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Black Sea Monitoring Guidelines

Microzooplankton

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1. Introduction

Microzooplankton plays an important role in the pelagic food web, consuming bacteria, small primary producers and heterotrophic nanoflagfellates, and is one of the main trophic links between nano and other zooplankton including fish larvae (Conover, 1982; Sherr et al., 1986; Pierce, Turner, 1992; Sanders, Wickham, 1993, etc.).

Microzooplankton mainly consists of protists (ciliates, heterotrophic flagellates, heliozoans, radiolarian and foraminifera) and the smallest invertebrates (those smallest invertebrates, which a zooplankton net with a mesh size of 200 microns fails to collect). The ciliates and some heterotrophic dinoflagellates are the dominant group, not only among protists but also in the microzooplankton as a whole and may represent up to 100 % of microzooplankton abundance in temperate coastal waters (Pierce, Turner, 1992), especially in the Black Sea, where planktonic radiolarian and foraminifera are probably absent (see Annex 1).

The general tasks of microzooplankton communities monitoring are:

a) Identification of species composition;

b) Estimation of abundance and biomass;

c) Description of community structure and spatial distribution;

d) Identification of temporal changes and trends of the above mentioned parameters.

It goes without saying that agreed methodology of microzooplankton sampling and enumeration is required in order to achieve comparable results in all the Black Sea littoral states. Because the Black Sea microzooplankton is still understudied, only general recommendations on sampling techniques and processes are considered in this manual. Questions concerning sampling site selection, sampling time, periodicity of material collection and sampling depth should be solved experimentally. It also concerns to the use of microzooplankton as indicator of environmental quality (Annex 4). Some of these factors, especially for metazoans, can be resolved using appropriate recommendations for mesozooplankton (see "Black Sea Monitoring guidelines. Mesozooplankton").

2. Sampling and treatment

The main problem in microzooplankton investigations is that protists and metazoans require different approaches in sampling and processing of samples. Protists and metazoans have different sensitivity to fixatives and species identification is also specific for each of these groups of organisms. For protists, in most cases unconcentrated 'native water' samples and their processing *in vivo* (without fixation to avoid cells destruction and distortion) are recommended, whereas invertebrates can be concentrated through net screens[1] and then fixed. Counting and species identification '*in vivo*' is often difficult and time consuming, because of organisms rapid and chaotic movement, and special cytological methods for species identification are suggested to use. Generally, sampling with nets could be used in both groups of organisms (Tumantseva, Sorokin, 1983). Yet, water bottles (bathometers) of various types and volume or pump samplers are recommended for collection of material[2]. Taking into account the above-mentioned differences between protists and invertebrates, investigative techniques for these groups are considered separately below.

[1] It is possible to filter not only through a net with a certain mesh size, but also through a sieve with further washing of samples onboard or in laboratory (depending on the type of sampling campaign), or through a gauze placed into the back filtration funnel when the reverse filtration method is used (see sub-chapter 2.1 for details on this method).

[2] Sampling with nets could be used to a limited extent only for qualitative and quantitative studies of separate groups of metazoans - copepods and loricate rotifers, as the rest of metazoans easily gets destroyed. Besides, nets get often clogged with phytoplankton and do not properly filter.

2.1. Metazoan microzooplankton

Most metazoan organisms fix well with common fixatives, such as Lugol's solution or formaldehyde and can also be easily concentrated before fixation through special microplanctonic nets (mesh size 55 µm) during vertical sampling from 0-10m layer (fig.1). For the smaller organisms several litters of sea water (1 – 2 l for biomass-rich coastal waters and up to 3 – 5 l for oligotrophic open sea waters) collected with bathometer should be filtered using a chamber for reverse filtration (fig.2) with 10 – 15 µm size nylon mesh screen.

Fig. 1 Small Planktonic net Apstein Hydro-Bios (55 µm mesh size nylon)

The volume of filtered water, as well as the sample volume in the chamber, must be accurately measured using a graduated cylinder. After each sample is prepared a waterproof label should be attached to the sample jar (do not place inside the jar!) detailing the name of the research vessel, cruise number, station number, data, time, volume of filtered water and sample etc. Scotch tape is useful to protect the label from chafing, discoloration or other physical damage during transportation and storage.

Fig. 2 Chamber for reverse filtration

The samples should be preserved in 2–4 % final concentration formaldehyde (1 part of 37 % formaldehyde solution to 8–14 parts of sample water) buffered with hexamethylenetetramine or another buffer (sodium acetate or borax). Samples should be stored in a dark cool place for at least several years to allow retrospective investigations of abnormal species occurrence, including alien forms or unusual numbers. For long-term storage, after settling of all organisms and gentle decantation, samples can be re-fixed with Bouin solution (saturated aqueous picric acid, 37 % formaldehyde and glacial acetic acid in ratio 15:5:1).

Subsequent procedures, such as sub-sampling, microscopy, taxonomical identification and estimation of abundance and biomass, do not differ from those for mesozooplankton[3] (see Black Sea Monitoring Guidelines. Mesozooplankton, eds. Alexandrov B. *et al*, 2015).

Samples should be viewed at a greater magnification (×25-40) under dissection microscope (binocular) due to the smaller sizes of microzooplankters, and a shallower Bogorov tray[4] (smaller volume, 5 ml is optional) should be used. Identification of some small animals (rotifers, nauplii etc.) requires a compound microscope[5] with high magnification (immersion oil) objective and special techniques: 5% $MgCl_2$ should be added to the sample to immobilize objects (4 drops to 10 ml of sample).

[3] They may differ in biomass calculations if biovolumes of metazoans are identified using the geometrical method (for details see sub-chapter 2.2.3 for details).

[4] It is also named 'Bogorov chamber', though it uses no lid.

[5] Light microscope with additional contrast devices, light filters and a condenser.

2.2. Protists

Further below methods of ciliates sampling and processing are given as ciliates are predominant group in microzooplankton, and the suggested methods are also applicable for other Protists.

Traditionally, pelagic ciliates are divided into two groups: "naked" forms and ones which form a more or less distinct lorica (tintinnids). Their functional role mainly depends on the trophic status of separate species, which may be mixotrophic, primary or secondary consumers.

Species composition and distribution of planktonic ciliates in the Black Sea are still understudied, except for the north-western coastal area and some adjacent water bodies (**Annex 1**) (Kurilov, 2007, 2010).

2.2.1. Processing of living material

There are many methods with a number of modifications for planktonic ciliates investigation, yet, worth mentioning are those satisfactory results can be achieved only with the study of living material (Petz, 1999). On the other hand, such processing onboard is hardly possible because the method is time consuming and requires certain skills, where large number of samples is usually collected. Procedures using "pencil box" chambers type with glass lid (fig. 3) (Tumantseva, Sorokin, 1983) are laborious even for experienced researchers. Moreover, due to air-deficit (in the chamber with a lid due to its small volume) some cells may burst or dissolve, so that identification of species composition in most cases is impossible. Thus, both quantitative and qualitative underestimation of ciliates can occur.

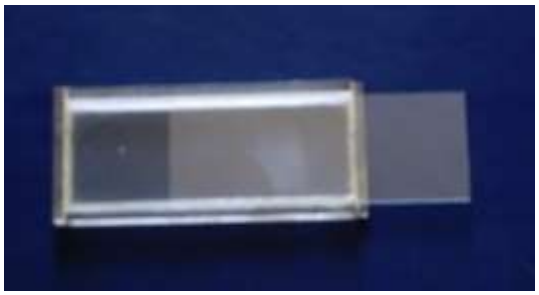

Fig. 3 "Pencil box" chamber for enumeration of ciliatoplankton.

A Bogorov counting tray is more appropriate for enumeration of ciliates, but it may be used only on land or on a large ship if sea conditions are fairly stable. A fresh (right after sampling) unconcentrated water sample should be carefully mixed before enumeration. One portion of the water from the sample (5 ml or less) should be placed into the counting chamber and examined using a dissection microscope at x25–45 magnification. To achieve the most accurate counts each cell may be picked up by micropipette and placed into a microaquarium (a small Petri dish or immunological well-plate)[6] with filtered sea water or into a slide with a cavity filled with a fixative (fig. 4).

Fig. 4 Bogorov tray, micropipette equipped with rubber hose with a mouthpiece and slide with cavity

[6] Every specimen caught is automatically considered counted and is included into a protocol (record of counts). Organisms are placed into a Petri dish for further taxonomic identification. This is to save time – first counting and fast preliminary identification in the Bogorov Tray, then consequent and more accurate species identification using temporal and permanent slides. Under the compound microscope, the sample in the Bogorov Tray can dry over if we spend time for accurate taxonomic studies.

Glass micropipettes are constructed in pairs from a piece of glass tubing 8–10 mm long and about 3–4 mm in diameter. The tubes are heated in their centres over a spirit burner and pulled out so that finely narrowed tips are formed. After cooling, the two pipettes are separated, and sharp edges are removed. Silicone rubber hose with a mouthpiece at one end can be utilized as a teat (Carey, 1992).

Several sub-samples should be examined until no new species appear in 3 consequent sub-samples. Usually, 20–50 ml of sample is enough to attain accurate counts. During this step, preliminary identification of species can be undertaken basing it on key properties such as body shape and colour, and type of movement. Verification of preliminary identifications must be carried out using a compound microscope equipped with interference contrast or differential-interference contrast (DIC). If not available, bright-field, oblique illumination or phase-contrast may be used. Specimens are picked out from the microaquarium under a dissecting microscope and placed in a very small drop of sea water on a slide. The droplet with the ciliate is then neatly covered by coverslip with small dabs of vaseline or wax applied to each of its four corners to prevent crushing of cell. Movement of the ciliate can easy be slowed down by pressing on the coverslip corners with a mounted needle. The major cell organelles (e.g., nuclear and oral apparatus, contractile vacuole, extrusomes, cortical granules, food vacuoles, etc.) can easily be observed under low (x100–400) and high (oil immersion objective) magnification (Foissner et al, 1999). At this step specimens can be measured for biomass determination (see sub-chapter 2.2.3 and Annex 2). The results are reported in counting protocols.

To shorten sample processing ciliates may be fixed in a slide with a cavity without live observation. In this case, after enumeration for consequent accurate taxonomic identification, all the fixed cells are transferred by micropipette onto a slide with a small droplet of glycerine or glycerine-methyl green (which stains the nuclear apparatus) and covered by coverslip as described above. For preparation of methyl green solution, small portions of powder of dry stain are dissolved in glycerine until the solution is still transparent, after which 3 drops of glacial acetic acid are added for each 10 ml of stain and mixed. A 10 % solution of glutaraldehyde is the most suitable fixative to use from which cells may be directly transferred onto drop of glycerine-methyl green without washing. Such temporary slides can be stored for up to 10 days or more, and are generally appropriate for species determination and measurement.

Final identification to species level must be undertaken using silver staining methods. Several protocols are available for this of which the improved modification of silver carbonate method (Ma et al., 2003) is the most suitable for the technique described above. Silver proteinate techniques (Montagnes, Lynn, 1987; Skibbe, 1994; Dieckmann, 1995; Foissner et al., 1999; Silva-Neto, 2000 etc.) are also appropriate but require costly reagents and some experience. Basic guidebooks for identification of microzooplankton species are listed in Annex 3.

2.2.2. Processing of preserved material

As mentioned above, during most research cruises the large number of samples collected does not allow *in vivo* processing of all materials. The samples should therefore be fixed. There are two general problems: cells losses due to fixation and changes in cell volume due to fixative-induced shrinkage. Additionally, fixation often distorts cells morphology.

Acid Lugol's iodine solution and modified Bouin's fluid are considered to be less destructive for planktonic ciliates than other common fixatives such as formaldehyde or glutaraldehyde (Stoecker at al., 1994; Petz, 1999). Acid Lugol's solution (10 g iodine and 20 g potassium iodine dissolved in 200 ml distilled water, followed by 20 ml glacial acetic acid), which is used at a final concentration of 1-2% (v/v) to sample, is a relatively inexpensive and safe method for planktonic ciliate fixation (Gifford, Caron, 2000). Nevertheless, cells shrinkage is relatively less in modified Bouin's solution, while both fixatives usually yield similar cell losses (Stoecker at al., 1994). Modified Bouin's fluid is prepared by saturating of buffered 37% formaldehyde with picric acid with glacial acetic acid added immediately before use so that the final concentration of acetic acid in the sample is 1% (v/v). This fixative is used at a ratio of 1:10 for sea water and of 1:19 for brackish water (Lee et al., 1985). So, for the Black Sea (S≈18‰) a ratio of 1:15 will be optimal.

Preservation of samples must be carried out by gently draining sea water from the bathometer or sampling bottle into a storage vessel containing the necessary amount of fixative (not the converse!). After fixation, samples should be allowed to sit undisturbed for at least 24 h because protists tend to be fragile immediately following preservation. A general settling time recommended by Gifford and Caron (Gifford, Caron, 2000) for fixed samples is 3 mm h^{-1}. Nevertheless, Claessens and Prast (2008) reported sufficient short settling times with a (probably) more reliable sinking rate of 1.7 mm·min^{-1} for fixed marine (41‰) ciliate samples and 2.4 mm·min^{-1} for fixed freshwater samples. With these rates settling times can be reduced up to 95% compared to old, experimentally-derived times. However, this time should not be less than 24 h.

Thus, for ciliates and any other protists the method of gravitational sinking to concentrate fixed samples with further discarding of water after settling is recommended. Inverted microscopy is the most common method used to enumerate planktonic ciliates in preliminary settled preserved samples according to the procedure of Utermöhl (1958). This method requires special plastic settling cylinders of different volumes (100–200 ml) with a detachable bottom plate, which is simultaneously a counting chamber. If not available, the following method is more accessible and easy. Samples in storage vessel should be concentrated on land by settling up to final volume of 5–7 ml or less. Then, after mixing well, 25 – 50 µl of subsample is removed with automatic pipette and placed onto a slide with droplet of glycerine. Both droplets are then thoroughly mixed with a mounted needle and covered by coverslip (18×18 or 24×24 mm, depending of subsample volume) as described above (see 2.2.1). Then each slide is examined under a compound microscope at x200–400 magnification. Adding glycerine prevents premature drying of the preparation during examination, which may take several hours.

A crucial drawback of using Lugol's iodine is dark staining of both cells and similar-sized particles of detritus, which usually mask ciliates. These samples have a short shelf life (because of iodine evaporation) and are usually unsuitable for silver staining methods. On the other hand, Bouin's preserved samples are the most common for different silver proteinate protocols and may be stored in dark cool place for long time, up to several years according to Alekperov (1992).

2.2.3. Biomass estimation.

Determination of biovolume of the unicellular organisms (and small metazoans) is generally based on their geometrical shape and dimensions measured by calibrated eye piece micrometer. The body shape of protists is usually equated to simple geometrical figures (ellipsoid, cone, truncated cone, sphere etc.) or their combinations (**Annex 2**). Useful formulas, especially for flagellates, can be obtained from Hillebrand et al., 1999; Sun and Liu, 2003; Bryantseva and Kurilov, 2003 and Bryantseva et al., 2005.

Live observations provide the most accurate biovolume estimations, but rapid mobility of organisms often makes cells difficult to measure. To slow down the swimming cells, cotton wool or glass wool fibres situated in the droplet with the organism measured is recommended. In the case of preserved material, measurement of cells may be facilitated by careful pattering on the coverslip with a mounted needle. Use of chemical immobilization (see Lindholm, 1982 for detail) or physical slowing down by increasing of the medium viscosity (e.g., methyl cellulose) is unsuitable, because those methods change the shape of the cells (Foissner et al., 1999).

As mentioned above, fixation can cause cells to shrink and may affect cell volumes. Therefore, data on biomass obtained from preserved material are underestimated. According to Choi and Stoecker (1989), the volume of fixed cells was found to be approximately 20 to 55% lower than the cell volume of live organisms. With ciliates, formaldehyde caused less shrinkage (13–20%) than glutaraldehyde (36–46%) or acid Lugol's solution (70% for *Favella* sp.). Changes in protozoan volume in response to fixation varied with species and fixatives, but responses of each species to a fixative were constant irrespective of its physiological state. Such shrinkage is common and must be taken into account in biomass estimations. Unfortunately, an overall conversion factor cannot be provided because the extent of shrinkage depends highly on many factors, such as the kind of species and the storage time of the sample. Thus, live specimens should be measured whenever possible (Foissner et al., 1999).

3. Data reporting

The following data should be reported:

N	Acronym	Name	Example
1	RV	Name of RV and cruise number	30 RV Vladymyr Parshin
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 sample collection	17:30
8	Ndec	Coordinate of station: Latitude (Degree)	45.6593
9	Edec	Coordinate of station: Longitude (Degree)	31.6113
10	Sampling	Type of the bathometer/net	Niskin/Apstein
11	Layer	Sampling depth/column, m	10/0-10
12	Filtrated volume (FV)	Volume of water filtered with chamber for reverse filtration, ml / volume of water filtered with planktonic net, l, for metazoan microzooplankton	5000/56,7
13	Volume 1	Volume of water sample for planktonic protists, ml	1.0
14	Volume F	Volume of concentrated sample for metazoan microzooplankton, ml	50
15	Volume S*	Volume of settled sample for planktonic protists, ml	3.7
16	Fixative	Fixative used for planktonic protists sample preservation	Acid Lugol
17	Volume 3FSS	Total volume of Stempel pipette sub-samples, which was used for counting procedure under binocular microscope and for calculation of the abundance of each individual taxon of metazoan microzooplankton, ml	7
18	Volume 3SSS	Total volume of automatic pipette sub-samples, which was used for counting procedure under compound microscope (or dissection microscope in case of live counting) and for calculation of abundance of each individual taxon of planktonic protists, ml	0.05
19	Coefficient F	Coefficient 2F = N14 / N17	7.143
20	Coefficient S	Coefficient 2S = N15 / N18	74
21	Taxon 1MN	Number of specimens counted in the subsample (Metazoa)	110
22	Taxon 1PN	Number of specimens counted in the subsample (Protista)	25
23	Taxon 1MAb	Specimens of metazoan taxa (ind./m^3) = N21 * (10^6 / N12) * N19	314292
24	Taxon 1PAb	Specimens of protistan taxa (ind./m^3) = N22 * (10^6 / N13) * N20	1850000
25	Taxon 1 MB	Biomass of metazoan taxa (mg/m^3) = ind./m^3 (N23) * Weight of single specimen	XXX.XX
26	Taxon 1 PB	Biomass of protistan taxa (mg/m^3) = ind./m^3 (N24) * Weight	XXX.XX

N	Acronym	Name	Example
		of single specimen	
…	Taxon NNM		
…	Taxon NNP		
…	Group 1 CM	Total concentration of metazoan taxonomic group (ind/m^3)	XXXXXX
…	Group 1 BM	Total biomass of metazoan taxonomic group (mg/m^3)	XXX.XX
…	Group 1 CP	Total concentration of protistan taxonomic group (ind/m^3)	XXXXXXXX
…	Group 1 BP	Total biomass of protistan taxonomic group (mg/m^3)	XXX.XX
…			
	Total CM	Total concentration of metazoan microzooplankton (ind/m^3)	XXXXXXXX
	Total BM	Total biomass of metazoan microzooplankton (mg/m^3)	XXX.XX
	Total CP	Total concentration of planktonic protists (ind/m^3)	XXXXXXXX
	Total BP	Total biomass of planktonic protists (mg/m^3)	XXX.XX
	Total C	Total concentration of microzooplankton (ind/m^3)	XXXXXXXX
	Total C	Total biomass of microzooplankton (mg/m^3)	XXXX.XX

* In case of live counting used Volume S (N15) is equal to Volume 1 (N13)

4. Recommended equipment

Preferably all organisations/laboratories should use standardized Black Sea microzooplankton sample collection/processing equipment. The following standard equipment should be used:

1. Bathometer (e.g. GoFlo® or Niskin®).
2. Chamber for reverse filtration (also used in phytoplankton investigations).
3. Nylon mesh screens (mesh size 10–15 μm).
4. Stempel-pipette (vol. = 1 ml).
5. Automatic pipette (vol. 0,025 – 0,1 ml).
6. Glass-lid chamber "pencil-box" type.
7. Bogorov's chamber.
8. Microscope slides: standard and with cavity.
9. Coverslips.
10. Mounted needles.
11. Graduated cylinders of different volume for sample volume determination.
12. Dissection microscope.
13. Compound microscope equipped with phase-contrast illumination.

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Annex 1 Species composition of microplankton and their distribution in national waters of Black Sea countries

TAXON		Bulgaria	Georgia	Romania	Russia	Turkey	Ukraine
CILIATOPLANKTON*							
Class Karyorelictea Corliss, 1974							
	Tracheloraphis spp.	?		?	?		+
	Loxodes vorax Stokes, 1884						+
Class Heterotrichea Stein, 1859							
	Blepharisma spp.	?		?	?		+
	Condylostoma arenaria Spieg., 1926	?		?	?		+
	Peritromus faurei Kahl, 1932			?			+
	P. ovalis F.-Fr., 1924				+		
Class Spirotrichea Butschli, 1889							
	Protocruzia adhaerens (Mansf., 1923)	?		?	?		+
	Euplotes balteatus (Duj., 1841)	?		?	?		+
	E. trisulcatus Kahl, 1932	?		?	?		+
	Euplotes spp.	?			?		+
	E. patella (O.F.M., 1973)	?					+
	Euplotopsis affinis (Duj., 1841)	?		?			+
	E. elegans (Kahl, 1932)			?			+
	Moneuplotes balticus (Kahl, 1932)	?		?	?		+
	Aspidisca baltica (Kahl, 1932)						+
	A. lyncaster (O.F.M., 1776)	?		?			+
	Aspidisca spp.	?		?	?		+
	Diophrys appendiculata (Ehr., 1838)	?		?	?		+
	D. scutum (Duj., 1841)	?		?	?		+
	Diophrys spp.				?		+
	Uronychia transfuga (O.F.M.,1776)	?		?	?		+
	Amphisiella capitata (Perej., 1886)	?			?		+
	Pseudokeronopsis rubra (Ehr., 1835)	?		?	?		+
	Cyrtohymena (Steinia) marina (Kahl, 1932)	?				?	
	Oxytricha spp.	?		?	?		+
	Halteria spp.	?					+
	Pelagohalteria cirrifera (Kahl, 1935)						+
	P. viridis (Fromentel, 1886)						+
	Meseres cardiformis (Schew., 1892)						+
	Cyrtostrombidium sp.						+
	Laboea strobila (Lohm., 1908)				+		+
	Spirostrombidium elegans (Flor., 1901)			?	?		+
	S. sauerbreyae (Kahl, 1932)	?		+			

TAXON		Bulgaria	Georgia	Romania	Russia	Turkey	Ukraine
	Strombidium acutum (Leeg., 1915)						+
	S. alveolare? Bullington, 1940						+
	S. arenicola Dragesco, 1960			?			+
	S. bilobum Lynn et Gilron, 1993						+
	S. calkinsi (Kahl, 1932)	+					
	Strombidium capitatum Kahl, 1932						+
	S. conicoides (Leeg., 1915)						+
	S. conicum (Lohm., 1908)			?	+		+
	S. constrictum (Meun., 1910)				+		
	S. coronatum (Leeg., 1915)						+
	S. dalum Lynn et al., 1988						+
	S. emergens (Leeg., 1915)						+
	S. epidemum Lynn et al., 1988						+
	S. faurei Drag., 1960						+
	S. filificum Kahl, 1932						+
	S. inclinatum Mont. et al., 1990						+
	S. lagenula F.-Fr., 1924			?			+
	S. macronucleatum Drag., 1960						+
	S. rhynchum Lynn et al., 1988						+
	S. obliquum (Kahl, 1932)						
	S. stylifer Levander., 1894						+
	S. sulcatum (Clap. et Lachm., 1858)			?	+		
	S. tressum Lynn et al., 1988						+
	S. ventropinnum Martin et Mont., 1993						+
	S. vestitum (Leeg., 1915)						+
	S. viride Stein, 1867				?		+
	S. wulffi (Wulff, 1919)						+
	Strombidium spp.	+		+	+		+
	Tontonia gracillima F.-Fr., 1924						+
	T. appendiculariformis F.-Fr., 1924				+		+
	Strombidinopsis elongata Song et Bradb., 1998						+
	S. cheshiri (=Strob. acuminatum) Snyder et Ohman, 1991				+		+
	S. minima Song et Bradb., 1998			?			+
	S. multiauris Mont.et Taylor, 1994						+
	S. gyrans (Kent, 1881)	+					
	Strombidinopsis sp.						+
	Strobilidium marinum (F.-Fr., 1924)				+		+
	S. mucotectum? (Bush, 1924)						+
	Strobilidium sp.						+

TAXON		Bulgaria	Georgia	Romania	Russia	Turkey	Ukraine
	Pelagostrobilidium spirale (Leeg., 1915)						+
	Pelagostrobilidium sp.						+
	Rimostrombidium caudatum (Kahl, 1932)	+					+
	R. conicum (Kahl, 1932)						+
	R. epacrum Lynn et Mont., 1988						+
	R. multinucleatum (Lynn et Mont., 1988)						+
	R. sphaericum (Lynn et Mont., 1988)						+
	R. velox (F.-Fr., 1924)						+
	Parastrombidium sp.						+
	Lohmanniella oviformis (Leeg., 1915)						+
	Leegardiella sp. Lynn et Mont., 1988						+
	Tintinnidium mucicola (Clap. et Lachm., 1858)	+			+		+
	Tintinnidium fluviatile (Stein, 1863)	+					
	Tintinnidium sp.				+		+
	Leprotintinnus pellucidus (Cleve, 1899)	+		+	+		+
	Codonella aspera Kofoid et Campbell, 1929					+	
	C. cratera Leidy, 1887	+			+		+
	C. lagenula (Claparede et Lachmann, 1858)				+		+
	Codonella sp.	+					
	Codonaria fimbriata (Meunier, 1919)						+
	Dictyocysta mitra Haeckel, 1873					+	
	Amphorellopsis acuta (Schmidt, 1901)				+		
	Tintinnopsis baltica Brandt, 1896	+	+		+		+
	T. beroidea Entz, 1884	+		+		+	+
	T. campanula Ehrenberg, 1840	+	+	+	+	+	+
	T. compressa Daday, 1887						+
	T. cylindrica Daday, 1886	+		+		+	+
	T. cylindrata Kofoid et Campbell, 1929	+					
	T. davidovi Daday, 1886	+	+	+			+
	T. directa Hada, 1932						+
	T. karajacensis Brandt, 1908	+					+
	T. kofoidi Hada, 1932				+		+
	T. lobiancoi Daday, 1886	+		+		+	+
	T. meunieri (Haeck., 1873)	+		+			+
	T. minuta Wailes, 1925	+		+			+
	T. parvula Jörgensen, 1912	+		+			+
	T. nudicauda Paulmer, 1995						+
	T. radix (Imhof, 1886)					+	
	T. rossolimi Morozovskaya, 1968						+

TAXON		Bulgaria	Georgia	Romania	Russia	Turkey	Ukraine
	T. strigosa Meunier, 1919					+	
	T. subacuta, Jörgensen, 1889	+					+
	T. tocantinensis Kofoid et Campbell, 1929				+		
	T. tubulosa Levander, 1900	+	+	+	+		+
	T. tubulosoides Meunier, 1910	+					
	T. urnula Meunier, 1910				+		+
	Tintinnopsis spp.	+	+		+		+
	Codonellopsis morchela (Cleve, 1900)					+	
	Stenosemella nivalis (Meun., 1910)	+	+			+	+
	S. ventricosa (Clap. et Lachm., 1858)	+	+	+	+		+
	Metacylis mediterranea (Mereschk., 1881)	+	+	+	+	+	+
	Helicostomella subulata Jörgensen, 1924	+		+	+		+
	Helicostomella sp.	+					
	Petalotricha ampulla (Fol, 1881)					+	
	Xystonella lohmanni (Brandt, 1906)					+	
	Favella cf. *ehrenbergii* (Clap. et Lachm., 1858)	+	+	+	+		+
	F. azorica (Cleve, 1900)					+	
	F. campanula (Schmidt, 1901)					+	
	F. serrata (=Parafavella denticulata) (Ehrenberg, 1840)				+	+	
	F. brevis (Laackmann, 1909)						+
	Eutintinnus lususundae (Entz, 1884)					+	+
	E. angustatus (Daday, 1887)						+
	E. apertus (Kofoid et Campbell, 1929)						+
	E. tubulosus (Ostenfeld, 1899)						+
	E. haslae Taniguchi et Hada, 1981						+
	Salpingella rotundata? Kofoid et Campbell, 1929						+
	S. decurlata Jörgensen, 1924				+		
	Nolaclusilis sp.						+
Class Litostomatea Small et Lynn, 1981							
	Sphatidium spp.	?		?			+
	Didinium gargantua Meun., 1907						+
	D. nasutum O.F.M., 1786				?		+
	Didinium sp.						+
	Monodinium balbianii Fab.-Dom., 1888	?			?		+
	M. nanum (Fab.-Dom., 1888)						+
	M. rostratum (Kahl, 1926)	?					+
	Lacrymaria pupula O.F.M., 1786						+
	Lacrymaria spp.	?		?	?		+
	Enchelydium sp.						+

TAXON		Bulgaria	Georgia	Romania	Russia	Turkey	Ukraine
	Enchelus spp.						+
	Litonotus fasciola (Ehr., 1838)	?			?		+
	L. lamella (Ehr., 1838)	?		?	?		+
	Litonotus spp.	?			?		+
	Loxophyllum setigerum Quenn., 1867	?		?	?		+
	L. uninucleatum Kahl, 1928	?		?	?		+
	Loxophyllum spp.	?		?	?		+
	Heminotus caudatus (Kahl, 1933)	?		?		?	
	Askenasia stellaris (Eichw., 1852)	?					+
	A. volvox (Clap. et Lachm., 1858)						+
	A. regina Earlander et Mont., 2002						+
	Rhabdoaskenasia sp.						+
	Cyclotrichium sphaericum F.-Fr., 1924						+
	Mesodinium acarus Stein, 1862						+
	M. pulex (Clap. et Lachm., 1858)	?		?			+
	Mesodinium sp.	?			+		
	M. rubrum (=Myrionecta rubra) (Lohm., 1908)	+		+	+		+
Class Phyllopharyngea de Puytorac et al., 1974							
	Chilodonella spp.	?		?	?		+
	Chlamydodon triquetrus (O.F.M., 1776)	?		?	?		+
	Dysteria spp.	?		?	?		+
	Ephelota coronata Kent, 1881						+
	Acineta tuberosa Ehr., 1834	?					+
Class Prostomatea Schewiakoff, 1896							
	Prorodon marinus Clap. et Lachm., 1858	?		?			+
	P. minutus Kahl, 1927						+
	Prorodon spp.	?					+
	Coleps tesselatus Kahl, 1930	?		?	?		+
	Tiarina fusus (Clap. et Lachm., 1857)			?	+		+
	Urotricha agilis Stokes, 1886						+
	U. farcta Clap. et Lachm., 1858						+
	U. globosa Schew., 1893						+
	U. ovata Kahl, 1927						+
	U. pelagica Kahl, 1935						+
	U. pusilla Penard, 1922						+
	Urotricha spp.	?		?			+
	Paraurotricha discolor (Kahl, 1930)						+
	Bursellopsis truncata (Kahl, 1927)						+
	Bursellopsis sp. (Balanion sp.?)						+

TAXON		Bulgaria	Georgia	Romania	Russia	Turkey	Ukraine
	B. nigricans (Laut., 1894)			?			+
	Balanion comatum (Wulff, 1919)						+
	Plagyocampa marina Kahl, 1930	?		?			+
	P. rouxi Kahl, 1926	?		?			+
	Plagiocampa spp.	?		?			+
	Metacystis crassa (Cohn, 1866)						+
	Vasicola gracilis Penard, 1922						+
	V. ciliata Tatem, 1869						+
	Holophrya marina Mansf., 1923						+
	H. pelagica Lohm., 1920						+
	Holophrya spp.	?		?			+
Class Oligohymenophorea de Puytorac et al., 1974							
	Frontonia acuminata Ehr., 1838						+
	F. acuminata var. angusta Kahl, 1932						+
	F. arenaria Kahl, 1933	?		?	?		+
	F. marina Fab.-Dom., 1891	?		?	?		+
	Frontonia spp.	?		?	?		+
	Loxocephalus spp.	?					+
	Cinetochilum spp.	?		?			+
	Helicostoma oblongum Cohn, 1866	+					+
	Uronema marinum Duj., 1841	?		?	?		+
	Uronema spp.	?		?	?		+
	Cyclidium spp.	?		?	?		+
	Cristigera minuta Kahl, 1928						+
	Pleuronema anodontae Kahl, 1932						+
	P. coronatum Kent, 1881	?		?	?		+
	P. marinum Duj., 1841	?		?	+		+
	Vorticella marina Greeff, 1870	?			?		+
	Vorticella microstoma Ehr., 1830	?					+
	Vorticella spp.	?			?		+
	Zoothamnium duplicatum Kahl, 1933						+
	Z. plumula Perej., 1883						+
	Zoothamnium spp.	?			?		+
	Thuricola valvata (Wright, 1858)						+
	*Ciliata taxa ordered according to Lynn, 2008 Note: pointed with question mark ciliates reported for the benthos						

TAXON		Bulgaria	Georgia	Romania	Russia	Turkey	Ukraine
HETEROTROPHIC FLAGELLATES		See check list of the Black Sea phytoplankton: http://phyto.bss.ibss.org.ua/wiki/Category:Species					
PLANKTONIC RHIZOPODS AND HELIOZOANS		No data available					
METAZOOPLANKTON							
Polychaete larvae (metatrohophora)							
Class Polychaeta Grube, 1850							
	Capitella capitata Fabricius, 1780				+		+
	Glycera tridactyla Schmarada. 1861				+		+
	Harmothoe imbricate Linne, 1767				+		+
	Lysidice ninetta Aud. Et Edw., 1834				+		+
	Nephthys sp.				+		+
	Pectinaria koreni Malmgren, 1865				+		+
	Pholoe synophthalmica Claparede, 1868				+		+
	Phyllodoce tuberculata Bobretzky, 1868				+		+
	Polydora spp.				+		+
	Protodrilus purpureus Schneider, 1868				+		+
	S.militaris Claparede, 1868				+		+
	Sabellaria taurica Rathke, 1837				+		+
	Spio filicornis Muller, 1776				+		+
	Spirorbis pusilla Rathke,1837				+		+
Gastropoda veligers							
Class Gastropoda Cuvier, 1795							
	Cerithium *vulgatum* Bruguière, 1789				+		+
	Bittium reticulatum da Costa, 1778				+		+
	Cerithidium pussilum Jeffreys, 1856				+		+
	Caecum elegans Perejaslawzewa, 1891				+		+
	Triphora perversa Linnaeus, 1758				+		+
	Rapana thomasiana Crosse, 1861				+		+
	Tritia reticulate Linnaeus, 1758				+		+
	Limapontia capitata Müller, 1774				+		+
	Doris ocelligera Bergh, 1881				+		+
	Tergipes tergipes (Forsskål in Niebuhr, 1775)				+		+
Bivalvia veligers							
Class Bivalvia Linne, 1758							
	Mytilus galloprovincialis Lamarck, 1819				+		+
	Modiolus adriaticus Lamarck, 1819				+		+
	Ostrea edulis Linne, 1758				+		+
	Flexopecten ponticus Bucquoy, Dautzenberg & Dollfus, 1889				+		+

TAXON		Bulgaria	Georgia	Romania	Russia	Turkey	Ukraine
	Loripes lucinalis Lamarck, 1818				+		+
	Mycella bidentata Montagu, 1803				+		+
	Fabulina fibula Gronovius, 1781				+		+
	Moerella donacina Linnaeus, 1758				+		+
	Gastrana fragilis Linne, 1758				+		+
	Abra alba occitanica Recluz,1843				+		+
	A.ovata Philippi, 1836				+		+
	Donax semistriatus Poli, 1791				+		+
	Spisula sp.				+		+
	Mactra stultorum Linne, 1758				+		+
	Pitar rudis Poli,1791				+		+
	Chamelea gallina Linnaeus, 1758				+		+
	Polititapes aurea Gmelin, 1790				+		+
	Petricola lithophaga Retzius, 1786				+		+
	Cerastoderma clodience Renieri,1804				+		+
	Parvicardiumexiguum Gmelin, 1790				+		+
	Phoals dactylus Linne,1758				+		+
	Barnea candida Linne, 1758				+		+
	Teredo navalis Linne, 1758				+		+
	Thracia papyracea Poli, 1791				+		+
Rotatoria (small rotifers only)							
Class Eurotatoria De Ridder, 1957							
	Anuraeopsis fissa Gosse, 1851	+					
	Asplanchna priodonta Gosse,1850	+		+			+
	Brachionus angularis Gosse,1851		+				+
	Br. a. angularis Gosse,1851						+
	Br. a. bidens Plate, 1886						+
	Br. calyciflorus Pallas, 1766	+		+	+		+
	Br. c. amphiceros Ehrenberg, 1838						+
	Br. c. calyciflorus Pallas,1776						+
	Br. c. dorcas Gosse, 1851						+
	Br. diversicornis Daday,1883						+
	Br. d. diversicornis Daday,1883						+
	B. plicatilis O.F.Müller, 1773			+	+		+
	Br. p. asplanchnoidis Charin, 1947				+		+
	Br. quadridentatus Hermann, 1783	+	+	+	+	+	+
	Br. q. ancylognathus Schmarda, 1859						+
	Br. leudigi leudigi Cohn, 1862						+
	Br. l. quadridentatus Rousselet, 1907						+

TAXON		Bulgaria	Georgia	Romania	Russia	Turkey	Ukraine
	Br .rubens Ehrenberg, 1838		+		+		+
	Br. urceolaris O.F.Müller, 1773				+		+
	Colurella adriatica Ehrenberg, 1831	+	+	+	+	+	+
	C. auricata O.F.Müller, 1773				+		+
	C. colurus (Ehrenberg, 1830)						+
	C. dicenta Gosse, 1887				+		+
	C. epitedia Myers, 1924				+		+
	C. gracilis Ehrenberg, 1832				+		
	C. monodactilos Althaus, 1957						+
	C. uncinata Ehrenberg, 1832				+		
	Colurella sp.			+			+
	Cephalodella catellina O.F.Müller, 1786				+		
	Encentrum marinum Dujardin, 1841						+
	Euchlanis dilatata Ehrenberg, 1832	+					+
	Filinina longiseta Ehrenberg, 1834						+
	F. terminalis Plate, 1886			+	+		+
	Hexarthra fennica (Levander, 1892)						+
	Kellicottia longispina (Kellicot, 1879)						+
	Keratella cruciformis Thompson, 1892						+
	K. cochlearis Gosse, 1851			+	+		+
	K. c. tecta (Gosse, 1851)						+
	K. qudrata O.F.Müller, 1786	+	+	+	+	+	+
	K. q. dispersa Carlin, 1943						+
	K. q. frenzeli (Eckstein, 1895)						+
	K. q. reticulata Carlin, 1943						+
	K. tropica tropica (Apstein, 1907)						+
	K. t. reducta Fadeev, 1927						+
	K. valga Ehrenberg, 1834				+		+
	K. v. valga (Ehrenberg, 1834)						+
	Lecane grandis Murray, 1913				+		+
	L. hastate Murray, 1913				+		
	L. luna O.F.Müller, 1776				+		
	L. nana Murray, 1913				+		
	L. paradoxa Steinecke, 1924				+		
	Lecane sp.						+
	Lepadella triptera Ehrenberg, 1830				+		+
	N. acuminata Ehrenberg, 1832				+		+
	N. a. extensa Olofsson, 1918						+
	N. squamula O.F.Müller, 1786				+		

TAXON		Bulgaria	Georgia	Romania	Russia	Turkey	Ukraine
	Notholca striata O.F.Müller, 1786				+		
	Philodina citrine Ehrtnberg, 1832	+			+		+
	P. halophila Remane, 1929				+		
	P. reinhardti Ehrenberg, 1834				+		
	P. remata, Skorikov,1896				+		
	Polyarthra vulgaris Carlin,1943						+
	P. remata Skorikov, 1896						+
	Polyarthra sp.						+
	Pompholys sulcata Hudson, 1885			+	+		
	Proales reinhardti (Ehrenberg, 1839)						+
	P. similis de Beauchamp, 1907				+		+
	Proales sp.						+
	Philodina roseola Ehrenberg, 1832						+
	Rotatoria elongata Weber		+				
	Rotatoria mertunia Ehrenberg		+				
	R. rotatoria Pallas		+				
	Rotaria tardigrada Ehrenberg, 1832						+
	R. tridens Montet		+				
	R. trisecata (Weber)		+				
	Synchaeta baltica Ehrenberg, 1834						+
	S. cecillia Rousselet, 1902	+		+			+
	S. curvata Lie-Pettersen, 1905				+		
	S. grimpei Remane, 1929						+
	S. gyrina Hood, 1887				+		
	S. littoralis Rousselet, 1902				+		+
	S. monopus Plate, 1889			+			+
	S. neapolitana Rousselet, 1902	+		+	+		+
	S. oblonga Ehrenberg, 1832						+
	S. pontica Rodewald-Rudescu, 1960			+	+		+
	S. pectinata Ehrenberg, 1832		+				+
	S. razelmi Rodewald-Rudescu, 1960			+	+		+
	S. stylata Wierz., 1893		+				+
	S. tavina Hood, 1893				+		
	S. tremula O.F.Müller, 1786				+		
	S. triophthalma Lauterborn, 1899						+
	S. vorax Rousselet, 1902	+	+				+
	Synchaeta sp.		+	+			+
	Testudinella clypeata O.F.Müller, 1786				+		+
	T. marina Daday, 1890				+		+

TAXON		Bulgaria	Georgia	Romania	Russia	Turkey	Ukraine
	T. patina Hermann, 1783				+		+
	T. rattus O.F.Müller, 1776				+		
	Trichocerca capucina Wierz. et Zach., 1893				+		+
	T. marina (Daday, 1890)		+				+
	T. rattus (Müller, 1776)						+
	Trichocerca sp.						+
	Tripleuchlanis plicata Levander, 1894				+		+
Nauplii of copepods							
	Acartia spp. (I-VI stage)				+		+
	Anomalocera patersoni Templeton, 1837 (I-II stage)				+		+
	Calanus euxinus Hulsemann, 1991(=*C. helgolandicus* Claus,1863), (I-III stage)				+		+
	Centropages ponticus Giesbrecht, 1889 (I-VI stage)				+		+
	Labidocera brunescens Czerniavsky, 1868 (I-IV stage)				+		+
	Oithona davisae Ferrari F.D. & Orsi, 1984 (I-VI stage)			+	+		+
	Oithona nana Scott T., 1894 (I-VI nstage)	+	+	+	+	+	+
	Oithona similis Claus, 1863 (I-VI stage)				+		+
	Paracalanus parvus Claus, 1863 (I-VI stage)				+		+
	Pontella mediterranea Claus, 1863 (I-III stage)				+		+
	Pseudocalanus elongatus Claus, 1863 (I-VI stage)				+		+
	Harpacticoida fam. gen. spp.	+	+	+	+	+	+
Nauplii of Cirripedia							
	Amphibalanus amphitrite (Darwin 1854)				+	+	+
	Amphibalanus improvisus (Darwin1854)				+	+	+
	Balanus eburneus Gould 1841				+	+	+
	Balanus perforatus Bruguiere 1789					+	
	Chthamalus stellatus (Poli 1791)				+	+	
	Euraphia depressa (Poli 1791)					+	
	Verruca stroemia (O.F.Muller 1776)					+	

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Annex 2 Figures and formulas for determination of the Black Sea protists and rotifers biovolume (Bryantseva & Kurilov, 2003)

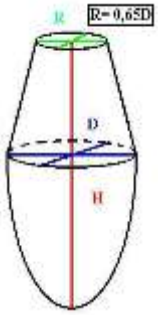
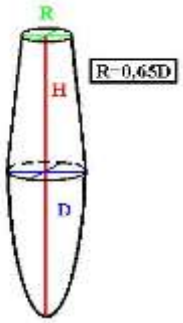
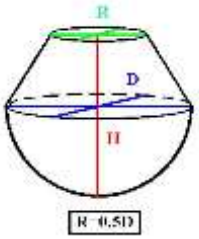
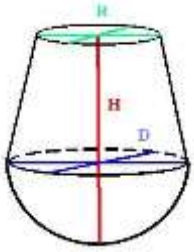
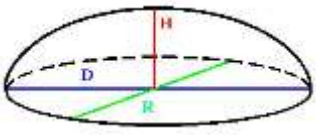
Figure	Formula	Examples of suitable species
	Truncated cone + 0,5 of round ellipsoid with equal heights $V = \frac{\pi H(1{,}5D^2 + 0{,}5DR + 0{,}5R^2)}{12}$ When R ≈ 0,65 D: $V = \frac{4{,}0725\,\pi HD^2}{24}$	Strombidinopsis spp. Balanion comatum Coleps spp. Rotifers
	Truncated cone + parabolic segment **with equal heights** $V = \frac{\pi H(1{,}25D^2 + 0{,}5DR + 0{,}5R^2)}{12}$ When R ≈ 0,65 D: $V = \frac{3{,}5725\,\pi HD^2}{24}$	Karyorelictid ciliates Lacrymaria spp. Rotifers
	Truncated cone + hemisphere When H ≈ 0,5 D; R ≈ 0,5 D $V = \frac{2{,}75\pi D^3}{12}$	Mesodinium spp. Askenasia spp. (Pelago-)Halteria spp. Dinoflagellates Rotifers
	Truncated cone + hemisphere $V = \frac{\pi(HD^2 + HDR + HR^2 + 0{,}5D^3 - 0{,}5D^2R - 0{,}5DR^2)}{12}$	Mesodinium spp. Rotifers
	Segment of ellipsoid $V = \frac{\pi H(4H^2 + 3DL)}{24}$	Euplotid ciliates

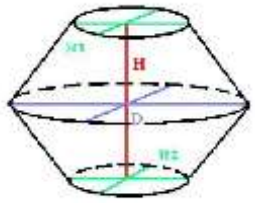
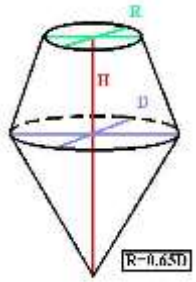
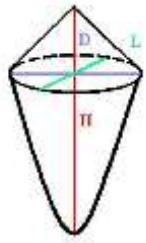
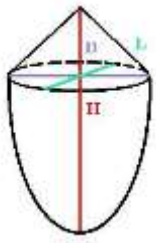
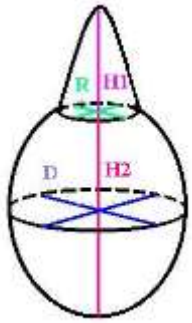
Figure	Formula	Examples of suitable species
	Two truncated cones $V = \frac{\pi H(D^2 + DR + R^2)}{12}$ When H ≈ D; R ≈ 0,5 H: $V = \frac{1{,}75\pi H^3}{12}$	Askenasia spp. Coleps spp. Dinoflagellates
	Cone + truncated cone with equal heights $V = \frac{\pi H(2D^2 + DR + R^2)}{24}$ When R ≈ 0,65 D: $V = \frac{3{,}0725\pi HD^2}{24}$	Tiarina fusus Rotifers
	Elliptic parabolic segment + elliptic cone When cone height ≈ 0,5 D: $V = \frac{\pi DL(1{,}5H - 0{,}25D)}{12}$ When cone height ≈ 0,5 L: $V = \frac{\pi DL(1{,}5H - 0{,}25L)}{12}$	Strombidiid ciliates *Loxophyllum* spp. Gyrodinium spp. Rotifers
	Elliptic cone + 0,5 elliptic ellipsoid When cone height ≈ 0,5 D: $V = \frac{\pi DL(2H - 0{,}5D)}{12}$ When cone height ≈ 0,5 L: $V = \frac{\pi DL(2H - 0{,}5L)}{12}$	Strombidiid ciliates Dinoflagellates Rotifers
	Parabolic segment + ellipsoid When H_1≈ 0,5 D; R ≈ 1/3 D: $V = \frac{\pi D^2(0{,}5D + 12H)}{72}$ or: $V = \frac{1{,}025\pi D^2 H}{6}$ When another combined:	Didiniid ciliates Tontonia spp. Rotifers

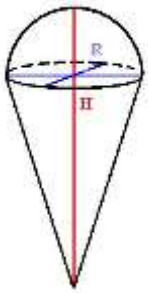
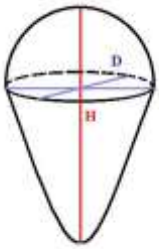
Figure	Formula	Examples of suitable species
	$V = \frac{\pi(2D^2H_2 + 1{,}5R^2H_1)}{12}$ 6)	
	Cone + hemisphere $V = \frac{\pi D^2(H + 0{,}5D)}{12}$	Strombidiid ciliates Rotifers
	Parabolic segment + hemisphere $V = \frac{\pi D^2(1{,}5H + 0{,}25D)}{12}$	Strombidiid ciliates Rotifers
	Cylinder + hemisphere $V = \frac{\pi D^2(6H - D)}{24}$	Tintinnid ciliates (lorica)
	Cylinder + 0,5 ellipsoid with equal heights $V = \frac{2{,}5\pi D^2 H}{12}$	Tintinnid ciliates (lorica)

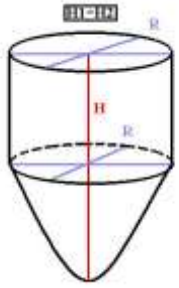
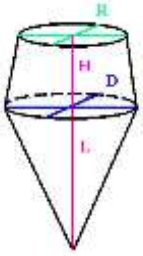
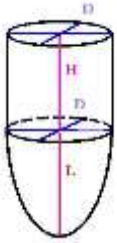
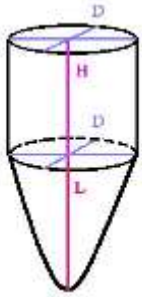
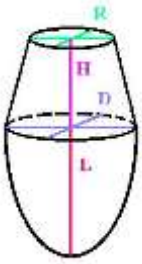
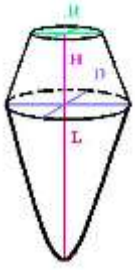
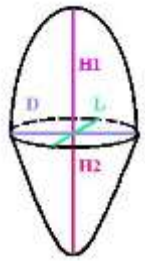
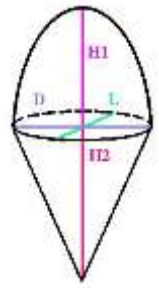
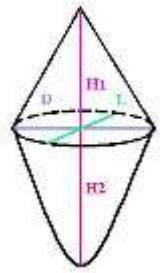
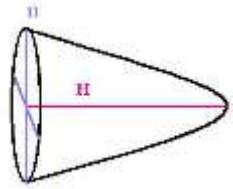
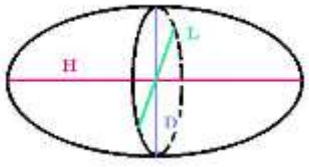
Figure	Formula	Examples of suitable species
	Cylinder + parabolic segment with equal heights $V = \frac{1{,}5\pi D^2 H}{8}$	Tintinnid ciliates (lorica) Rimostrombidium spp.
	Cone + truncated cone with unequal heights $V = \frac{\pi(HD^2 + HDR + HR^2 + D^2 L)}{12}$	Rimostrombidium spp.
	Cylinder + 0,5 ellipsoid with unequal heights $V = \frac{\pi D^2 (3H + 2L)}{12}$	Tintinnid ciliates (lorica) Rimostrombidium spp. Strombidinopsis spp.
	Cylinder + parabolic segment with unequal heights $V = \frac{\pi D^2 (2H + L)}{8}$	Tintinnid ciliates (lorica) Rimostrombidium spp. Strombidinopsis spp.
	Truncated cone + 0,5 ellipsoid **with unequal heights** $V = \frac{\pi(HD^2 + HDR + HR^2 + 2D^2 L)}{12}$	Tintinnid ciliates (lorica) Rimostrombidium spp. Strombidinopsis spp. Rotifers
	Truncated cone + parabolic segment with unequal heights $V = \frac{\pi(HD^2 + HDR + HR^2 + 1{,}5D^2 L)}{12}$	Tintinnid ciliates (lorica) Rimostrombidium spp. Strombidinopsis spp. Rotifers

Figure	Formula	Examples of suitable species
	Elliptic parabolic segment + 0,5 elliptic ellipsoid with unequal heights $V = \frac{\pi DL(2H_1 + 1{,}5H_2)}{12}$	Amphileptid ciliates Prostomatid ciliates Rotifers
	Elliptic cone + 0,5 elliptic ellipsoid with unequal heights $V = \frac{\pi DL(2H_1 + H_2)}{12}$	Strombidiids ciliates Amphileptid ciliates Rotifers
	Elliptic cone + elliptic parabolic segment with unequal heights $V = \frac{\pi DL(H_1 + 1{,}5H_2)}{12}$	Strombidiids ciliates Amphileptid ciliates Rotifers
	Parabolic segment $V = \frac{\pi D^2 H}{8}$	Rimostrombidium epacrum Rotifers
	Ellipsoid (sphere) $V = \frac{\pi DLH}{6}$	Different ciliates, flagellates and heliozoans Rotifers (mainly fixed)

Biomass (wet weight (WW), mg) of some rotifers also can easily be calculated using equations derived for certain rotifers genera:

Genus	Equation	Reference
Anuraeopsis	$0,030 \cdot L^3$	Sherstiyk, 1971; Ruttner-Kolisko, 1977
Ascomorpha	$0,120 \cdot L^3$	Sherstiyk, 1971; Ruttner-Kolisko, 1977
Asplanchna	$0,230 \cdot L^3$	Sherstiyk, 1971; Ruttner-Kolisko, 1977
Brachionus	$0,120 \cdot L^3$	Sherstiyk, 1971; Ruttner-Kolisko, 1977
Euchlanus	$0,100 \cdot L^3$	Sherstiyk, 1971; Ruttner-Kolisko, 1977
Filinia	$0,130 \cdot L^3$	Sherstiyk, 1971; Ruttner-Kolisko, 1977
Gastrops	$0,200 \cdot L^3$	Sherstiyk, 1971; Ruttner-Kolisko, 1977
Hexarthra	$0,130 \cdot L^3$	Sherstiyk, 1971; Ruttner-Kolisko, 1977
Kellikottia	$0,030 \cdot L^3$	Sherstiyk, 1971; Ruttner-Kolisko, 1977
Keratella quadrata	$0,220 \cdot L^3$	Sherstiyk, 1971; Ruttner-Kolisko, 1977
K. cochlearis	$0,020 \cdot L^3$	Sherstiyk, 1971; Ruttner-Kolisko, 1977
Notholca	$0,035 \cdot L^3$	Sherstiyk, 1971; Ruttner-Kolisko, 1977
Polyarthra	$0,280 \cdot L^3$	Sherstiyk, 1971; Ruttner-Kolisko, 1977
Synchaeta	$0,120 \cdot L^3$	Vinogradov & Shushkina, 1987
Testudinella	$0,080 \cdot L^3$	Sherstiyk, 1971; Ruttner-Kolisko, 1977
Trichocerca	$0,520 \cdot L \cdot D^2$	Sherstiyk, 1971; Ruttner-Kolisko, 1977

Where L – length of organisms, mm; **D** – diameter of organisms, mm;

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Annex 4 Indicators of environmental quality assessment using microzooplankton

This annex will be useful during field work since indicators for microzooplankton are still not fully developed because of the complexity and poor knowledge of the subject. For this reason only common indicators, usually applied in ecological investigations may be recommended here. Some of them were successfully implemented during study of planktonic ciliates in the North Western Black Sea and adjacent water bodies (Kurilov, 2010).

The main parameters obtained after samples treatments are abundance (ind. per m^3 of water), biomass (mg per m^3 water) and species composition. Thus, the following indices include these data. They should be divided on two categories: α-diversity (information from a sample) and β-diversity (information from series of samples).

α-diversity

Species richness (Margalef index, d):

$$D = \frac{S - 1}{log_2 N},$$

where S is number of species, N is abundance.

Species diversity (Shannon index):

$$H = -\sum_{i}^{S} \frac{N_i}{N} log_2 \left(\frac{N_i}{N} \right),$$

where N_i is abundance of species i, N is total abundance in the sample that consists of S species.

Species evenness (equitability), Pielou's index:

$$J' = \frac{H}{H_{max}} = \frac{H}{log_2 S},$$

where H is Shannon index, H_{max} is maximal value of Shannon index, which can be calculated at the available N and S.

Size structure. It can be easily calculated by dividing B by N. The most bacterivorous microzooplankters have small sizes, especially protists, so this index may be interpreted as level of bacterial pollution. Size structure could also be revealed well using **Meire-Dereu index** based on Warwick et al. (1987) abundance-biomass comparison method:

$$ABC = \sum_{i=1} \frac{B_i - N_i}{S},$$

where B_i and N_i are per cents of accumulation abundance and biomass of the i-order species, S – number of species. In this case index is reflecting correlation of size groups, which is convenient for comparative analysis. The averaged ABC indexes can serve for estimation of community position in *r* - *K* continuum that gives an opportunity to get an idea about changeability of its state in space and time.

Trophic structure. It is determined as a proportion of different trophic categories of microplankters in abundance or biomass and reflects food diversity and their abundance. However, identification of trophic status or preferred food requires some skills.

Taxonomic structure. R. M. Warwick, K. R. Clark (2001) proposed indices revealing community taxonomic structure: Average Taxonomic Distinctness (AvTD) and Variation of Taxonomic Distinctness (VarTD). However these indices require strong taxonomical skills, may be recommended to the experienced specialists only and are not considered here. See reference for more details.

β-diversity

To compare samples the most common indices can be recommended.

Bray-Curtis index (for quantitative samples**):**

$$I_{BC} = \frac{2 \cdot \sum_{i=1}^{n} min(y_{ij}, y_{ik})}{\sum_{i=1}^{n} (y_{ij} + y_{ik})} \cdot 100\%$$

where y_{ik} and y_{ij} are abundance/biomass *i*-species in the *k* and *j* samples, *n* is the total number of species. For qualitative samples the most common is **Czekanovsky-Sorrencen index:**

$$I_{Cz} = \frac{2a}{2a + b + c} \cdot 100\%$$

where a is the number of species common for compared samples, b and c are numbers of species found in one sample and absent from the other.

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Microzooplankton

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