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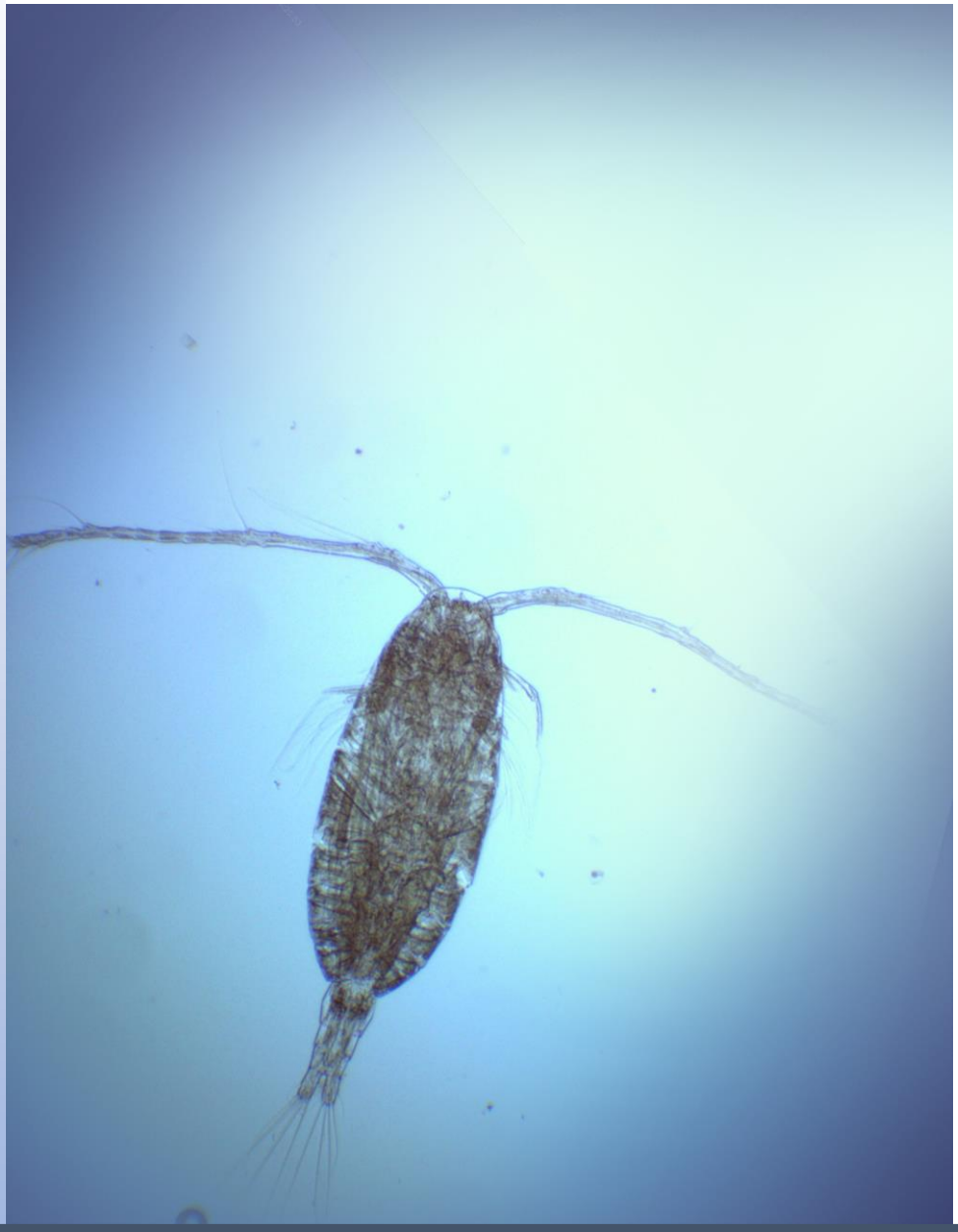

# Black Sea Monitoring Guidelines

# Mesozooplankton

This document has been prepared in the frame of the EU/UNDP Project: Improving Environmental Monitoring in the Black Sea – EMBLAS.

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# 1 Introduction

Mesozooplankton plays a pivotal role in the pelagic food web since it links primary producers and higher trophic levels (mainly fishes). Assessment of mesozooplankton community is the essential component of descriptors of good environment status established by the Marine Strategy Framework Directive (European Commission, 2008).

In general, zooplankton is divided into three size classes: microzooplankton with length range 20-200 μm; mesozooplankton with 0.2-20 mm and macrozooplankton > 20 mm (Sieburth et al., 1978). This classification is now widely accepted (Raymont, 1983; ICES Zooplankton Methodology manual, 2000). This is a conventional division and different developmental stages of some species can belong to different size classes (for example, the early nauplii of copepods and their older copepodite stages according to their size belong to micro- and mesozooplankton, respectively).

All size classes play an important role in the functioning of pelagic ecosystems. For their investigation different sampling equipment and methodological approaches are used: microzooplankton is mainly collected with water-samplers, mesozooplankton is usually sampled using different models of plankton nets, while for macrozooplankton studies large plankton nets, trawls, underwater observations and video technique are usually recommended.

This manual is devoted to methods and approaches applicable for monitoring of mesozooplankton. For other zooplankton groups the relevant manuals on "Microzooplankton" and "Macrozooplankton", published together with "Mesozooplankton", have been elaborated for the Black Sea regional monitoring. Beside methodological recommendations all zooplankton Manuals contain their relevant Black Sea species List.

Mesozooplankton consists of holoplanktonic and meroplanktonic animals. The former spend their entire life cycle in the pelagial (e.g. copepods, cladocerans, chaetognaths, rotifers), the latter (usually larvae of benthic invertebrates) spend only the larval or early stages of their lives as part of the plankton and then as adults they live on the sea bottom. The list of benthic animals having pelagic larval stages will be included into the "Zoobenthos" Manual check-list[1].

Demersal zooplankton constitutes mobile benthic organisms, which periodically emerge from the benthos and move up into the water column. The Black Sea demersal mesozooplankton consists mainly of Mysidacea, Isopoda and Cumacea (Anokhina, 2006). At night they can amount up to 90% of the total mesozooplankton biomass (Anokhina, 2005).

The heterotrophic dinoflagellate *Noctiluca scintillans* is a 'special' component of the Black Sea mesozooplankton, since this species is not metazoan. *Noctiluca* plays an important role in the Black Sea pelagic community forming massive blooms when its concentration can reach millions cells per square meter, exceeding the total abundance of mesozooplankton metazoans (Konsulov and Kamburska, 1998). This omnivorous species consumes intensively a wide range of food particles - from small flagellates and coccolithophorids (< 5 μm) to large diatoms, copepod eggs and nauplii (200 μm and more) (Nikishina et al., 2011) and can successfully compete for food with other mesozooplankters, thereby resulting in reduction of their abundance. Traditionally the distribution of *Noctiluca* is studied together with mesozooplankton because of its omnivory and large size (200-800 μm), but its abundance and wet biomass are usually reported separately and not included in the total abundance and biomass of zooplankton metazoans (so called "fodder zooplankton"). In terms of dry and carbon biomasses *N. scintillans* rarely exceeds 10% of the total Black Sea mesozooplankton biomass.

A special group of mesozooplankton consists of organisms inhabiting the surface layer or moving on the surface film. This group forms a very numerous global scale association together with bacteria, fungi, algae, fish eggs, larvae and fry. All of them are well adapted to the specific environmental conditions of the surface habitat, conventionally the microlayer 0-5 cm, named "neustal" (Zaitsev, 1970). The inhabitants of neustal, or neuston, have intensive protective

[1] Planned for development in 2015-2016.

coloration against UV radiation and predators from the water and air, appropriate behavioural reactions. Therefore, neustonic organisms are very rare or lacking in the water column. The marine neuston plays an important role in the food ration of many organisms, including commercially important species of invertebrate and fish. Because of its surface position, the marine neuston proved to be an ecological target for different kinds of man-made impacts *inter alia* of chemical and radioactive pollution, which adversely affects the abundance of neustonic species. The monitoring of marine neuston is an efficient method of assessment of the ecological status of the marine environment (Zaitsev, 1997, 2012a).

# 2 Purposes of zooplankton monitoring

The main goal of zooplankton monitoring is assessing of the state of pelagic ecosystem and its changes under natural and anthropogenic impacts. Within the frame of this general goal zooplankton monitoring has the following explicit aims:

- To portray the species composition and spatial distribution of mesozooplankton abundance and biomass;
- To diagnose the early introduction of non-indigenous species in the region;
- To determine interannual, decadal and long-term changes in mesozooplankton abundance and community structure;
- To forecast the state of zoobenthic communities (success of reproduction, changes in the ratio of main taxonomic groups etc.) based on quantitative assessment of meroplankton abundance and composition.

To achieve comparability of the data obtained during monitoring program in the different Black Sea littoral states a standard methodology for mesozooplankton sampling and processing is required. At present, a comparison of quantitative results over the entire Black Sea is complicated due to the differences in methodology and equipment used. Therefore, the Manual suggests the recommended technics and approaches an adherence to which will provide the comparable results on zooplankton abundance and biomass obtained by the different laboratories. Other methods and equipment can be used as well, but the extended intercomparison with the suggested standard technics is strongly recommended.

# 3 Sampling

## 3.1 Equipment

**Vertical hauls**

Mesozooplankton should be sampled by means of vertical hauls using a plankton closing net. Juday net (Fig. 1a) is an appropriate instrument for this aim. The results of most zooplankton investigations in Russia, Ukraine, Georgia, Bulgaria and Romania are based on the Juday net tows. This is a biconical net with a non-filtering upper part and filtering lower part. The original net suggested by Juday in 1916 had the following parameters: diameter of opening 12 cm, diameter of middle ring 17 cm, length of upper cone 40 cm and length of filtering cone 47 cm (Bogorov, 1947). Later V.G. Bogorov proposed a modified model of this net for marine investigations, which had the same proportions but was enlarged: diameter of mouth 36 cm (i.e. mouth area 0.1 $m^2$), diameter of medium ring 50 cm, length of upper cone 120 cm and length of filtering cone 150 cm (Kiselev, 1969). This type was used widely for a long time under the name of Large Juday Net in most of the Black Sea countries. Since 1960s, the majority of long-term data sets on the Black Sea mesozooplankton were based on the samples taken with this type of net. The Juday net has good filtration capacity (up to 100%), simple and reliable mode of closing, lack of loss of plankton during closing (Kiselev, 1969) and has been considered as optimal in size for coastal and shallow waters, being easier to operate onboard small vessels. However, in Turkey and Romania together with

Juday net WP-2 net (Fig. 1b) and Nansen net (Fig. 1c) were sometimes used. In Turkey: 1) WP-2 closing net with 200 μm mesh size (EU project SESAME); 2) Nansen net, 50 cm mouth diameter, 200-212 μm mesh size ; 3) Nansen net, 70 cm diameter, 112 μm mesh size was used in the 1990s, together with the WP-2 net equipped with 300 μm mesh size. In Romania: 1) Nansen closing net, with mouth area 0.385 $m^2$ (70 cm diameter), the upper part is nonfiltering, the middle part with 100 μm mesh size, and the lower part with 55 μm mesh size (model: Nansen closing net produced by HYDRO-BIOS) (EU project SESAME); 2) Juday net with mouth area 0.1$m^2$ and mesh size 150 μm.

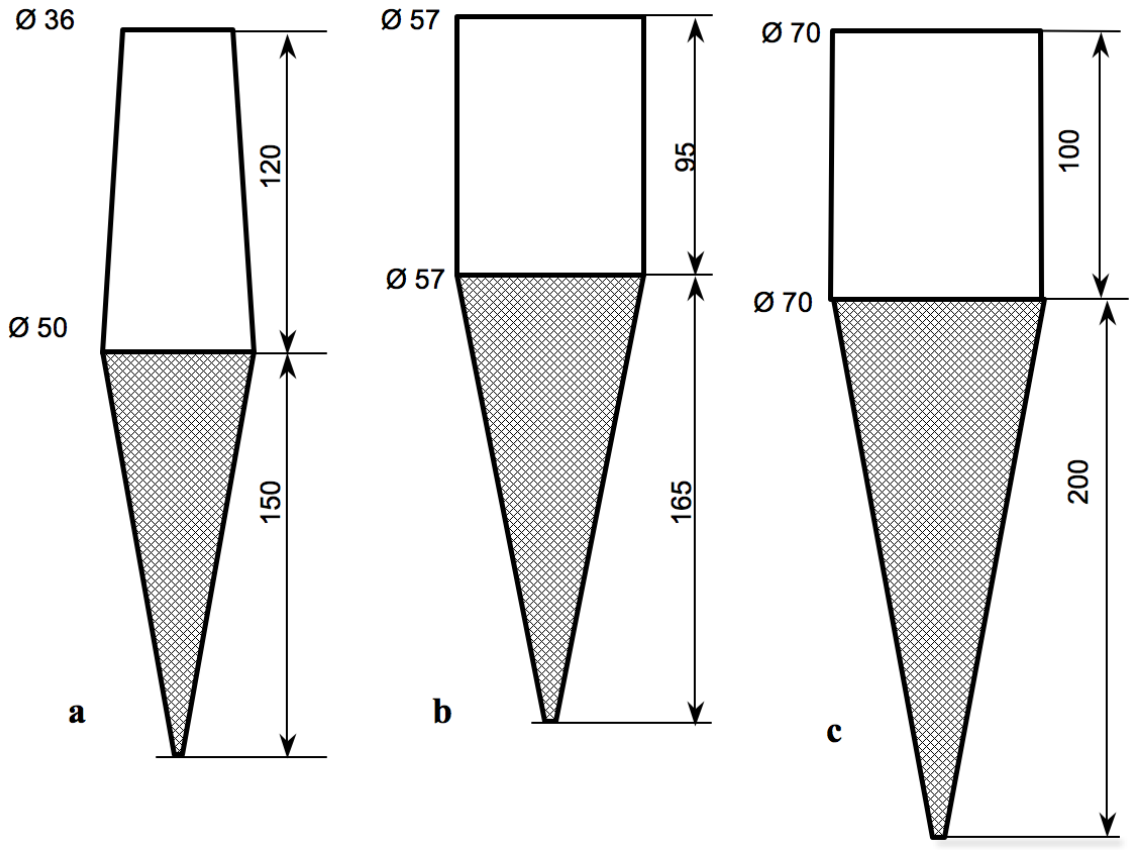


**Fig. 1. Types of zooplankton nets used in the Black Sea countries (a – Juday net, b – WP-2 net, c – Nansen net).**

Taking into account the technical parameters of the Juday closing net with mouth diameter of 36 cm and 150 μm mesh size, as well as the long history of its usage, this net is recommended as a standard net for mesozooplankton monitoring. The 150 μm mesh nylon was used rather often in the Black Sea investigations by different Institutions and was a compromise between window 100-110-120 μm, commonly used in the brackish waters dominated by small rotifers and cladocerans, for instance in the Baltic Sea or North-West shelf of the Black Sea, and 180-200 μm, typical for the marine and ocean waters dominated by large zooplankters, for instance the Northern Atlantic.

For stratified sampling the net should be equipped with a releaser. Any type of a releaser can be used, although widely distributed since 1960s PVR-60 of SIO RAS construction have the advantage of a swivel protecting wire against twisting.

In order to take the tows as vertically as possible, a lead weight of 10 kg is recommended when sea conditions are relatively calm. When the ship drifts rapidly, a lead weight of 20 kg or more may be required to keep the wire angle below the suggested maximum of 25° (UNESCO, 1968).

A measure of the volume of water filtered during a plankton tow is essential in quantitative sampling. The simple and so far most widespread calculation is based on length of tow and area of mouth: V=S·d, where V is the volume of water filtered by the net, S is the mouth area, and d is the distance of the tow. However, clogging of the net introduces an error in this calculation and the use of flowmeter is strongly recommended. The flowmeter should have a stop to prevent reversing and another stop to prevent turning in air. The best position for the flowmeter is midway between the center and the net rim. Whenever possible a second flowmeter should be placed outside the rim. The ratio of the inner to outer flowmeter readings will yield the integrated filtration efficiency for each tow. Filtration efficiency less than 85 per cent would indicate that clogging has occurred and the tow should not be regarded as quantitatively accurate (UNESCO, 1968). In this case a new sample should be taken after rinsing the net.

The flowmeters are commonly supplied with a calibration curve from the manufacturer. However, the field calibration is also recommended. The standard method is to tow the flowmeter free of the net over a known distance and equate impeller revolutions to a measure of distance. Calibration should be performed in the calm windless day by the following procedure:

- to attach the flowmeter to the net's rim free of the net;
- to tow the rim with the attached flowmeter vertically from the depth of 100 m to the surface at the same velocity as the one used for the actual sampling and to record the number of impeller revolutions;
- to repeat the procedure five times to obtain a reliable average number of impeller revolutions without net, e.g. without clogging;
- to equate the number of impeller revolutions to a measure of distance.

This number can be compared with the real revolutions during the sampling for estimation of the filtration capacity of the net under certain conditions.

To lower the net at the desired depth it is necessary to measure the length of wire with a meter wheel and the cable angle with a clinometer. To compute the wire length needed to release in order to take sample from the desired depth, one can use the equation: L = D/cos α, where L is length of the wire; D is the desired depth, α is the wire angle. In Table 1 the examples of estimation of wire length needed to achieve some standard sampling depths at different wire angles are shown.

If the wire angle exceeds 40º, the sample should be discarded. Records of wind speed should be kept.

**Table 1. Length of the wire (*L*, m) to be released to achieve the desired depth (*D*, m) at different wire angles (α) estimated as: *L = D/cos a**.**

| Desired depth (D) | Length of the wire (L) at different angles (*a*) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *a*=5° | *a*=10° | *a*=15° | *a*=20° | *a*=25° | *a*=30° | *a*=35° | *a*=40° |
| 10 | 10 | 10 | 10 | 11 | 11 | 12 | 12 | 13 |
| 25 | 25 | 25 | 26 | 27 | 28 | 29 | 31 | 33 |
| 50 | 50 | 51 | 52 | 53 | 55 | 58 | 61 | 65 |
| 75 | 75 | 76 | 78 | 80 | 83 | 87 | 92 | 98 |
| 100 | 100 | 102 | 104 | 106 | 110 | 115 | 122 | 131 |
| 150 | 151 | 152 | 155 | 160 | 166 | 173 | 183 | 196 |
| 175 | 176 | 178 | 181 | 186 | 193 | 202 | 214 | 228 |
| 200 | 201 | 203 | 207 | 213 | 221 | 231 | 244 | 261 |
| 225 | 226 | 228 | 233 | 239 | 248 | 260 | 275 | 294 |

* The cosine values for some angles: cos 5°= 0.996; cos 10°= 0.985; cos 15°= 0.966; cos 20°= 0.940; cos 25°= 0.906; cos 30°= 0.866; cos 35° = 0.819; cos 40°= 0.766

**Horizontal hauls**

Neustonic zooplankton should be sampled by means of horizontal hauls using Marine Neuston Trawl (MNT). Unlike drifting nets, which only occasionally catch a few specimens of the most motile neustonic zooplankters, such as Pontellidae copepods, isopods, large decapod larvae, fish larvae and fry, a moving gear can be used for the quantitative sampling of these organisms. The MNT neuston trawl is especially suitable for this purpose. The frame of the trawl is an ellipse with axes 100 and 50 cm long, made of bronze or brass rod, 10-12 mm thick (Fig. 2). A belt of solid fabric 10 cm wide joins the net to the frame. The net is 400 cm long and made of 180-300 μm mesh sieve. A belt fastens a cylindrical brass vessel to the filtering part of the MNT. Four lines, 5-6 mm in diameter, lead from each side of the frame to the brass vessel. Grooved, prism-shaped plastic foam floats measuring 25 x 12 x 8 cm are fitted firmly to each side of the frame (Zaitsev, 1983).

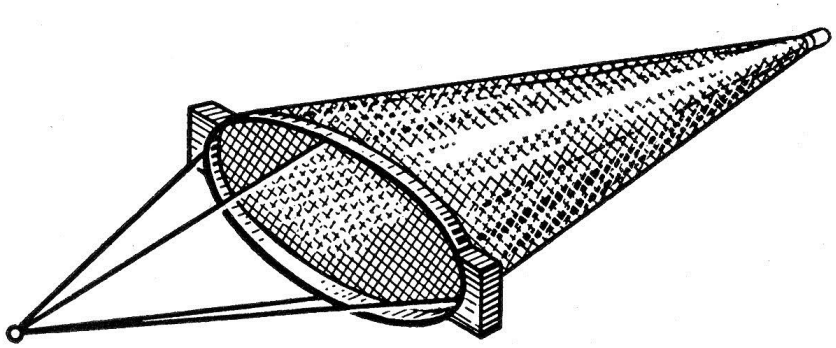

**Fig. 2. Marine neustone trawl.**

The MNT is operated from a circling ship and towed at a speed of 2 m/sec (Fig. 3). The trawl is attached by the shackle to the steel cable of the winch. As the ship moves, the MNT is shot, paying out 50-100 m of line; then the winch is locked and the time count begins. After 10 min the trawl is hauled by slowly turning the winch. Because of the floats, the trawl mouth is submerged in the water only to a depth of 25 cm and covers a width of 1 m. Since the ship moves in a circle, the trawl does not enter wake and operates in a zone where turbulence of the hull and propeller is absent or negligible. The monitored area of the sea surface ($m^2$) is equal to the distance of the MNT hauling (m). For more correct calculation of the catching area the use of a flowmeter is recommended.

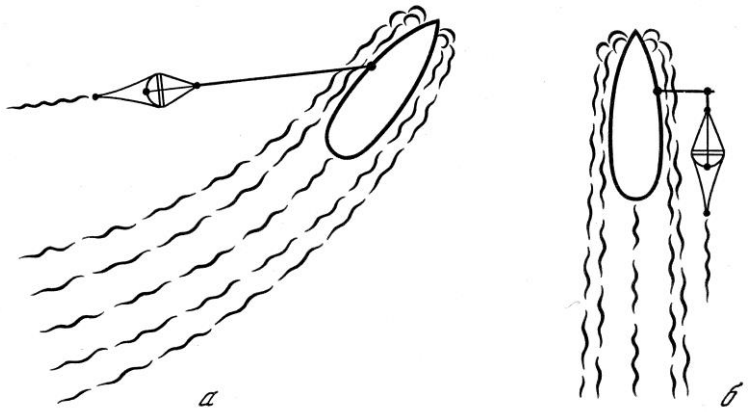


**Fig. 3. Two methods of sampling by marine neustone trawl: a - circling ship, b - towed.**

In addition to large organisms, the MNT catches various small organisms. Some of them are damaged by the high speed of trawling. For example, many fish eggs at the early stages of embryogenesis are so deformed that they can be determined only by experts. Since the MNT is immersed in water up to 25 cm, zooplankton species not obligatory associated with the neuston layer of 0-5 cm can be found in the samples. Therefore, only obligate neustonic species should be taken into account, namely copepods Pontellidae, isopod *Idothea ostroumovi*, zoea and megalop stages of Decapoda, larvae and fry of grey mullets (*Mugil, Liza*), red mullets (*Mullus*), garfish (*Belone*), and shore rockling (*Gaidropsarus*).

**Remark**

For environmental monitoring the neustone samples from coastal waters and shelf zones are most representative, because the inhabitants of the sea surface are the first to react on human influence, such as chemical contamination (Zaitsev, 2012b). Due to the fact that neustone has not yet become obligatory monitoring object, although such proposals exist (The sea surface…, 2005), investigation of neustone can be recommended as a complementary to mesozooplankton vertical hauls.

Zaitsev, Yu.P., 2012. Accumulation of matter and energy at the sea surface and the marine neustone phenomenon // Marine Ecological Journal.- Vol. XI, No. 1.- P. 5-23 (in Russian).

The sea surface and global change, 2005. / Ed. by Peter S. Liss, P.S. Liss, Robert A. Duce.- Cambridge Univ. Press.- 519 pp.

## 3.2 When to sample

Water temperature is one of the main factors that influence aquatic organisms and determine their functional activity (Portner, 2002, Hoffmann and Todgham, 2010). Temperature determines 'biological seasons' of the sea. The Black Sea is the temperate continental basin with the high amplitude of seasonal temperature variations. In winter, the surface temperature drops to 4-6 °C in the northeastern part and to 6-8 °C in the central open sea; in summer the temperature can reach 27 °C and more. The formation of seasonal thermocline starts in April-May depending on the regional climatic conditions. This period is usually associated with hydrological and biological spring accompanied by phytoplankton bloom and reproductive activity of many pelagic and benthic invertebrates (with development of their pelagic larvae). In summer, as the upper layer gets warmer, the thermocline deepens and by autumn the thickness of the upper mixed layer can reach 30-50 m depending on the water dynamics. Below thermocline the temperature equals to 7-8 °C. Sharp seasonal thermocline serves a boundary for vertical distribution of thermophilic and psychrophilic zooplankters. Autumn storms and drop in the temperature destroy the thermocline and the winter temperature becomes practically homogenous in the entire water column at most parts of the sea except nearshore areas.

The hydrophysical and temperature conditions essentially affect the timing of biological seasons. The period before the formation of thermocline and with the temperature below 8 °C can be defined as biological winter; biological spring starts after thermocline formation and warming of the surface water from 8 to 16 °C; the surface temperature above 16 °C corresponds to biological summer; biological autumn coincides with the period of deepening of thermocline and fall of temperature from 16 to 8 °C (Vinogradov et al., 1966; Vinogradov et al., 1992). Seasonality of hydrophysical characteristics can slightly vary depending on regional climatic conditions and geographical setting but in a general way it has the common pattern for the entire Black Sea.

The minimum sampling frequency for mesozooplankton is four times per year. It is recommended to perform monitoring surveys in the periods corresponding to four described above biological seasons. The onset and duration of these seasons differ between coastal areas of the Black Sea countries, so it is the water temperature rather than pre-defined dates that should be the signal for sampling activity. Whenever possible mesozooplankton sampling should be accompanied by hydrophysical and hydrochemical studies to keep the complex approach to ecosystem monitoring and minimize sampling costs.

## 3.3 Sampling site

Choosing the location of monitoring stations, special attention should be given to areas of high risk or/and sites of special scientific interest.

The recommended sites for monitoring are:

- "Hot spots" – the areas with land-based and sea-based sources of pollution or any other sources of environmental degradation;
- The areas under the influence of river runoff;
- The reference areas with good environmental status (to monitor the natural changes in zooplankton community);
- Long-term transects or polygons (to sustain time-series data collected for decades).

For the correct determination of the status of the zooplankton community, monitoring stations should be located not only in coastal waters, but also in the deep sea.

The spatial arrangements of sampling stations (polygon, transect, random design, preferential design etc.) depend on the objective of the monitoring.

## 3.4 Sampling depths

The Black Sea is characterized by strongly stratified water column. In the warm seasons the thermocline separates the warmed-up upper layer from the cold intermediate layer (CIL) with the temperature 7-8 °C. Below CIL the sharp halocline causes the permanent anoxia. The depth of the oxic/anoxic boundary coincides with a density σ-theta=16.2 (Vinogradov and Nalbandov, 1990; Tugrul et al., 1992). Both the depth of the boundaries and extension of the layers vary at temporal and spatial scales, depending on the water dynamics. In this connection, the depth of zooplankton sampling should be chosen based on data of CTD casts.

For fractionated hauls the following depth intervals are suggested:

1. The upper mixed layer (if the thickness is more than 10 m) - from the upper boundary of the thermocline to the surface.
2. Thermocline (if the thickness is more than 10 m) - from the lower thermocline boundary to the upper thermocline boundary.
3. From the bottom or from the oxic/anoxic boundary to the lower boundary of the thermocline.

In case of weak temperature stratification or when the sampling is not accompanied by CTD cast, two standard layers, 25-0 m and 200 m (bottom) - 25 m, should be sampled. If the depth of the sampling station is less than 10 m a single vertical haul should be undertaken from the bottom to the water surface.

The volume of filtered water has to be sufficient to get the number of plankton animals appropriate for statistically significant counting. In case the sample contains low number of zooplankters, the additional sample should be taken to increase the number of collected animals.

## 3.5 Sampling procedure

The Juday net should be hauled vertically with a speed not exceeding 1 m/s (UNESCO, 1968). After taking the net onboard, the content of cod-end should be poured in the large jar or bucket. After each tow, the net should be rinsed two times with the closed cod-end using a gentle flow of sea water from a hose. The rest of the sample collected in the cod-end should be added to the main part already placed in the jar. Alternatively, the lower part of the net might be rinsed by lowering it into the sea consequently two times with the closed cod-end. After sampling the net should be rinsed without cod-end or with open tap by hosing or by lowering into the sea. The

sample should be concentrated through the sieve with mesh size smaller than the mesh size of the filtering cone of the net, and then thoroughly washed down to the sample bottle. When jelly-fish are present in the sample, it is recommended to discard the sample and collect a new one, if possible. In order to save the filtration capacity, after each cruise the net must be washed with detergent in warm fresh water.

A waterproof label or piece of paper (preferably in a small plastic bag) should be placed inside the sample bottle, detailing the name of RV, cruise number, station number, data, time, sampling layer, and number of hauls written by a lead pencil. The small plastic bag protects the label from chafing, discoloration or other physical damage during transportation and storage. An additional outside label can be attached to the bottle.

# 4 Preservation

The samples should be preserved in 4% seawater formalin solution (1 part of 40% formaldehyde and 9 parts of sea water). The formaldehyde should be buffered to pH 8-8.2 with sodium borate (borax) ($Na_2B_4O_3.10H_2O$). Ensure that a sample volume is correctly measured. The volume of 50-100-150-200 ml is the most suitable for further analysis and storage. It is convenient to mark the levels of 50, 100, 150, and 200 ml on the wall of empty bottles before sampling. The graduated pipette or syringes are usually used to add formaldehyde to the sample bottles.

Samples should be stored for at least 5 years. Other different methodologies used for preservation of microzooplankton and large gelatinous animals are described in the appropriate Manuals.

# 5 Processing of samples

Different types of microscopes with an ocular scale may be required. The typical magnifications used for counting procedure in Bogorov's chamber under dissecting stereo-microscope are x16 or x32. The width of the track in the Bogorov's chamber has to correspond to the diameter of field of vision under the microscope. For taxonomical identification a light microscope with higher magnification is often required.

## 5.1 Taxonomical identification

The detailed taxonomic analysis of species composition is crucial for any ecological study including community dynamics and variability of external factors influence, extinctions and invasions. Therefore, correct species identification is an important aspect of zooplankton monitoring. It should be based on the Guidebook for Marine Fauna of the Black Sea and the Sea of Azov (Mordukhay-Boltovskoy, 1968; Mordukhay- Boltovskoy, 1969; Mordukhay- Boltovskoy, 1972). However, since the second half of the twentieth century studies have revealed serious changes both in the taxonomic status of some species and in the structure of zooplankton community. These changes are reflected in the references presented in Annex 2. An updated list of the most important taxonomic groups of the Black Sea mesozooplankton is presented in Annex 1.

For biological monitoring a particular attention is paid to indicator species and indices that help to determine trends in environmental status (Annex 3). The task of the near future should be to develop quantitative methods for assessing the water quality with the help of indicator species.

Special attention should also be paid to taxonomic identification of non-indigenous species (synonyms: alien, exotic, non-native, allochthonous, invader, foreign) which may affect the native biodiversity, ecosystem functioning, commercial marine resources, etc (Olenin et al., 2010). First record of a new non-native species should provide photo (or taxonomic description with illustrations) and information on number of individuals, stage of development for crustaceans and coordinates of location. The species should be kept in a small tube with a leak-proof lid for future validation of identification.

Only established (naturalized) species were included in the list of the Black Sea mesozooplankton species (Annex 1).

## 5.2 Sub-samples

At first a sample preserved with formalin must be flushed with water through a sieve with a mesh size smaller than that of the sampling net. Filtered tap or marine water should be added. Organisms suspended in water are ready for analysis. Then the large-sized organisms (chaetognaths, malacostracans, fish larvae, large jelly-fish etc.) are recommended to be removed from the sample and enumerated in the whole sample. This procedure provides accurate counting of large zooplankters and guarantees random mixing of the sample before sub-sampling. Some organisms (cladocerans, small copepods etc.) can float in the surface film. A few drops of a detergent should be added to allow cladocerans to sink to the bottom.

The rest of the sample should be poured in a graduated beaker of appropriated size for measurement of the sample volume. Samples are brought to volume of 100, 200 or 250 ml depending upon zooplankton density. The sample should be mixed thoroughly until the organisms are distributed randomly before taking an aliquot. To achieve this, the Stempel-pipette should be moved in a figure-of-eight manner from the bottom to the top of the water. Round movement/stirring should be avoided since such stirring results in non-homogenous distribution of plankton within the sample - a very wide-spread error. The pipette should be closed in the middle part of the beaker. Sub-sample picked up with a Stempel-pipette should be released into a Bogorov's chamber for further quantitative and qualitative processing.

Folsom or Motodo splitters could be used for sub-sampling. They split sample half-and-half. The splitting may be repeated to obtain 1/4, 1/8 etc. part of sample if necessary. However, it should be remembered that error increases from step to step.

## 5.3 Abundance

For each sample 2 aliquots should be totally counted. Namely, all specimens should be identified and counted in the first two 1 ml Stempel pipette aliquot. The results are to be reported in the protocol of counting. If there are dominant species (in particular any species of copepods present with at least 100 specimens: sum of males, females, juv/aliquot), these will be counted only in the first 2 aliquots, and in the following aliquots the count will proceed only for the other abundant copepods and taxonomic groups in a sample. Those taxonomic group(s) that reached 100 specimens in the previous sub-samples are not needed to be counted in the next sub-sample(s). The precision of calculated abundance for organisms that are counted up to 100 specimens is equal to 20%. The estimation of abundance for other groups ("tail") is less precise (Cassie, 1971; HELCOM, 1988; ICES Zooplankton Methodology manual, 2000; Proceedings of the workshop on zooplankton…, 2008). The data for taxa numbering less than 10 specimens should be marked in results and considered as qualitative in description.

The abundance of nauplii, rotifers and tintinnids should be estimated semi-quantitatively from the first sub-sample because their small size results in unpredictable losses through the mesh. Although macrozooplankton, nauplii, rotifers and tintinnids fall outside the size range of mesozooplankton, as do many of the meroplankton, there is a considerable amount of historical data on these groups. Thus, they should be reported for qualitative assessment of their abundance.

Presence of large macrozooplankton organisms and rare species can be noted after an overview of the whole sample. This step is very important for biomass calculation and estimation of biological diversity.

Aggregations of organisms should be taken out of the sub-sample, divided into the constituent organisms and counted.

## 5.4 Biomass

According to the Working Groups Reports of the NATO Advanced Research Workshop, Constanta, Romania, 6-10 October 1997, "…biological methods need to be standardized between all Black Sea countries. Standard methods for biomass and primary productivity should be the first goal" (Environmental degradation..., 1999).

One of the main reasons for this decision was the large differences in calculations of zooplankton biomass obtained not only by different Black Sea countries, but also by different specialists within a single country. Historically determination of zooplankton biomass was based on the tables of constant weight of the Black Sea zooplankters (Mordukhai-Boltovskoi, 1954; Petipa, 1957). For biomass determination it was assumed that the wet mass of pelagic organisms (g) was equal to their volume ($cm^3$).

Afterwards special scientific investigation showed significant differences in the size of some species in different Black Sea areas. For example, *Noctiluca scintillans* (= *N.miliaris*), an abundant species in the Black Sea, has an average diameter in coastal zone of the north-western shelf, which is some 16% smaller than in the open sea, but when expressed in terms of biomass, the open sea *Noctiluca* are 1,5 times bigger than their NW shelf counterparts (Polyschuk et al., 1981). Other large differences in biomass are registered for bivalve larvae. During their pelagic life stages, the length of mussel larvae vary between 0.120 and 0.500 mm, corresponding to the differences in their biomass of up to 62 times (0.0003-0.0185 mg). Significant mistakes in biomass calculations arising from measurement of their average length were removed with the help of "Chislenko nomogramms" (Chislenko, 1968). The nomogramms are based on the allometric dependence $V = a \cdot L^b$ that relates the volume (or biomass) of an organism to its length (L). Another source of error during the calculation of biomass is the failure to account for different concentrations of organic matter ($C_{org}$) in the same taxa collected from different areas. For example, organic carbon content in zooplankton from the Panama Canal is about 5% of wet weight (Smayda, 1966) and in the Black Sea zooplankton – 4,5±1,0% (Aleksandrov, 2001).

At present zooplankton biomass is often recorded as energy-equivalents in calories, referred to as caloricity. For different taxonomic groups of planktonic organisms, caloricity is proportional to wet or dry mass, albeit with some seasonal differences. Different methods of estimating the caloricity of zooplankton can introduce errors of up to 28% (Vollenveider, 1965; Sprung, 1984). Measurement of the energy content of different species enables the investigation of energy transfer in food webs, and allows the significance of zooplankton in organic matter transfer within aquatic ecosystems to be assessed. For the Black Sea zooplankton taxa the equations for biomass and organic carbon measurement are developed and could be used as first approximation to very complicated subject; they have to be thoroughly investigated further on (Annex 4).

# 6 Meta data and Data reporting

With the purpose of unification of Meta data and data recording, the following Format is proposed:

| N | Acronym | Name | Example |
|---|---|---|---|
| 1 | RV | Name of RV 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 |

| N | Acronym | Name | Example |
|---|---|---|---|
| 16 | Flowmeter | Volume of water filtered by the net estimated on the base of flowmeter reading ($m^3$) | 2.0 |
| 17 | Volume | Volume of sample (ml) | 150 |
| 18 | Taxon 1 SS | Total volume of aliquots taken for counting under binocular microscope and to calculate the 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 taxon enumerated in aliquots (ind.) | 65 |
| 21 | Taxon 1 Ind | Number of taxon in the whole sample = K (N19) * N (N20) (ind.) | 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) * Individual weight of taxon ($mg/m^3$) | 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 mesozooplankton ($ind./m^3$) | XXXX |
| | Total C | Total biomass of mesozooplankton ($mg/m^3$) | 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.

# 7 Quality assurance

Throughout a year, zooplankton monitoring results tend to be highly variable. While much of this variability is normal/natural, it is necessary to employ strict quality assurance procedures to ensure that observed variability is genuine, and not the result of poor methodological practices. Quality assurance procedures therefore need to encompass the whole process of sampling site/depth selection, sampling and sub-sampling procedures, sample preservation, analysis (identification) and reporting. Quality assurance procedures (starting with good and systematic record keeping) need to be followed strictly by all the monitoring organizations/laboratories (internal QA).

## 7.1 Use of standardized equipment

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

1. Juday net (diameter of net mouth 36 cm, mesh size 150 μm).
2. Planktonic releaser.
3. Flowmeter.
4. Messenger load for net closing (weight about 0.2-0.5 kg, inlet diameter for wire = 6-8 mm).

5. Stempel-pipette.
6. Bogorov's chamber.
7. Graduated cylinder for sample volume determination.
8. Dissecting binocular microscope.

## 7.2 Sampling methodology

Good 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.

## 7.3 Sample storage

Samples should be stored in sealed containers for at least 5 years to allow subsequent validation of species composition or other details.

## 7.4 Sample analysis (identification and counting)

The precision of zooplankton numbers and biomass estimation depends mainly on the number of specimens from the sample counted under a microscope, but other characteristics, like sample volume and total volume of sub-samples, are also important. The total volume of sub-samples is used to measure the abundance and biomass of each taxon (for details see Section 6). Around 3-5-10% of all samples analyzed should be re-analyzed (within one month or year) by a suitably experienced colleague [this could be a colleague from another laboratory/organization or a colleague from the same laboratory]. Results should be compared. Where large differences in biomass/enumeration (>40%) or taxonomic identification are reported by different workers, the underlying reasons should be investigated and steps taken to ensure that such inaccuracies do not re-occur.

Regional leading experts should be selected for particular taxonomic groups and consulted over taxonomic issues. The inter-laboratory exchange of samples/photographs with unclear taxonomical composition is highly recommended.

## 7.5 Meta data and Data reporting

The regional database should be comparable with other databases. Therefore, the proposed above Format of Meta data and data recording is recommended.

## 7.6 Inter-laboratory proficiency testing

All laboratories should participate in at least one intercomparison exercise every 1-3 year where subsamples of the same original sample are analyzed individually by members of all the laboratories. Successive exercises should be organized by different laboratories on a rotation basis.

## 7.7 Staff training

Workers should participate in taxonomical workshops (as funding allows). The results of the internal quality assurance schemes (re-analysis of at least 3-5-10% of the samples by colleagues) and inter-laboratory proficiency tests should be used to prioritize training requirements/programmes.

## 7.8 Quality control (data checking)

Quality control (QC) is based on the information of quality assurance (QA). Considering the steps of the whole procedure it could be possible to assess the errors on each stage in *per cent*. The assessment could not be done automatically but only manually.

Stages of mesozooplankton studying procedure: **S** - sampling, **C** - counting, **T** - data treatment, **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 1000%) **T**

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

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

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

Data presentation units (0% up to 1000%) **P**

Database column titles (0% up to 1000%) **P**

Flag Description / SEADATANET Flag application Yes/No

| | | |
|---|---|---|
| no quality control | 0 | Y |
| good value | 1 | Y |
| probably good value | 2 | Y |
| probably bad value | 3 | Y |
| bad value | 4 | Y |
| changed value | 5 | ? |
| value below detection | 6 | N |
| value in excess | 7 | ? |
| interpolated value | 8 | ? |
| missing value | 9 | ? |
| value phenomenon uncertain | A | ? |

Final results of QA/QC procedures would be:

- Data Quality Flag
- Error percentage for each group of organisms

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# Annex 1 Taxonomic composition of the most important groups of mesozooplankton and their distribution in the national waters of the Black Sea countries

| | Taxa | Bulgaria | Georgia | Romania | Russia | Turkey | Ukraine |
|---|---|---|---|---|---|---|---|
| **ARTHROPODA (**Phylum**)** | | | | | | | |
| | **CRUSTACEA (**Subphylum**)** | | | | | | |
| | **MAXILLOPODA** (Class) | | | | | | |
| | **COPEPODA (**Subclass) | | | | | | |
| | **CALANOIDA (**Order) | | | | | | |
| | **Acartiidae** (Family) | | | | | | |
| 1 | *Acartia (Acartiura) clausi* Giesbrecht, 1889 | + | + | + | + | + | + |
| 2 | *Acartia tonsa* Dana, 1849 | + | + | + | + | + | + |
| | **Calanidae** (Family) | | | | | | |
| 3 | *Calanus euxinus* Hulsemann, 1991<br>Syn.: *C. helgolandicus* Claus, 1863; C. *ponticus* Karavaev, 1894 | + | + | + | + | + | + |
| | **Centropagidae** (Family) | | | | | | |
| 4 | *Centropages ponticus* Karavaev, 1894<br>Syn.: *C. kroyeri var. pontica, Karavaev, 1894* | + | + | + | + | + | + |
| | **Clausocalanidae** (Family) | | | | | | |
| 5 | *Pseudocalanus elongatus* (Boeck, 1865) | + | + | + | + | + | + |
| | **Paracalanidae** (Family) | | | | | | |
| 6 | *Paracalanus parvus* (Claus, 1863) | + | + | + | + | + | + |
| | **Pontellidae** (Family) | | | | | | |
| 7 | *Labidocera brunescens* (Czerniavsky, 1868) | | + | | + | + | + |
| 8 | *Pontella mediterranea* (Claus, 1863) | + | + | + | + | + | + |
| 9 | *Anomalocera patersoni* Templeton, 1837 | + | + | + | | | + |
| | **Pseudodiaptomidae** (Family) | | | | | | |
| 10 | *Calanipeda aquaedulcis* (Kritczagin, 1873 | | | + | | | + |
| | **Temoridae** (Family) | | | | | | |
| 11 | **Eurytemora* sp. (Giesbrecht, 1881) | | | | | | + |
| | **Cyclopoida (Order)** | | | | | | |
| | **Cyclopidae** (Family) | | | | | | |
| 12 | *Euryte longicauda* (Philippi, 1843) | | | | | | + |
| | **Oithonidae** (Family) | | | | | | |
| 13 | *Oithona davisae* (Ferrari F.D. and Orsi, 1984)<br>(At first was identified as *O. brevicornis* Giesbrecht, 1891 ) | + | + | + | + | + | + |
| 14 | *O. similis* Claus, 1866 | + | + | + | + | + | + |
| | **BRANCHIOPODA (**Class)<br>**PHYLLOPODA** (Subclass)<br>**DIPLOSTRACA (**Order)<br>**ONYCHOCAUDATA (**Suborder)<br>**CLADOCERA (**Infraorder) | | | | | | |
| | **Bosminidae** (Family) | | | | | | |
| 15 | **Bosmina (Eubosmina) coregoni* Baird, 1857 | | | | | | + |
| 16 | **Bosmina (Bosmina) longirostris* (O. F. Müller, 1785) | | + | + | | | + |
| 17 | **Bosmina (Eubosmina) longispina* Leydig, 1857 | | + | | | | |
| | **Chydoridae** (Family) | | | | | | |
| 18 | **Alona rectangula* Sars, 1962 | | | | | | + |
| 19 | **Alona quadrangularis* (O.F. Müller, 1785) | | | | | | + |
| 20 | **Chydorus sphaericus* (O.F. Müller, 1785) | | | + | | | + |
| 21 | **Graptoleberis testudinaria* (Fischer, 1848) | | | | | | + |

| | Taxa | Bulgaria | Georgia | Romania | Russia | Turkey | Ukraine |
|---|---|---|---|---|---|---|---|
| | **Daphniidae** (Family) | | | | | | |
| 22 | **Daphnia cucullata* Sars, 1862 | | | + | | | + |
| 23 | **D. longispina* O.F. Müller, 1785 | | | + | | | + |
| 24 | **D. magna* Straus, 1820 | | | | | | + |
| 25 | **D. pulex* De Geer, 1778 | | | | | | + |
| | **Sididae** (Family) | | | | | | |
| 26 | **Diaphanosoma brachyurum* (Lievin, 1848) | | | | | | + |
| 27 | *Penilia avirostris* Dana, 1849 | + | + | + | + | + | + |
| | **Gercopadidae** (Family) | | | | | | |
| 28 | **Cercopagis (Cercopagis) pengoi* (Ostroumov, 1891) | | | | | | + |
| 29 | **Ceriodaphnia reticulata* (Jurine, 1820) | | | | | | + |
| | **Leptodoridae** (Family) | | | | | | |
| 30 | **Leptodora kindtii* (Focke, 1844)<br>Syn.: *L. hyalina* Lilljeborg, 1900 | | | | | | + |
| | **Moinidae** (Family) | | | | | | |
| 31 | **Moina brachiata* (Jurine, 1820) | | | | | | + |
| | **Podonidae** (Family) | | | | | | |
| 32 | **Cornigerius maeoticus maeoticus* Pengo, 1879 | | | | | | + |
| 33 | *Evadne spinifera* O.F. Müller, 1867 | + | + | + | + | + | + |
| 34 | *P. leuckartii* (G.O. Sars, 1862) | | | + | | | + |
| 35 | *Pleopis polyphaemoides* (Leucart, 1859) | + | + | + | + | + | + |
| 36 | *Podon intermedius* Lilljeborg, 1853 | | + | | + | + | + |
| 37 | *Pseudevadne tergestina* (Claus, 1877)<br>Syn.:*Evadne tergestina* Claus, 1864, *Podon tergestina* (Claus, 1877) | + | + | + | + | + | + |
| 38 | **Podonevadne trigona* (G.O. Sars, 1897) | | + | + | | | + |
| 39 | **MONSTRILLOIDA** (Order ) | + | + | | + | | + |
| 40 | **HARPACTICOIDA (**Order) | | + | | + | + | + |
| | **MALACOSTRACA** (Class) | | | | | | |
| | ****MYSIDA** (Order)<br>**Mysidae** (Family) | | | | | | |
| 41 | *Diamysis mecznikowi* (Czerniavsky, 1882) | | | | + | | + |
| 42 | *Gastrosaccus sanctus* (van Beneden, 1861) | | | | | | + |
| 43 | *Hemimysis anomala* G.O. Sars, 1907 | | | | | | + |
| 44 | *H. lamornae pontica* Czerniavsky, 1882 | | | | + | | |
| 45 | *Leptomysis lingvura* (Sars G.O., 1866) | | | | + | | |
| 46 | *Mesopodopsis slabberi* (Van Beneden,1861) | | + | + | + | | + |
| 47 | *Paramysis (Occiparamysis) agigensis* Bacescu, 1938 | | | | + | | + |
| 48 | *P. (Longidentia)kroyeri* (Czerniavsky,1882) | | + | | | | + |
| 49 | *P. (Serrapalpisis) lacustris* (Czerniavsky, 1882) | | | | | | + |
| 50 | *P. pontica (Pseudoparamysis)* Bacescu, 1938 | | | | | | + |
| 51 | *Siriella jaltensis* Czerniavsky, 1868 | | | | + | | + |
| | ****CUMACEA** (Order) | | | | | | |
| | **Bodotriidae** (Family) | | | | | | |
| 52 | *Bodotria scorpioides* (Montagu, 1804) | | | | + | | + |
| 53 | *Cumopsis goodsir* (van Beneden, 1861) | | | | | | + |
| 54 | *Iphinoe elisae* Bacescu, 1950 | | | | + | | + |
| 55 | *I. maeotica* Sowinsky, 1893 | | | | | | + |
| 56 | *I. tenella* Sars,1873 | | + | | + | | + |
| | **Leuconidae** (Family) | | | | | | |
| 57 | *Eudorella truncatula* (Bate, 1856) | | | | | | + |

| | Taxa | Bulgaria | Georgia | Romania | Russia | Turkey | Ukraine |
|---|---|---|---|---|---|---|---|
| | **Nannastacidae** (Family) | | | | | | |
| 58 | *Cumella (Cumella) limicola* Sars, 1879 | | | | + | | + |
| 59 | *C. (Cumella) pygmaea euxinica* Bacescu, 1950 | | | | + | | + |
| 60 | *Nannastacus unguiculatus* (Bate, 1859) | | | | + | | |
| | **Pseudocumatidae** (Family) | | | | | | |
| 61 | *Pseudocuma (Pseudocuma) longicorne* (Bate, 1858) | | | | + | | + |
| 62 | *Pterocuma pectinatum* (Sowinsky, 1893) | | | | | | + |
| 63 | *P. rostratum* (Sars, 1894) | | | | | | + |
| | **ISOPODA** (Order)<br>**Idoteidae** (Family) | | | | | | |
| 64 | *Idotea ostroumovi* Sowinsky,1895 | | + | + | + | | + |
| **CHAETOGNATHA** (Phylum) | | | | | | | |
| | **SAGITTOIDEA** (Class)<br>**APHRAGMOPHORA** (Order)<br>**Sagittidae** (Family) | | | | | | |
| 65 | *Parasagitta setosa* (Müller,1847)<br>Syn.: *Sagitta euxina* Moltschanoff, 1909 ; *Sagitta setosa* J. Müller,1847 | + | + | + | + | + | + |
| **CHORDATA** (Phylum) | | | | | | | |
| | **APPENDICULARIA** (Class)<br>**COPELATA** (Order)<br>**Oikopleuridae** (Family) | | | | | | |
| 66 | *Oikopleura dioca* Fol, 1872 | + | + | + | + | + | + |
| | **VERTEBRATA (**Subphylum) | | | | | | |
| 67 | **Pisces**: ova, larva | + | + | + | + | + | + |
| 68 | **ROTIFERA**(Phylum)<br>Syn. Rotatoria | + | + | + | + | + | + |
| **CTENOPHORA** (Phylum) | | | | | | | |
| | **NUDA (**Class)<br>**BEROIDA** (Order)<br>**Beroidae** (Family) | | | | | | |
| 69 | *Beroe ovata* Bruguière, 1789: ova, larvae | + | + | + | + | + | + |
| | **TENTACULATA** (Class) | | | | | | |
| | **CYCLOCOELA** (Subclass)<br>**LOBATA** (Order)<br>**Bolinopsidae** (Family) | | | | | | |
| 70 | *Mnemiopsis leidyi* A. Agassiz, 1865 ova, larvae | + | + | + | + | + | + |
| | **TYPHLOCOELA** (Subclass)<br>**CYDIPPIDA** (Order)<br>**Pleurobrachiidae** (Family) | | | | | | |
| 71 | *Pleurobrachia pileus* (O.F. Müller, 1776)<br>Syn.: *Pleurobrachia rhodopis* Chun, 1879 | + | + | + | + | + | + |
| **7.8.1.1.1.1.1.1** | **CNIDARIA** (Phylum)<br>**HYDROZOA** (Class)<br>**HYDROIDOLINA (**Subclass)<br>**ANTHOATHECATA** (Order) | | | | | | |
| | **Corymorphidae** (**Family)** | | | | | | |
| 72 | *Corymorpha nutans* M. Sars,1835 | + | | | | | + |
| | **Corynidae** (Family) | | | | | | |
| 73 | *Sarsia tubulosa* (M. Sars,1835) | + | + | + | + | | + |
| | **Cladonematidae** (Family) | | | | | | |
| 74 | *Cladonema radiatum* Dujardin, 1843 | | | | | | + |

| | Taxa | Bulgaria | Georgia | Romania | Russia | Turkey | Ukraine |
|---|---|---|---|---|---|---|---|
| 75 | *Eleutheria dichotoma* Quatrefages, 1842 | | | | | | + |
| | **Hydractiniidae** (Family) | | | | | | |
| 76 | *Hydractinia carnea* (M. Sars, 1846)<br>Syn.: *Podocoryna carnea* M. Sars, 1846 | | | | | | + |
| | **Moerisiidae** (Family) | | | | | | |
| 77 | *Moerisia maeotica* (Ostroumov, 1896)<br>Syn.: *Odessia maeotica* (Ostroumoff, 1896) | + | + | | | | + |
| | **Rathkeidae** (Family) | | | | | | |
| | *Rathkea octopunctata* (M. Sars, 1835 ) | + | | | | | + |
| | **Tubulariidae** (Family) | | | | | | |
| 80 | *Tubularia prolifer* (L.Agassiz, 1862 )<br>Syn.: *Hybocodon prolifer* Agassiz, 1860 | | | | | | + |
| | **LEPTOTHECATA** (Order) | | | | | | |
| | **Blackfordiidae** (Family) | | | | | | |
| 81 | *Blackfordia virginica* Mayer, 1910 | + | | | | | + |
| | **Campanulariidae** (Family) | | | | | | |
| 82 | *Campanularia johnstoni* (Alder, 1856) | | | | | | + |
| 83 | *Obelia longissima* (Pallas, 1766) | +\| | | | | | + |
| | **TRACHYLINAE** (Subclass)<br>**LIMNOMEDUSAE** (Order) | | | | | | |
| | **Olindiidae** (Family) | | | | | | |
| 84 | *Maeotias marginata* (Modeer,1791)<br>Syn.: *Maeotias inexpectata* Ostroumov, 1896 | + | | + | | | |
| | **DINOPHYCEAE** (Class)<br>**NOCTILUCALES** (Order)<br>**Noctilucaceae** (Family) | | | | | | |
| 85 | *Noctiluca scintillans* (Macartney) Kofoid & Swezy, 1921<br>Syn.: *Noctiluca miliaris* Suriray, 1816 | + | + | + | + | + | + |
| | | | | | | | |
| | **M E R O P L A N K T O N** | | | | | | |
| 86 | **Ascidiacea** larvae | + | | + | | + | + |
| 87 | **Gastropoda** larvae: veliger | + | + | + | + | + | + |
| 88 | **Bivalvia** larvae: veliger | + | + | + | + | + | + |
| 89 | **Cirripedia** larvae: nauplius, cypris | + | + | + | + | + | + |
| 90 | **Decapoda** larvae: protozoea, zoea, megalope | + | + | + | + | + | + |
| 91 | **Nemertea** larvae: pilidium | + | + | + | + | + | + |
| 92 | **Polychaeta** larvae: nectochaeta | + | + | + | + | + | + |
| 93 | **Bryozoa** larvae: cyphonautes | | + | | + | | + |
| 94 | **Phoronida** larvae: actinotrocha | | + | + | + | | + |

Classification and species name are given in accordance with the WoRMS (Word Register of Marine Species) http://www.marinespecies.org/index.php.

*Freswater and brackish species

**Mysida and Cumacea species are demersal zooplankton (mobile benthic organisms, which periodically emerge from the benthos and move up into the water column)

Annotation:

- Hydrozoa species are given by Boris Anninsky (Institute of Biology of the Southern Seas, Sevastopol);
- check-list of Scyphozoa species is presented in the "Macrozooplankton" Manual;
- check-list of Ciliophora and Rotatoria species is presented in the "Microzooplankton" Manual.

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# Annex 3 Zooplankton indicator species

Indicator species. A species that is of narrow ecological amplitude with respect to one or more environmental factors and which is, when present, therefore indicative of a particular environmental condition or set of conditions. Indicator species could be divided into three groups: indicators of hazardous contaminant pollution (stable trace elements, organic substances, radionuclides, indicators of nutrient enrichment (eutrophication) and indicators of altering ecosystems (establishment of non-indigenous species).

1. Indicators of hazardous contaminant pollution – organisms that decrease their number (mass mortality or stop of reproduction activity) as the result of environmental conditions worsening.

Typical representatives of this group are neuston organisms inhabiting upper sea layer. In the Black Sea those are: copepods of Pontelidae family (*Pontella mediterranea*, *Anomalocera patersoni*, *Labidocera brunescens*), neistonic isopod *Idothea ostroumovi*, larvae of bottom invertebrates, first of all larvae of shrimps and crabs (zoea), and fish (mulets *Liza saliens*, *Lisa aurata*, *Mugil cephalus*; *Solea*, *Callionymus*, *Belone* et al.) (Zaitsev, 1997; Zaitsev, Mamaev, 1997).

2. Indicators of nutrient enrichment (eutrophication) – organisms that increase their abundance with the increase in concentration of nutrients, dissolved and particulate organic matter.

According to the results of the Workshop on developing indicators of Eutrophication for the Black Sea; PIU, Istanbul, 25-30 September, 2000 (Support for the regional activity centre for pollution monitoring and assessment, Odessa, Ukraine, EU TACIS Project: ENVRUS9602: Phase 2) indicators of eutrophication have been identified, which can be successfully used for monitoring programmes. In particular:

Zooplankton:

- Total mesozooplankton biomass, $mg \bullet m^{-3}$.
- Biomass of *Noctiluca scintillans* in total mesozooplankton, %.
- Number of neustonic copepods (Pontelidae Family: *Pontella mediterranea*, *Anomalocera patersoni*, *Labidocera brunescens*), $ind \bullet m^{-3}$.
- Number of Polychaeta larvae in total number of meroplankton, %.
- Specific production of dominant species, $d^{-1}$.

Others:

- Average biomass of jellyfish *Aurelia aurita*, $g \bullet m^{-2}$.
- Total biomass of exotic ctenophore species (*Mnemiopsis leidyi* and *Beroe ovata*), $g \bullet m^{-2}$.
- Number of fish eggs and larvae with special attention to commercially important species, $ind \bullet m^{-3}$.
- Ratio between the total biomass of phyto- and zooplankton.

In addition to the organisms that increase in number during eutrophication it is necessary to mention jellyfish *Rhizostoma pulmo* and cladoceras *Pleopis polyphemoides* that indicate high concentration of organic matter. At the same time, Romanian and Ukrainian specialists noted the species that decreased in their number and practically disappeared from the north-western part of the Black Sea during hypertrophic period of 1970s-90s. Among them are: cladocerans *Penilia avirostris*, *Pseudoevadne tergestina*, *Evadne spinifera*; copepods monstrilloid (*Monstrilla grandis*, *M. helgollandica*, *M. longiremis*) (Zaitsev et al., 1987; Petranu et al., 1999).

3. Indicators of the ecosystem alterity – established non-indigenous species (NIS) that are the indicators of unstable ecosystem with destroyed equilibrium (Olenin et al., 2010). During biological monitoring attention should be paid to recent invaders that were registered during past decades and started increasing in their numbers. Moreover, it is necessary to establish a year of the first registration of the NIS in the lists of each Black Sea country.

The recommended list of zooplankton indicator organisms includes the following species.

1) Indicators of worsening environmental conditions – organisms, whose increasing numbers indicate worsening of environment conditions

DYNOPHYCEAE
*Noctiluca scintillans* (=*N. miliaris*)

SCYPHOMEDUSA
*Aurelia aurita*
*Rhizostoma pulmo*

CLADOCERA
*Pleopis polyphemoides*

2) Indicators of improving conditions – organisms, whose registration in several or increasing number indicates improvement of environment conditions

CLADOCERA
*Penilia avirostris*
*Pseudoevadne tergestina*
*Evadne spinifera*

MONSTRILOIDA
*Monstrilla grandis*
*Monstrilla helgollandica*
*Monstrilla longiremis*

CALANOIDA
*Pontella mediterranea*
*Anomalocera patersoni*
*Labidocera brunescens*
*Centropages ponticus*

ISOPODA
*Idothea ostroumovi*

DECAPODA larvae

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# Annex 4 Individual weights of the Black Sea zooplankton taxa

*Symbols*: WW – wet weight, mg; L – total body length, mm; D – diameter of organisms, mm; T – trunk length, mm; C- organic carbon, µg; FGS – formula of geometric similarity.

| Taxon | **WW**, mg | **References** | **C**, µg | **References** |
|---|---|---|---|---|
| **Copepoda Calanoida** | | | | |
| Calanoida ova | 1/6* π*·$D^3$ | FGS | 140·WW | Huntley, Lopez, 1992 |
| Calanoida nauplii | 0.0758·$L^3$ | Chislenko, 1968 | 4.906·$L^{2.505}$ | Rey et al., 2001 |
| *Acartia clausi* | 0.03·$L^3$ | Petipa, 1957 | 2.4·$L^3$ | Hagen, 2000 |
| *Acartia tonsa* | 0.0235·$L^3$ | Chislenko, 1968 | 1.88·$L^3$ | Hagen, 2000 |
| *Calanus euxinus*, C I-IV | 0.0324·$L^{2.999}$ | Petipa, 1957 | 2.592·$L^{2.999}$ | Hagen, 2000 |
| *C. euxinus*, C V-VI | 0.0324·$L^{2.999}$ | Petipa, 1957 | 2.652·$L^3$ | Arashkevich et al., 2014 |
| *Centropages ponticus* | 0.035·$L^3$ | Chislenko, 1968 | 2.8·$L^3$ | Hagen, 2000 |
| *Pseudocalanus elongatus* | 0.03·$L^3$ | Chislenko, 1968 | 2.4·$L^3$ | Hagen, 2000 |
| *Paracalanus parvus* | 0.042·$L^{3.253}$ | Petipa, 1957 | 3.36·$L^{3.253}$ | Hagen, 2000 |
| Pontellidae | 0.172·$L^{4.49}$ | Aleksandrov, 2001 | 21.309·$L^{4.49}$ | Aleksandrov, 2001 |
| *Eurythemora affinis* | 0.012·$L^{2.466}$ | Kankaala, Johansson, 1986 | 12.955·$L^{2.71}$ | Vasama, Kankaala, 1990 |
| *Eurythemora velox* | 0.170·$L^{3.628}$ | Kankaala, Johansson, 1986 | 12.955·$L^{2.71}$ | Vasama, Kankaala, 1990 |
| *Diaptomus* | 0.229·$L^{3.628}$ | Kankaala, Johansson, 1986 | 8.599·$L^{4.436}$ | Kankaala, Johansson, 1986 |
| Copepoda Cyclopoida | | | | |
| *Oithona similis* | 0.019·$L^{2.336}$ | Petipa, 1957 | 1.52·$L^{2.336}$ | Hagen, 2000 |
| *Cyclops* | 0.039·$L^{2.313}$ | Alimov, 1989; Sherstiyk, 1971 | 2.524·$L^{2.313}$ | Alimov, 1989; Sherstiyk, 1971 |
| **Harpacticoida** | 0.033·$L^3$ | Chislenko, 1968 | 2.167·$L^3$ | Vinogradov, Shushkina, 1987 |
| **Cladocera** | | | | |
| Carnivorous cladocerans | 0.070·$L^3$ | Vinogradov, Shushkina, 1987 | 3.261·$L^3$ | Vinogradov, Shushkina, 1987 |
| Herbivorous cladocerans | 0.060·$L^3$ | Vinogradov, Shushkina, 1987 | 2.814·$L^3$ | Vinogradov, Shushkina, 1987 |
| *Penilia avirostris* | 0.302 $L^{3.743}$ | Chislenko, 1968 | 55.238·WW | Aleksandrov, 2001 |
| *Bosmina* | 0.200·$L^{2.062}$ | Larson, 1986 | 9.337·$L^{2.062}$ | Larson, 1986 |
| *Moina* | 0.074·$L^{3.050}$ | Alimov, 1989; Umnov, 1986 | 3.462·$L^{3.050}$ | Alimov, 1989; Umnov, 1986 |
| *Chydorus* | 0.203·$L^{2.771}$ | Alimov, 1989; Umnov, 1986 | 9.493·$L^{2.771}$ | Alimov, 1989; Umnov, 1986 |
| *Daphnia* | 0.083·$L^{0.369}$ | Larson, 1986 | 3.864·$L^{0.369}$ | Larson, 1986 |

| Taxon | **WW**, mg | **References** | **C**, µg | **References** |
|---|---|---|---|---|
| *Leptodora kindtii* | $0.006 \cdot L^{2.85}$ | Alimov, 1989; Umnov, 1986 | $0.290 \cdot L^{2.85}$ | Alimov, 1989; Umnov, 1986 |
| **Mysidacea** | $0.007 \cdot L^{3}$ | Vinogradov, Shushkina, 1987 | $0.447 \cdot L^{3}$ | Vinogradov, Shushkina, 1987 |
| **Chaetognatha** | | | | |
| *Parasagitta setosa* | $0.0013 \cdot L^{3.123}$ | Petipa, 1957 | $C=0.0473 \cdot L^{3.14}$ | Convey and Robins, 1991 |
| **Noctilucales** | | | | |
| *Noctiluca scintillans* | $0.200 \cdot D^{3}$ | Polyschuk et al., 1981 | $0.938 \cdot D^{3}$ | Aleksandrov, 2001 |
| ***Rotatoria** | $0.120 \cdot L^{3}$ | Vinogradov, Shushkina, 1987 | $5.606 \cdot L^{3}$ | Vinogradov, Shushkina, 1987 |
| **Hydrozoa** (medusa) | $0.140 \cdot L^{3}$ | Vinogradov, Shushkina, 1987 | $0.402 \cdot L^{3}$ | Vinogradov, Shushkina, 1987 |
| **Scyphozoa** | | | | |
| *Aurelia aurita* | $0.0007 \cdot D^{2.899}$ | Bømstedt, 1990 | $0.0009 \cdot D^{2.899}$ | Larson, 1986 |
| **Ctenophora** | | | | |
| *Pleurobrachia pileus* | $4/3 \cdot \pi \cdot (L/2) \cdot (D/2)^{2}$ | FGS | $9.81 \cdot L^{2.65}$ | Hirota, 1972; Hoeger, 1983 |
| ***Beroe ovata* | | | | |
| ***Mnemiopsis leidyi* | | | | |
| **Appendicularia** | | | | |
| *Oikopleura dioica* | $0.09 \cdot T^{2.49}$ | Paffenhofer, 1976 | $C=9 \cdot T^{2.49}$ | Gorsky et al., 1988 |
| **Pisces**: ova | $1/6\ \pi \cdot D^{3}$ | 7.8.1.1.2 FGS | $46.750 \cdot D^{3}$ | Vinogradov, Shushkina, 1987 |
| **MEROPLANKTON** | | | | |
| Cirripedia larvae | $0.056 \cdot L^{2.75}$ | Aleksandrov, 2001 | $3.953 \cdot L^{2.862}$ | Aleksandrov, 2001 |
| Polychaeta larvae | $0.010 \cdot L^{2.136}$ | Aleksandrov, 2001 | $0.759 \cdot L^{2.136}$ | Aleksandrov, 2001 |
| Bivalvia larvae | $0.135 \cdot L^{2.87}$ | Sprung, 1984 | $11.593 \cdot L^{3.02}$ | Sprung, 1984 |
| Gastropoda larvae | $0.868 \cdot L^{3.459}$ | Pechenik, 1980 | $29.707 \cdot L^{3.459}$ | Pechenik, 1980 |

*Individual weight of Rotatoria is given for *Synchaeta* as the most common species in the brackish parts of the northwestern Black Sea. Weights of the other Rotifers species are given in the Microzooplankton guidelines.

**Weights of *Beroe ovata* and *Mnemiopsis leidyi* are given in the Macrozooplankton guidelines.

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