of references on relevant literature. For hydromedusae, identification guidance from special taxonomic publications (Baullion et al, 2006) or description in WoRMS should be used (in electronic version of Manual all Hydromedusae species are linked to WoRMS).

During biological monitoring assessment, particular attention should be focused on species indicators of ecosystem state and their abundance and biomass. Most of gelatinous species belong to indicators, which help to determine trends in an environmental status of the basin. Special attention should also be given to taxonomic identification of non-native species (non-indigenous, alien, exotic species) which in most cases are recent invaders to the Black Sea ecosystem. They are also often used as indicators of disturbed ecosystems (ANNEX IV).

6. Calculation of gelatinous plankton abundance and biomass

6.1 Ctenophores

6.1.1. Measurements of ctenophores (Mnemiopsis leidyi, Beroe ovata and Pleurobrachia pileus) length, wet weight and length/weight ratio

Individuals of ctenophores ***M. leidyi*** and ***B. ovata***, obtained by vertical 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 (Mutlu, 1999. Shiganova et al., 2000; Finenko et al., 2001).

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*** in the Black Sea:

<0.5 mm hatched larvae

>0.5-5 mm cydippid larvae

6-10 mm lobate larvae

11-30 mm juvenile individuals

31-40 mm stage of beginning maturity accepted as adult

41> adult individuals

We suggest to measure length as a 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 at the bottom of Petri dish. This procedure allows more accurate length measurement.

Recommended size groups for ***B.ovata*:**

<0.8 mm hatched larva

>1-8 mm larva,

9-40 mm juvenile,

41-50 mm stage of mature, accepted as adult

>51 adult

In some circumstances, *in situ* measurements of the wet weight with balance could not be quite precise. Therefore, weight estimates is recommended to be done by using the length-weight (L-W) or volume-weight (V-W) equations.

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

Equations for estimation of wet weight or biovolume of ***Mnemiopsis leidyi*** and ***Beroe ovata*** were obtained by several researchers in different areas of the Black Sea. Some equations were derived using measurements of the total length of *Mnemiopsis* (Shiganova), while other was based on length, measured without lobes (oral-aboral length) (Table 1). A special equation should be used for larvae of *M.leidyi* and *B.ovata* (Anninsky et al., 2007).

All individuals of ***Pleurobrachia pileus*** should be measured alive if they collected by BR net. From Juday net in zooplankton samples they should be preserved in 2% solution of buffered formaldehyde.

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

6.2 Scyphomedusae

6.2.1. Measurements of length and weight of jellyfish *Aurelia aurita*

Recommended intervals for medusa stage of ***Aurelia aurita*** grouping and measurement are 1 cm. In each size group medusae are counted and their diameters are measured. The umbrella diameter of medusa is measured on the glass plate between ropalia at the moment of maximal relaxation of individuals. The medusae ***A. aurita*** can be fixed in 2% buffered formaldehyde.

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

6.2.2. Measurements of length and weight of jellyfish *Rhizostoma pulmo*

Medusa ***Rhizostoma pulmo*** should be pulling out carefully from the net. Length and bell diameter of each animal should be measured and then weighted on balance.

6.3. Calculation of large gelatinous plankton species abundance and biomass

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

Quantitative characteristics of species include their abundance and biomass calculated from vertical hauls per square meter under the sampled water column and per cubic meter of filtrated water column. 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, that 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 V. If gelatinous species are not found in a sample, 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

Table 1. Equations for calculating the biomass and carbon content of gelatinous zooplankton

Organisms	Wet weight (WW), mg·ind^{-1}	Carbon,·mg g^{-1} of WW	Carbon,· mkg ·ind^{-1}	Reference
Cnidaria, Hydrozoa (medusa stage)	0.14·L^3	2.81	0.39·L^3	Vinogradov & Shushkina, 1987
Hydromedusae	-	0.95-3.40	-	Larson, 1986
Cnidaria, Scyphozoa				
Aurelia aurita (D 2-247 mm)	0.05 D $^{2.99}$	-	1.24 D$^{2.33}$	Anninsky, 2009
Aurelia aurita ephyrae (D 2-4 mm)	-	10.68	-	Anninsky, 2009
Aurelia aurita (D 5-50 mm)	-	19.26 D $^{-0.77}$	-	Anninsky, 2009
Aurelia aurita (D 50-250 mm)	-	0.79	-	Anninsky, 2009
Rhizostoma pulmo	-	~3.5		Tinta et al., 2012
Ctenophora:				
Beroe ovata (L 10-120 mm)	0.85 L $^{2.47}$	1.54	1.31 L $^{2.47}$	Finenko et al., 2003
Beroe ovata (L 8-162 mm)	0,88 L $^{0.967}$	-	-	Joint results of Shiganova et al, 2000 Kamburska, 2004.
Beroe ovata (L 15-95 mm)	-	1.32	-	Anninsky et al., 2005
Beroe ovata larvae (L 1-10 mm)	0.20 L $^{2.7}$	-	-	Anninsky et al., 2007
Bolinopsis vitrea		0.20		Kremer et al., 1986

Organisms	Wet weight (WW), mg·ind^{-1}	Carbon,·mg g^{-1} of WW	Carbon,· mkg ·ind^{-1}	Reference
Mnemiopsis leidyi (WW (g), L (2-160 mm) (total length)	0043·L $^{1.9}$ R^2=0.944 (n=300,p< 0.01)	1.02	3.2·L$^{2.22}$	Shiganova et al., 2000, 2001a;2004, North-eastern Black Sea (spring, summer, autumn)
Mnemiopsis leidyi (without lobes) (L 2-10 mm)	1.07 L $^{2.7}$	-	-	Finenko et al., 2003,South-Western Black Sea (winter, spring, summer, autumn)
Mnemiopsis leidyi (without lobes) (11-70 mm)	1.31 L			Finenko et al., 2003,South-Western Black Sea (winter, spring, summer, autumn
Pleurobrachia pileus (L 3-25 mm)	0.68 L $^{2.5}$	1.19	0.81 L $^{2.5}$	Mutlu, 1994; Anninsky, 1994
Pleurobrachia pileus	0.25·L^3	-	-	Vinogradov & Shushkina, 1987
Pleurobrachia pileus	-	0.50 -1.08	-	Schneider,1989
Pleurobrachia sp	-	1.40	-	Clarke et al., 1992

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).

Table 2. Equations for calculating the WW (wet weight, biomass, biovolume) of Cnidaria

Organisms	WW (Biomass) , mg	Reference
Cnidaria, Hydrozoa (medusa stage)		
Hydromedusae	*0.140·L^3	Vinogradov & Shushkina, 1987
Cnidaria, Scyphozoa (medusa stage)		
Aurelia aurita (D* 5- 235 mm)	WW=0.058D $^{1.91}$ (R^2=0.93)	Shiganova (unpublished)
Aurelia aurita (D* 2-247 mm)	WW=_0.051 D $^{2.99}$	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 individual.

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. Hydromedusae

7.1. Taxonomic composition of the Hydromedusae

Table 3.Taxonomic composition of the Hydromedusae 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 Dujardin, 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 proloifer Agassiz, 1860 Syn.: *Tubularia prolifer* (L.Agassiz, 1862)						+
Order LEPTOTHECATA						
Family BLACKFORDIIDAE Bouillon, 1984						
Blackfordia virginica Mayer, 1910 (non-native)	+		+			+
Family CAMPANULARIIDAE Johnston, 1836						
Clytia hemisphaerica (Linnaeus, 1767) (Syn.*:Campanularia johnstoni* (Alder, 1856))						+
Order LEPTOLIDA						
Family Bougainvilliidae Lütken, 1850	+		+			+
Calyptospadix cerulea Clarke, 1882 (non-native) **(Syn.Bougainvillia megas (Kinne,1956))**	+		+			+

*All mentioned hydromedusae species are linked to WoRMS electronic version, providing easy species identification

Hydromedusae are still poor studied in the Black Sea and therefore they should be preserved and identified during processing. Special attention should be paid to widely dispersed non-native species, which have medusa stage such as ***Calyptospadix cerulea*** and Ponto-Caspian species ***Odessia maeotica***, which penetrated in the Sea of Azov, Caspian Sea and found in the San-Francisco Bay.

After estimations, results of the analysis of macroplankton species should be presented in the same way as in the Table 3 and tables in ANNEXES.

8. Sampling information on gelatinous plankton collection note

After taking a sample, the information on sampling should be recorded during survey 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:

Table 4. Example of table to be filled during survey of gelatinous plankton

N	short description	explanation	example
1	RV	Name of R/V and cruise number	RV Ashamba
2	Station	Station number	5
3	Depth	Depth (m)	38
4	Year	Year	2019
5	Month	Month	7
6	Day	Day	1
7	Time	Time of sampling	17:30
8	N deg	Coordinate of station: Latitude (Degrees)	45.6593
9	E deg	Coordinate of station: Longitude (Degrees)	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 (degrees)	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 counted 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 abundance of determined taxonomic group ($ind.m^3$)	XXXX
…	Group 1 B	Total biomass of certain taxonomic group ($mg.m^{-3}$)	XXX.XX
	Total C	Total abundance 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. The value excessing 10 $ind.m^{-3}$ should be rounded to integer number.

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

- Average length of each species 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 ANNEX 4.

9. Quality control

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

9.1. Usage 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 (200) 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.

9.2. 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 mucus) should be discarded and sampling repeated.

9.3. 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.

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

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

9.5. Staff training

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

9.6. Data control

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 percents. 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, buffered 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**

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ANNEX I. Taxonomic composition of the most important groups of gelatinous meso- and macrozooplankton

Table AI_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)	+					+
Order LEPTOLIDA						
Family BOUGAINVILLIDAE						
Calyptospadix cerulea Clarke, 1882 (non-native) **(Syn.Bougainvillia megas (Kinne, 1956)**	+		+			+

Taxa	Bulgaria	Georgia	Romania	Russia	Turkey	Ukraine
Class SCYPHOZOA						
Subclass DYSCOMEDUSAE						
Order RHIZOSTOMEAE						
Family RHIZOSTOMATIDAE Cuvier, 1799						
Rhizostoma pulmo (Macri, 1778)	+	+	+	+	+	+
Order SEMAEOSTOMEAE						
Family ULMARIDAE						
Aurelia aurita (L., 1758) (Aurelia sp.)	+	+	+	+	+	+
Phylum CTENOPHORA						
Class NUDA						
Order BEROIDA						
Family BEROIDAE Eschscholtz, 1829						
Genus BEROE Gronov, 1760						
Beroe ovata Bruguière, 1789 (non-native) Syn.:*Beroe ovata* Chamisso and Eysenhardt, 1821 *Beroe ovata* Mayer, 9012	+	+	+	+	+	+
Class TENTACULATA						
Subclass CYCLOCOELA						
Order LOBATA						
Family BOLINOPSIDAE Bigelow, 1912						
Genus BOLINOPSIS L. Agassiz, 1860						
Bolinopsis vitrea (L. Agassiz, 1860) (non-native)	+				+	
Genus MNEMIOPSIS L. Agassiz , 1860						
Mnemiopsis leidyi A.Agassiz 1865 (non-native) 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 II. Identification of the Black Sea native and non-native gelatinous species

TYPE COELENTERATA

Class Scyphozoa:

Fam. Ulmaridae

***Aurelia aurita* (L, 1758) (Fig. AII_1).**

A. aurita is one of the most abundant species among the native gelatinous species (Fig.A2_1). However, as it was mentioned above, recent genetic analyses showed that the Black Sea ***Aurelia aurita*** belongs to clade Borealis (Ramsak et al., 2012) and should be referred as ***Aurelia sp.*** until the final global molecular analyses will be done.

The medusa *Aurelia aurita* 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.AII_1. Adult medusa stage of *Aurelia aurita* (photo Tihomir Makovec)

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

Larva stage of *planula* develops in specific lobes of mouth of female and then lives in water about one week, then sinking to 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 exist for over 3 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.A2_2).

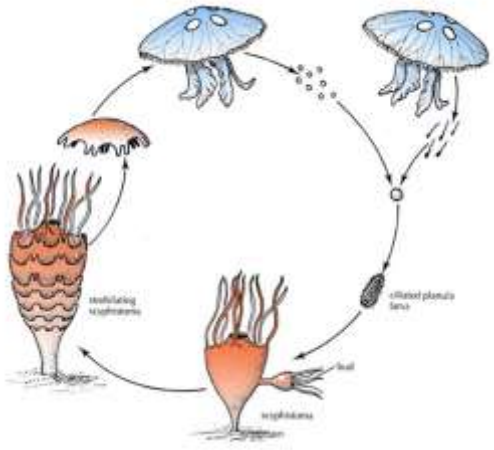


Fig.AII_2. Life cycle of *Aurelia aurita* (http://jellieszone.com/scyphomedusae.html)

Distribution.

Spatial distributions of medusae are extremely irregular in the Black Sea and globally. It is caused by their transport with currents and is manifested in the form of aggregations, observed as local patches or bands, sometimes extended along the shore or, in open regions, along the wind direction. The size of those aggregations may be extremely variable.

In the Black Sea the bulk of the animals usually are concentrated in the subsurface layers or at depths of 30–50 m, where up to 90% of individuals may occur; meanwhile, accumulations may also be observed in the 70–80 m layer. In the near-shore zone, the numbers of medusae that prefer dwelling in the near-bottom layers in the warm periods exceed that in cold ones (Lebedeva and Shushkina, 1983).

Order Rhizostomeae (Cuvier, 1799)
Fam. Rhizostomidae Cuvier, 1800
***Rhizostoma pulmo* (Macri, 1778)**

Rhizostoma pulmo (Fig. **AII_3**) is a common species for the Black Sea; it typically is up to 40-60 cm in diameter in the Black Sea, but can be larger.

The convex umbrella and massive mouth blades with numerous appendages give the jellyfish a distinctive appearance. On the lace blades are located poisonous stinging cells. The poison of ***Rhizostoma pulmo*** does not pose a serious danger to humans. Only sensitive people, when in contact with the oral lobes, can get severe irritation.

The normal life cycle is metagenesis: the alternation of asexual generation (polyps) and sexual generation (medusa).

Fig. AII_3 *Rhizostoma pulmo* (Macri, 1778) (photo Tihomir Makovec)

Distribution

Rhizostoma pulmo occurs in the Black and Mediterranean Seas and in the north-eastern and southern Atlantic off the west coast of South Africa. It feeds on plankton. It dwells mainly in the near-shore regions of the Black Sea and sometimes penetrates with currents into its open part, where only single individuals are encountered. Meanwhile, outbursts of the abundance may also be featured, as it was observed in the north-western part of the sea in the 1960s–early 1970s.

During the last years ***Rhisostoma pulmo*** population began to increase. For instance, in September 2017 its large aggregation was observed in inshore waters off Batumi. Regular blooms are recorded in the Kerch Strait and in the Sea of Azov after its salinity increase during last years (Shiganova, pers. observations; Mirsoyan et al., 2019).

Order Semaeostomeae

Fam. Pelagiidae Gegenbaur, 1856

Chrysaora hysoscella (Linnaeus, 1767)

Fig AII_4 Scypomedusa

Chrysaura hysoscella

Chrysaora hysoscella (Fig.AII_4) Compass jellyfish inhabits the Mediterranean Sea. It is sting jellyfish, which has stinging cells at each tentacle for capturing prey and defense from predators. Recently it has been recorded in the Sea of Marmara and Turkish waters of the Black Sea in a few numbers (Shiganova, Ozturk, 2010). His jellyfish has a benthic polyp stage before developing into a pelagic adult medusae. In adult stage, the bell of the compass jellyfish typically has a diameter of 15–25 cm. It usually has 16 brown elongated V-shaped markings on the translucent yellow-white bell. The markings surround a central brown spot and resemble the face of a compass, hence justifying the common name "compass" jellyfish. It is usually colored yellowish white, with some brown. Its 24 tentacles are arranged in eight groups of three. Compass jellyfish consume a variety of marine invertebrates and plankton.

Field observation should be focused on discovering of this species.

TYPE CTENOPHORA

Order Cydippidea

Fam.Pleurobrachiidae

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

Fig.AII_5 Hunting ***Pleurobrachia pileus*** (https//www.flickr.com)

Pleurobrachia refers to the most primitive order of ctenophores **Cydippidea** and before the 1980s, ***Pleurobrachia pileus*** (Fig. AII_5) was the only species of Ctenophora in the Black Sea. They are small ctenophores; in the Black Sea, their length ranges from 0.1-0.2 mm for hatched larva - and up to 25 mm for adults. 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 a prey, the mouth of Pleurobrachia widely opens. ***P. pileus***, similarly to all the ctenophores, has 8 rows of swimming combs that commence at a distance from the aboral pole.

The length of the swimming combs in the meridional direction comprises two-thirds of the total body length. ***P. pileus*** moves using the swimming combs, located ahead of its oral part. The aboral end hosts the aboral organ. Near it, on both sides, two tentacular bulbs are located, where the ctenophore can retract its tentacles. ***P. pileus*** can move with its tentacles completely hidden. When the tentacles are outside, they may stretch reaching a length of 20 times longer than the length of the animal proper. Extended tentacles feature a row of numerous elongated lateral filaments. At the outer shell, sticky cells or colloblasts are located; they help the animal to catch its prey. ***P. pileus*** is capable of long-term hanging with its oral end up (feeding position); in this position, its tentacles are extended downward and on the sides, forming a kind of catching net. ***P. pileus*** is zooplanktivorous comb-jelly, inhabiting mainly the intermediate layer of the Black Sea.

Invasive Ctenophores in the Black Sea.

Mnemiopsis leidyi **A.Agassiz 1865** (Fig. AII_6 A,B)
Phylum Ctenophora Esch
Class Tentaculata Chun
Order Lobata Esch
Fam. Bolinopsidae Bigelow, 1912
Genus Mnemiopsis L. Agassiz, 1860
Mnemiopsis leidyi **A. Agassiz, 1865**

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

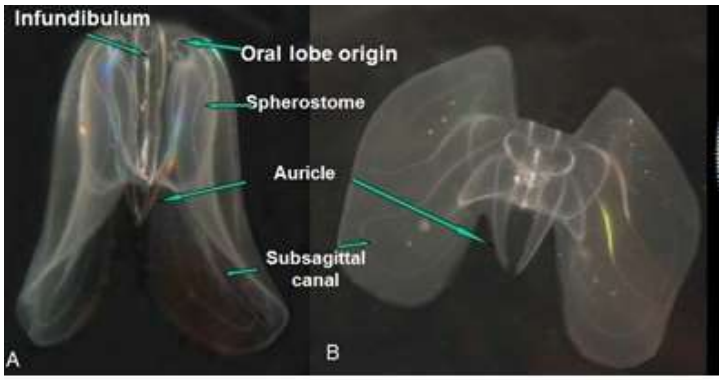


Fig. AII_6 A,B. ***Mnemiopsis leidyi*** from the Black Sea (photo T.Shiganova).

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 – auricles, are situated under the two principal oral lobes.
Closed down with one another by their distal edges, they completely envelop the mouth area of the animal (Agassiz, 1860; Seravin, 1994). The length of this ctenophore in the Black Sea appears to be variable (adult from 40 to maximal 180 mm), depending on the environmental conditions and prey availability.

M. leidyi is a hermaphrodite with two fascicles of gonads (ovaries and testicles) in its gastrodermis. The gonads are located along eight meridional canals of the gastrovascular system. They are localized in the spaces between the ctenes (comb flappers). The rows of ovaries face the principal symmetry planes: sagittal and testicular. On the opposite side of each meridional canal, rows of testicles extend. Thus, in adjacent canals, the rows of testicles are arranged in a mirror-like manner, which is characteristic of Lobata. Gonads are formed in the central parts of the canals from the level of the statocyst almost up to the extreme ctenes toward the oral edge of the body.
It is important to concentrate on collection of early stages of ***M.leidyi*** and ***B.ovata*** development: ovae and larvae, which could be collected with mesozooplankton samples and can be preserved with formaldehyde (Fig.AII_7-9).

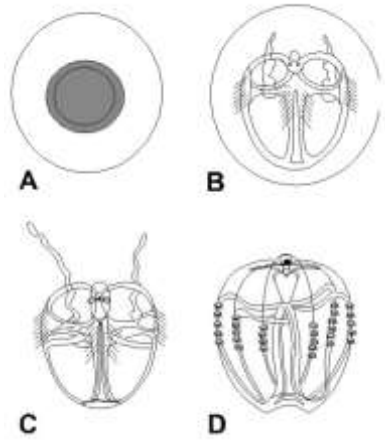


Fig.II_7. A – Ovae of ***Mnemiopsis leidyi***, (size 0.3-0.4 mm), B - embryo, C-D - early stages of larvae development (drawing by T. Shiganova). ***M.leidyi*** ovae are spherical (0.3–0.4 mm in diameter) with a thin non-structured membrane. Average dry mass of egg is 0.0005 mg, with 2% of organic carbon content (Reeve et al., 1989).

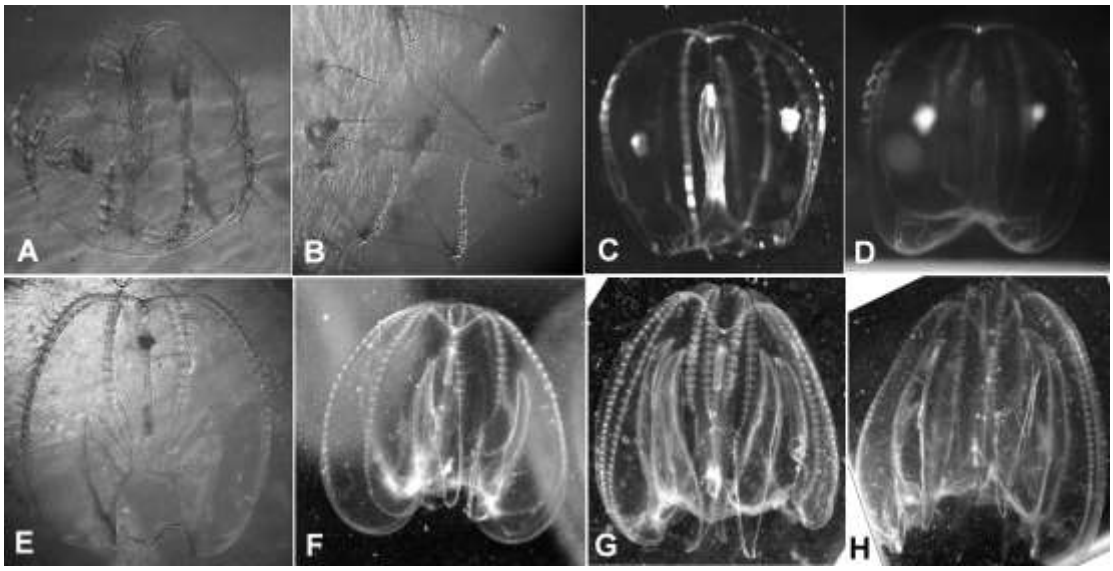


Fig.II_8. A,B **-** Cydippid larvae of ***M. leidyi***; C,D - initial stage of lobes development; E - transition stage of lobes' development; F - transition stage with lobes and auricles' development; G,H - formed individuals with continuation of lobes development (Photo by Shiganova T; Fedorov A).

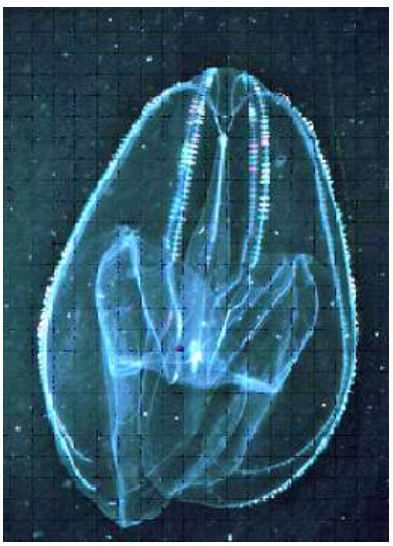
Duration of growth until early maturity depends on temperature and food availability. The average development time from egg to maturity is 19±5 days at temperature 23-25°C (experimental data in the Black Sea, Shiganova, 2009). This is consistent with the data of Baker and Reeve (1974), who reported duration of growth 13-23 days.

Native distribution. The lobate ctenophore ***Mnemiopsis leidyi*** A. Agassiz 1865 is native to estuaries and bays along temperate and subtropical coastal waters of North and South America where it occurs at a wide range of temperature and salinity (Harbison et al., 1978; Kremer, 1994; Purcell et al., 2001; Costello et al., 2012, Mianzan, 1999; Oliveira et al., 2016)

Non-native distribution. This assessment revealed that there are at least two eco-types (Southern and Northern) in the recipient seas of Eurasia with features specific for their donor areas. The range of thresholds for ***M. leidyi*** establishment, occurrence and life cycle in both eco-types depends on variability of environmental parameters in their native habitats (Shiganova et al.,2019).

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

In 2007 and 2010 another ctenophore ***Bolinopsis vitrea***, 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 here we provide information on features that are specific for ***Bolinopsis vitrea*** and allow distinguishing the two mentioned species (Fig. AII_9).



Representative of ***B. vitrea*** can be easily distinguished morphologically from ***M .leidyi*** (Fig. AII_6), based on their 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 two principal oral lobes. The main difference between ***B. vitrea*** and ***M. leidyi*** is in a position of the oral lobes. In ***M. leidyi***, the oral lobes originate near the level of infundibulum (Fig. AII_6), whereas in ***B. vitrea*** they originate approximately half-way between the mouth and the infundibulum (Fig.AII_10). In addition, ***M. leidyi*** may have papillae on the body, while ***B. vitrea*** does not (Shiganova and Malej, 2009).

Fig. AII_9. *Bolinopsis vitrea* (photo of Tihomir Makovec)

***Beroe ovata* Bruguière, 1789**

Phylum Ctenophora Eschscholtz, 1829

Order Beroida Eschscholtz, 1829

genus *Beroe* Browne, 1756

syn.Beroe ovata (sensu) Mayer 1912

Beroe ovata is another species of Ctenophora, non-native for the Black Sea. When species of genus Beroe was introduced in the Black Sea, the problem of species identification became relevant. Detailed analyses of historical data as well as morphology of a new species conducted by Seravin et al. (2002) allowed us to define it as *Beroe ovata,* which was introduced in the Black Sea with ballast waters of the ships from the western Atlantic coast of Northern America as well as previous invader *Mnemiopsis leidyi*. Therefore, identification of another species named *B.ovata,* which inhabits the Mediterranean Sea, became questionable (Seravin et al., 2002). Later Bayha et al. (2004) supported Seravin et al. (2002) identification after genetic analyses using ITS-1. However ITS-1 length variation, sequence divergence and molecular phylogenetic analysis indicated existence of two well-differentiated species in the Black Sea and the Mediterranean both named *B.ovata*. Bayha et al. (2004) gave preliminary names to these two different species of *Beroe*: *B. cucumis* sensu Mayer (=*Beroe ovata* sensu Chun) in the Mediterranean and *B. ovata* sensu Mayer, in the Black Sea, where it was introduced from western Atlantic (Bayha et al., 2004). Now we made genetic analyses of both species with inclusion of Beroe cucumis Fabricius 1780 from the North Sea. As a result we re-name Mediterranean species, initially labeled as *Beroe ovata* Eschscholtz, 1829 (Chun, and later as *B.cucumis* sensu Mayer 1912 (Bayha et al., 2004), to ***Beroe pseudocucumis sp.****nov*. The name ***Beroe ovata*** in the Black Sea should be used with the authority of Bruguiere 1789 but not of sensu Mayer (Shiganova & Abusova,in press 2021).

Beroe ovata has mitten-shaped body, 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. AII_10) (Mayer, 1912).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 coloured 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 ***B. 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.

Fig. AII_ 10. *Beroe ovata* from the Black Sea (photo T. Shiganova)

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 rimming 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).

Beroe ovata, like most species of ctenophores, is a hermaphrodite, capable of self-fertilization. In *B. ovata* individuals of medium to large size, when examined under binoculars, one can see the ribbon-shaped gonads (testes and ovaries) running along the meridional canals and containing mature reproductive elements. The testes are located in the peripheral parts of the canals; the eggs are in the central part. Often eggs can be seen in diverticula (Fig. AII_10). At night, some individuals caught in the coastal area were observed to throw eggs when they were placed in the aquarium. Data on the time of sperm washout are contradictory; some authors indicate that the sperm is washed out first (Carre et al., 1991), others believe that the sperm washout occurs a little bit later (Oliveira, Migotto, 2006). The second conclusion looks more reasonable and is also indicated for M. leidyi (Pianka, 1974). Washout of eggs observed in the gap, formed temporarily by gonatopus, after the spawning of the eggs gonatopus closes, without any injury to the Ctenophora. There may be cases of internal fertilization and even embryonic development, up to the appearance of a larva inside the diverticula (Oliveira, Migotto, 2006). One or more spermatozoa enter the *Beroe* egg and fertilization occurs in less than one minute (Yatsu, 1911). The bulk of the eggs and sperm are usually swept out, and fertilization takes place outside the body of the ctenophores. Carre and Sardet believe that only cross-fertilization exists **in *B. ovata*** (Carre & Sardet, 1984, Carre et al., 1991). However, in our experiments on Beroe fecundity, specimens were kept in isolation, one at a time, and we regularly performed fertilizing and developing of eggs, and received larvae, although in this case the percentage of unfertilized eggs was very high (Shiganova, unpubl.).

Beroe individuals larger than 40 mm and with a raw weight of more than 15 g, actively reproduces. The quantity of released eggs varied from 24 up to 2.945, the average number of spawned eggs amounted to 1989±886 eggs $ind^{-1}day^{-1}$.

The spawned eggs of *B. ovata* have a round shape, sometimes slightly oval, the diameter of the eggs is from 800 to 1000 μm, the inner diameter is 300 μk.

Images of ovae and larvae of *B.ovata* are shown below (Fig. AII_11)

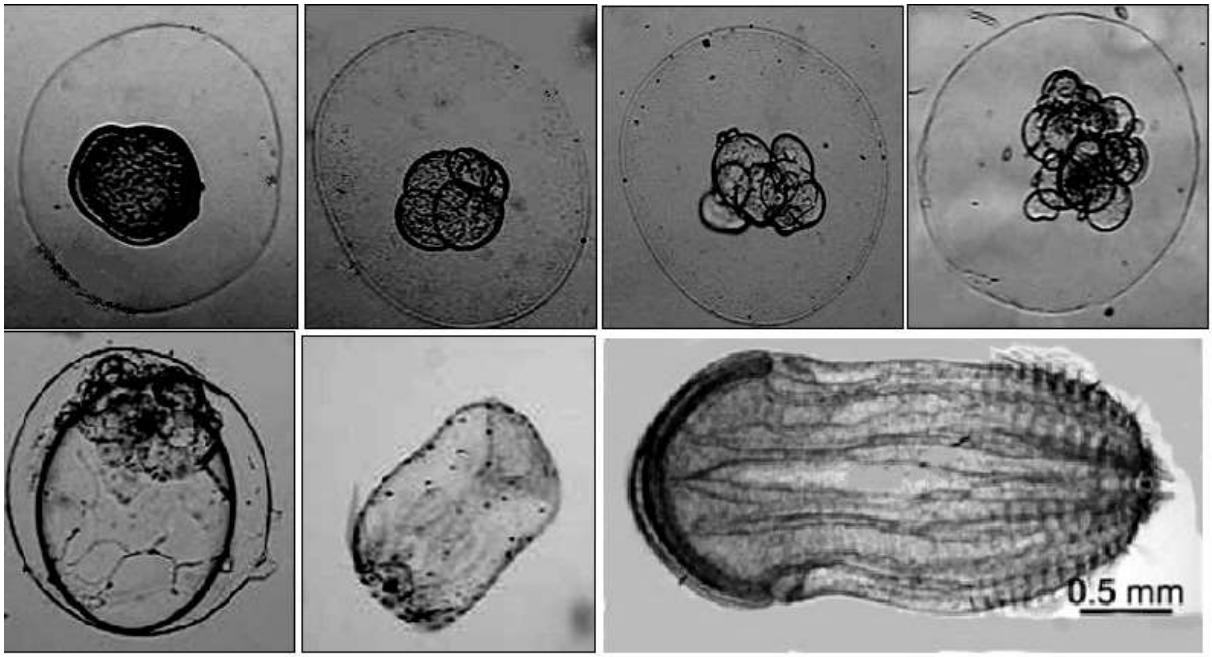


Fig.AII_11. Ovae (size 0.8-1.0 mm) and hatched larvae of ***B.ovata*** *(*size 0.4-0.8 mm) (photo 1-6 T. Shiganova; 7 – O.Oliveira)

Native distribution. In the Southern America – western coasts of the Atlantic Ocean from Colombia to Argentina (10°N to 42°S) (Domaneschi, 1976; Genzano & Zamponi, 1993; Mianzan, 1986, 1999; Oliveira & Migotto, 2006; Oliveira et al., 2007; Nogueira Jr. , 2012; Nogueira Jr. et al., 2015); and in the Northern America - Narragansett Bay, Rhode Island; Chesapeake Bay, Maryland (only in high salinity waters); Biscayne Bay, Florida, coastal waters along the Gulf of Mexico (Kremer, 1994).

Recently it was found in the Indian Ocean off Kollam Coast of the Arabian Sea (09°0.3279'N 76°23.4594'E) (Haripraved et al., in press).

Non-native distribution. ***B. ovata*** has been reported in the Black Sea, Sea of Azov, Caspian Sea, Sea of Marmara, Aegean Sea, Levantine Sea and the Danish waters (Great Belt) (Table 4), where in the most cases it is able to control the abundance of harmful invasive ctenophore M. leidyi (Konsulov & Kamburska, 1998; Shiganova et al., 2000; Shiganova et al., 2001; Seravin et al., 2002; Finenko et al., 2003; Isinibilir et al., 2004; Shiganova et al., 2007; Shiganova & Malej, 2009; Galil et al., 2011: Shiganova et al., 2014a, b; Roohi et al.,2020; Mamish et al.,2020; Badreddine et al.,2020).

Hydromedusae

There are two non-native hydromedusae recorded in the Black Sea: *Blackfordia virginica* and *Calyptospadix cerulea* Clarke, 1882 (Syn. *Bougainvillia megas* (Kinne, 1956)), who were brought first to the Black Sea, and then to the Sea of Azov and the Caspian Sea.

Fig. AII_12 *Blackfordia virginica* (non-native species) (photo Faasse, M.A. & M. Melchers 2014).

Fig. AII_13 *Odessia maeotica* (Ostroumoff, 1896). Mature medusa (diameter about 12 mm) from Portiragnes Plage, France.

Blackfordia virginica was introduced in the Black Sea from the brackish waters of the North America and then widely invaded the other seas (Shiganova, 2009). *Odessia maeotica* is a Ponto-Caspian species, introduced in the Azov and Caspian seas (Shiganova, 2009).

Blackfordia virginica together with the Ponto-Caspian ***Odessia maeotica*** were introduced from the Black Sea in other areas, including the San Francisco Bay (Rees and Gershvin, 1999).

ANNEX III. List of relevant publications on gelatinous species identifications

Baullion Jean, Cinzia Gravini, Francesc Pages, Josef-Maria Gili and Ferdinando Boero (2006) An introduction to Hydrozoa. Publication Scientifiques du Museum.T.124. 593 pp.

Claudia E. Mills and Steven H. D. Haddock. (2007). Ctenophora. GRBQ188-2777G-CH11[189-199].qxd 5/3/07 04:05 PM Page 189 Techbooks (PPG Quark).

Mills, C. E. (1998-present**).** Phylum Ctenophora: list of all valid species names. Available online at: http://faculty.washington.edu/cemills/Ctenolist.html. Published by the author, web page established March 1998, last updated January 2017.

Guide of fauna of the Black and Azov seas. T.1 Free living invertabrata. Ed. V.A.Vodyanitsky. 437 p. (In Russian)

Kramp P.L. (1961). Synopsis of the medusae of the World. J. Mar. Biol. Ass. U.K. 40 (1). 469 p.

Mayer A.G. (1912). Ctenophores of the Atlantic coast of North America // Publ. Carnegie Inst. Washington. V. 162: 1-58.

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ANNEX IV. Criteria and indicators for identification of an environmental status

1. Indicator: *Mnemiopsis leidyi* and *Aurelia aurita* biomass (g·m^{-3}) and abundance (ind·m^{-3}) are proposed as environmental pressure indicators

Gelatinous species are commonly used as an indicator of environmental conditions. We considered here first of all two most abundant gelatinous predators: the most harmful invader ctenophore *Mnemiopsis leidyi* and native jellyfish *Aurelia aurita* (Table 5).

The indicators are also consistent with Descriptor 1 (Biodiversity) and Descriptor 3 (Food web).

Development of *M.leidyi* directly influence mesozooplankton biomass, since it preys upon zooplankton, therefore negatively affecting the biomass of zooplankton and their species diversity, the structure of the community, and the regularities of the functioning of the pelagic ecosystem (Konsulov & Kamburska, 1998, Shiganova et al., 2001; 2004; Kideys & Romanova, 2001, Kamburska, 2004, Finenko et al., 2013). As a key factor controlling the mesozooplankton, *M. leidyi* becomes a reliable indicator of the pelagic ecosystem state and food web functioning. *M.leidyi* biomass growth induces trophic cascades, as it directly affects the population size and composition of the zooplankton and, indirectly the planktonofagous fish and primary producers in the food web (Shiganova et al., 2000; 2004; Daskalov, 2002; Daskalov et al., 2007).

Since 1997, *M.leidyi* population is controlled by *Beroe ovata*. After that, the duration of *M. leidyi* impact on trophic zooplankton structure was limited by two months (July-August) of the year, instead of 6-8 months before *B.ovata* arrival (Shiganova et al., 2014).

To assess *M.leidyi* effect on zooplankton, we take that its population, which consumes less than 10% of the zooplankton biomass per day, cannot reduce their abundance and biomass (Burrell & Van Engel, 1976; Larson, 1979; Purcell, 1994, Finenko et al., 2009). However, higher consumption rate (more than 20 % of zooplankton biomass per day) results in a sharp reduction of the prey abundance (Deason, 1982; Matsakis & Conover, 1991; Shiganova et al., 2004). Based on calculated critical biomass of ctenophore *M.leidyi* that does not affect mesozooplankton abundance, 4 g·m^{-3} or 120 g·m^{-2} (Vinogradov et al. 2005, Finenko et al., 2006) or abundance less that <5 ind.m^{-3} (Shiganova et al, 2014) was identified as a threshold for GES (Good Environmental Status) (Table AIV_1).

Indicator for present state: *M. leidyi* biomass varied in a widely during the last years, like the most zooplankton metrics, but the summer population during the period 2007–2013 has never reached a size as high as at the end of 1980s – mid-1990s. Nevertheless, coastal and shelf habitats manifested larger fluctuations and higher biomass compare to the open sea ones (Shiganova et al., 2014; Stefanova, 2014, Stefanova et. al, 2016). Obviously, during last 6 years recorded concentrations of M.leidyi exceeded the GES threshold almost in all study area, except at open sea stations in Romanian part (Moncheva and Boicenco, 2014).

Table AIV_1. Values of environmental state indicators for ***M. leidyi*** and ***A.aurita***

Indicator	Environmental status				
	High	Good	Moderate	Poor	Bad
M.leidyi					
Daily grazing rate (%)	0	<10	<20	>50	>100
Biomass (g.m^{-3})	0	1-4	5-10	10-30	>30
Abundance (ind.m^{-3})	0	<5	5-10	10-20	>20
A.aurita					
Daily grazing rate (%)	0	<10	>10	>20	
Biomass (g.m^{-3})	0	<1	<5	5-10	>10

2. Indicator: non-native to native species ratio

Gelatinous plankton species include a significant number of established invasive species. Among ctenophores, its share comprises 66.7% and among Hydromedusae - 9%. The proposed GES threshold for the proportion of abundance (or biomass, when appropriate) of alien species to native ones is less than 10% (Moncheva and Boicenco, 2014).

3.Indicator: Abundance/biomass of gelatinous species – related to primary criteria D4C2

The abundance and biomass of Scyphozoa and Ctenophora undergo significant seasonal and interunnual fluctuations, and their biomass development and aggregations may cause trophic cascades [Mills, 2001; Lucas, 2001]. Most of these species (except *B. ovata*) are mesozooplankton consumers and exhibit a considerable variety of strategies.

4.Indicator: *Mnemiopsis leidyi* abundance/biomass - related to secondary criteria D2C2

Some of non-native species are identified as invasive and their abundance or biomass, trends in population, temporal occurrence and spatial distribution are an important indicators for defining the status of the species and, respectively, its opportunity to achieve good status. Among them invader *M. leidyi* is a key controlling factor for the mesozooplankton stocks and a reliable indicator of the pelagic ecosystem dynamic and food web functioning.

5.Indicator*: Noctiluca scintillans* biomass (N.sci %) related to secondary criteria D5C3contribution of N. scintillans biomass to total mesozooplankton biomass.

The wide feeding spectrum (phytoplankton, zooplankton and detritus) of the species, developing in high bloom concentrations, usually after the mass development of phytoplankton, determines its ecological importance for the pelagic ecosystem (Kiørboe, Titelman 1998; Dela-Cruz et al., 2003). *Noctiluca* expanded in bloom concentrations under favorable conditions (calm weather, phytoplankton blooms, low salinity) (Adnan AlAzri et al., 2007). *N. scintillans* density is usually higher in coastal areas where maxima of phyto- and zooplankton concentrations are registered.

6.Indicator: Mesozooplankton abundance/biomass related to primary criteria D1C6

Assessments as a rule start with the evaluation of a single element (e.g. species, habitat) for which there is a dataset for the assessment area in the assessment period defined in the national monitoring programs. These then pass through several steps of an assessment process. Indicators need setting threshold values at national or regional level, testing and validation, with associated target values or classification boundaries.

The importance of zooplankton as an indicator of ecological conditions stems from its position in the food web, sandwiched between the top-down regulators (fish or jellyfish) and bottom-up factors (phytoplankton), thus providing information about the relative significance of top-down and bottom-up controls and their impact on water clarity (Jeppesen et al., 2011). Zoooplankton is mentioned in the WFD CIS Monitoring Guidance (2003) as a 'supportive/interpretative parameter''. Nevertheless, the WFD approach could be applied to zooplankton for development of classification system for the ecological state assessment of coastal marine waters. The list of zooplankton metrics (indicators) could include: a) Mesozooplankton biomass [mg/m^3], b) *Noctiluca scintillans* biomass [mg/m^3], c) *Mnemiopsis leidyi* biomass [g/m^3], d) Shannon-Weaver index [ind/bit^1].

Additionally, for implementing of MSFD, indicators relevant to Descriptor 1, 4 (Biodiversity and Food web), Descriptor 2 (Non-indigenous species) and Descriptor 5 (Eutrophication) are as follow:

1.The indicators reflecting composition of zooplankton community.

Mezozooplankton indirectly exposed to eutrophication process (with changes of the amount of edible zooplankton, its composition and size) and scale of catches of commercially exploited fish (through changes in the pelagic food web), while the direct impact is shaped by climate change (temperature) and predation on fish and gelatinous plankton, which decrease their stocks.

2.Indicator: Copepoda biomass [mg.m^{-3}] or [%] related to primary criteria D1C6.

Key group of Copepods has a crucial role in the pelagic food web dynamics by transferring energy from the primary producers into the form, available for fish consumption. The copepods species composition affects directly both the phytoplankton and zooplankton species composition and has a potential to affect the biodiversity in these communities (HELCOM, 2012).

3.Indicator: Mesozooplankton mean size (MeanSize) related to secondary criteria D4C3

The mean size of zooplankton individual is defined as a ratio between the total biomass and zooplankton abundance (ICES Advice, 2015; HELCOM, 2015).

Zooplankton community mean size is indicative for both fish feeding intensity and the grazing pressure on phytoplankton. This core indicator employs zooplankton mean size and total stock to evaluate pelagic food web structure, with particular focus on the lower level of webs (HELCOM, 2015). Thus, the indicator represent a synthetic descriptor of zooplankton community structure i.e. a high concentration of zooplankton with a large average size of individuals predetermines good feeding conditions for zooplanktonivorous fish as well as a high grazing potential. Combinations like as a small total stock or an average small size, or both, would indicate limitations in the ability of the zooplankton community to transfer energy from primary producers to higher trophic levels. The indicator has a solid scientific basis and addresses the relevant aspects of zooplankton as a mediator of energy from primary producers to fish.

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ANNEX V. Examples of data reporting / Dataset description format

Dataset description format (example)

Cruise information			
Area of cruise	Vessel name	Ashamba	Dates of cruise
Country	Russia		
Organization	P.P.Shirshov Institute of Oceanology Russian Academy of Sciences		

Index cruise	Cruise	Date beginning	Date end
Cruise 1	Ashamba_2016_05_05	05.05.2016	05.05.2016
Cruise 2	Ashamba_2016_06_21	21.06.2016	21.06.2016
Cruise 3	Ashamba_2016_07_25	25.07.2016	25.07.2016
Cruise 4	Ashamba_2016_08_22	22.08.2016	22.08.2016
Cruise 5	Ashamba_2016_11_02	02.11.2016	02.11.2016
Cruise 6	Ashamba_2016_11_25	25.11.2016	25.11.2016

LABORATORY DETAILS

Organization performing the analysis	Name:	RU-P.P.Shirshov Institute of Oceanology, RAS
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	Contact person:	
	Contact person Phone:	
	Contact person E-mail:	
Laboratory performing the analysis	Name:	
	Contact person:	
	Contact person Phone:	
	Contact person E-mail:	

Metadata format (example)

Sampling information

Index cruise	Sampling number	Day	Month	Year	Lat [North]	Lon [East]	Sampling time	Up Depth	Low Depth	Bottom depth	Tool Net	Note
Cruise 1	Sample 1	5	5	2016	44°31´259N	37°53´992E	10:35	0	160	500	BR	
Cruise 1	Sample 2	5	5	2016	44°31´259N	37°53´992E	10:35	0	27	500	BR	
Cruise 2	Sample 3	21	6	2016	44°31´284N	37°54´060E	14:40	0	160	500	BR	
Cruise 2	Sample 4	21	6	2016	44°31´284N	37°54´060E	14:40	0	7	500	BR	
Cruise 3	Sample 5	25	7	2016	44°31´269N	37°54´012E	13:30	0	160	500	BR	

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

Data format (example)

Species	ind.m^{-2}	mg. m^{-2}	cal.m^{-2}	ind.m^{-3}	mg.m^{-3}	cal.m^{-3}	aver.mm	aver.mg	aver.cal
Aurelia aurita 10-30 mm	20	400	12	0,8	16	0,48	10	20	0,6
Pleurobrachia pileus	20	5000	250	0,8	200	10	10	250	12,5
Aurelia aurita 10-50 mm	20	317000	9520	0,4	6350	190	92,5	15900	476
M.leidyi 10-30 mm	50	292000	2920	1	5830	58,3	41,4	5830	58,3
Pleurobrachia pileus	80	22000	1100	1,6	440	22	10,1	275	13,7
Aurelia aurita >50 mm	10	123000	3680	0,0625	768	23	85	12300	368
M.leidyi <10 mm	40	245000	2450	0,25	1530	15,3	41	6110	61,1
Pleurobrachia pileus	700	185000	9230	4,38	1150	57,7	9,69	264	13,2
Aurelia aurita <10 mm	40	139000	4160	0,25	866	26	51,5	3460	104
M.leidyi 2-10 mm	10	42400	424	0,0625	265	2,65	37	4240	42,4
Pleurobrachia pileus	820	220000	11000	5,13	1370	68,7	9,73	268	13,4
Aurelia aurita 10-30 mm	20	84700	2540	0,8	3390	102	43,5	4240	127
M.leidyi 2 mm	10	62800	628	0,4	2510	25,1	45	6280	62,8
Pleurobrachia pileus	80	35900	1800	3,2	1440	71,8	11,8	449	22,5
Aurelia aurita 10-30 mm	40	23300	698	0,8	465	14	26	582	17,4
M.leidyi 2-10 mm	10	87100	871	0,2	1740	17,4	53	8710	87,1
Pleurobrachia pileus	120	38400	1920	2,4	768	38,4	10,5	320	16

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Macroplankton (Gelatinous plankton)

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