Principles of collection and plankton sample handling from ballast tanks to assess the efficiency of ship ballast water treatment systems

Zh P Selifonova¹, P R Makarevich³, S I Kondratiev¹, E Z Samyshev², A L Boran-Keshishyan¹

¹Admiral Ushakov Maritime State University, 93 Lenin avenue, Novorossiysk, 353924 Russia
²A.O. Kovalevsky Institute of Biology of the Southern Seas, Russian Academy of Sciences, 2 Nakhimov avenue, Sevastopol, 299011, Russia
³Murmansk Marine Biological Institute Russian Academy of Sciences, 17 Vladimirskaya street, Murmansk, 183010, Russia

e-mail: Selifa@mail.ru

Abstract. Based on the material collected in 2004–2006 and 2009–2010 in the Novorossiysk Sea Port from the ships of the merchant fleet as well as the literature data, the collection and sample handling methods of phyto- and zooplankton from ballast tanks were analyzed. To assess the efficiency of the ballast water treatment systems (BWTS), there should be introduced correct definitions of the number, size and taxa of viable phyto- and zooplankton organisms in the IMO BWM documents. It is recommended to use closed Sorokin-type chambers of different sizes for express analysis (microscopy) of nanoplankton and microplankton on board a ship. To control compliance with the ballast water discharge standard, the mortality ratio of aquatic organisms should be 100% in the decontaminated ballast water. Effective decontamination of ballast water using UV irradiation is not possible unless BWTS is installed on the ballast water flow (draining) from the vessel. It is necessary to develop instructions for carrying out the procedure for obtaining ballast water samples for different types of ships and analyzing them for the facilities under the technical supervision of the Russian Maritime Register of Shipping. The aim of the study is to analyze the basic principles of collecting and sample handling of phyto- and zooplankton from the ballast tanks to control their compliance with the standard for ballast water discharge into the territorial waters of the Russian Federation.

1. Introduction
To combat biological invasions, the International Convention for the Control and Management of Ships’ Ballast Water and Sediments (BWM) was adopted in 2004, which entered into force on 8 September 2017 [1]. The Convention provides mandatory standards: Standard D-1: Ballast Water Exchange on the High Seas and Standard D-2: Ballast Water Quality. One of the requirements of the BWM Convention is to have a ballast water management system on board to ensure that such waters are treated so that pests in water discharged overboard do not exceed certain concentrations (Standard
D-2). The D-2 Standard is a performance indicator that defines the minimum number of viable organisms in the drained water. The subject of management is planktonic organisms, which are divided into two size groups: 1) ≥ 50 µm, 2) from 10 to 50 µm, and 3) three types of bacteria (<10 µm). The first group includes metazoan zooplankton, large cells of phytoplankton and ciliates (mesoplankton); the second group includes phytoplankton, ciliates, protozoa (microplankton). This rule applies to both counting the number of planktonic organisms of two size groups: 10 - 50 µm and over 50 µm and taxonomic composition (at least 5 species of three taxonomic types). According to the D-2 Standard, the number of organisms in the first size group should be <10 ind./m³, and in the second size group <10 cells/mL in the minimum size. The ballast water quality standard also includes certain indicators of microbes harmful to human health, such as toxigenic cholera vibrios O1 and O139 serogroups, Escherichia coli, intestinal enterococci. It is believed that the emergence of technological capabilities for the treatment of isolated ballast waters allows on special onboard equipment, a floating or stationary base to carry out treatment to a level corresponding to the Standard D-2 of the International Maritime Organization [2].

Currently, all ships under construction must comply with the D-2 Standard, while the vessels already in operation must comply with the D-1 Standard. For new ships (keel laid on or after 8 September 2017), Ballast Water Treatment Systems (BWTS) are required prior to the ship’s commissioning. For non-MARPOL vessels, the BWM D-2 compliance deadline is determined by the Administration, but no later than 8 September 2024. For most vessels this means the installation of special avionics. BWTS must be approved by national authorities in accordance with regulations developed by IMO.

The authorized organization for the survey of ships for compliance with the BWM Convention [1] is the Russian Maritime Register of Shipping (RS). In order to comply with the provisions of the Convention, RS has issued appropriate guidance [3]. Currently, four laboratories located in the cities of Riga, Odessa, Rostov-on-Don, Novorossiysk have the technical competence in accordance with the RS requirements for testing BWTS in accordance with IMO Resolution MEPC. 173 (58) Guidelines for Taking Ballast Samples [3]. However, there is still no approved national methodology for collecting and sample handling of phyto- and zooplankton from ship ballast tanks in the ports of the Russian Federation.

The aim of the study is to analyze the basic principles of collecting and sample handling of phyto- and zooplankton from the ballast tanks to control their compliance with the standard for discharge of ballast water into the territorial waters of the Russian Federation.

2. Results and discussion

2.1 Plankton sample handling

In case of non-compliance with the requirements of the Convention, the express analysis of phyto- and zooplankton on board the ship allows the port state control officers to stop the discharge of ballast water. Several sampling and processing techniques have been developed for hydrobiological analysis to assess the diversity and abundance of phyto- and zooplankton organisms in ballast water. The main ones among them are microscopy and flow cytometry [4, 5]. However, these methods are still not considered adequate for testing the effectiveness of ballast water treatment systems and monitoring compliance with the IMO standard for the discharge of ballast water into ports and bays. Microscopy and flow cytometry have their own advantages and disadvantages. It is believed that microscopy is more suitable for detailed taxonomic analysis of large planktonic organisms (magnification ×40–×100) ≥50 µm in size, and flow cytometry is more suitable for rapid quantitative analysis of small organisms (magnification ×200–400) in a size range 2–50 µm [6]. To increase the abundance, metazoan zooplankton (multicellular zooplankton) (≥50 µm) can be concentrated using plankton nets, which reduces the counting error. Protozoa (10–50 µm) are more difficult to concentrate and adequately count. Flow cytometers are expensive, cumbersome, difficult to operate, and, above all, do not provide visual verification of enumerated organisms [5]. Dead organic particles (detritus) that have a fluorescent glow with such a device can be mistakenly attributed to living organisms [7]. Colonial
cells (<10 µm) are identified by the flocytometer as a single organism of a larger size group (10–50 µm). Another disadvantage of a flow cytometer is the use of special dyes to distinguish dead cells from living cells. Floating microheterotrophs and plant cells are certainly alive, but not all immobile protists should be considered dead. Plant cells can be in a cyst state, some of which are potentially toxic.

Potentially toxic algae, being both in active and in cystic state in ballast water of tanks, can retain viability even after treatment of ballast water with UV irradiation [8]. Therefore, the effectiveness of BWTS in decontaminating ballast water using UV irradiation depends on the location of the plant. Effective decontamination of ballast water using UV irradiation is not possible without installing the BWTS on the ballast water flow (drain) from the vessel. It is well known that even if a small amount (<10 ind./m³ or <10 cells/mL in the minimum size) of potentially toxic phytoplankton species (phytoplankton) enters a habitat with optimal living conditions, the invasive species can be introduced and naturalized into the marine ecosystem of the recipient port.

In the port waters, with appropriate biogenic stimulation of cyst germination, “ecological explosions” in the number of harmful non-native phytoplankton and ciliates reaching the level of “red tides” have been repeatedly observed [9, 10]. Therefore, in the disinfected ballast water there should not be a single living organism of phyto- and zooplankton. In this regard, the accuracy of determination of the number of viable organisms of phyto- and zooplankton, stated in the Convention [1], raises doubts. According to Standard D-2, the number of organisms in the first size group should be <10 ind./m³, and in the second size group <10 cells/mL in the minimum size. The boundaries described in Standard D-2 for counting the number of planktonic organisms of two size groups (≥ 50 µm and 10–50 µm) and their taxonomic composition are conditional and cannot be considered proved either ecologically or physiologically [11]. It is of serious concern that the Standard D-2 does not include marine protists ranging in size from 2 to 10 microns, which may include potentially hazardous species.

In 2004–2006 and 2009–2010 at the oil terminals of the Novorossiysk Sea Port (berth 5, the Sheskharis Oil Harbor) by the Murmansk Marine Biological Institute of the Russian Academy of Sciences and the State Marine University named after Admiral F.F. Ushakov, studies of the environment and population of ballast waters of commercial ships were carried out [12, 13]. The study of phyto- and zooplankton (381 samples) in the ballast waters of commercial ships was carried out using an original method developed by Professor Zh.P. Selifonova. Plankton size groups were classified in the following way: nanoplankton are organisms of 2–20 µm (zooflafellates, ciliates, phytoplankton and other protozoa); microplankton are organisms of 20–200 µm (phytoplankton, ciliates, rotifers, amoeba, radiolarians, pelagic larval stages of bottom invertebrates, meroplankton, larvae of copepods; mesoplankton are organisms with the size of > 200-500 µm (crustacean, benthic larvae, rotifers, meroplankton, appendicularia, Parasagitta, ctenophores and other organisms).

Nanoplankton. The method of epifluorescence microscopy is successfully used for the quantitative registration and identification of this group of plankton in the coastal laboratory. The technique of performing this procedure in the field is laborious and rather complicated without special training. On board the ship, it is more convenient to view nanoplankton in closed Sorokin chambers with a volume of 1 to 5 ml (chamber depth is 1.5 mm, chamber dimensions are 23 × 60 mm) [14] (Fig. 1). Cameras are made of plexiglass. The sample is mixed gently after which the chamber is filled so that no air bubbles remain. Nanoplankton are counted in 10–20 fields of view of a phase-contrast microscope with a magnification of ×100×200. At the same time, the size of the organisms is determined using an eyepiece ruler. When working with oligotrophic ballast waters, view half or all of the chamber volume. To facilitate viewing, longitudinal lines are applied to the bottom of the chamber with a corundum needle, the distance between which is close to the size of the microscope field of view. The use of the Sorokin chamber ensures the immobility of the liquid in it during the rocking of the vessel, which makes it possible to microscop the samples in the ship’s conditions.
Figure 1. Sorokin’s counting chamber: A – small chamber for counting phytoplankton and nanoheterotroph organisms (23×60 mm, depth is 1.5 mm); B – medium and large chambers for counting microplankton ciliates (60×90 mm, depth is 3.5 mm) and metazoic microplankton (80×120 mm, depth is 5 mm). a - movable cover, b - fixed bottom, c – plexiglass frame, d – water sample, f – internal partitions of the chamber is 0.5–1 mm thick [15].

Microplankton. Protozoal microplankton are viewed in a 10–20 ml middle chamber (60×90 mm, 3.5 mm deep), metazoan microplankton are vied in large 30 ml chambers with longitudinal partitions spaced 1 cm apart (80×120 mm, 5 mm deep). Organisms are counted in 10–20 fields of view of a stereomicroscope with a magnification of ×40–×50.

Mesoplankton is scanned in Bogorov’s chamber. Bogorov’s modified counting chamber makes it possible to more accurately analyze larger organisms on board while the ship is moving or rolling [4]. Organisms are viewed in 10–20 fields of view of a stereomicroscope with a magnification of ×16–×32 and ×40–×50. The indices of the number of planktonic organisms obtained by counting in the chamber are converted into ind./m³. After three chamber counts, the average value is calculated from one sample, and this value is recalculated over the entire sample volume. Under a microscope, the state of planktonic algae and zooplanktonic organisms is noted (destruction of cells, colonies, loss of flagella, membranes, mobility, wrinkling or twisting of dead rotifers or larvae of polychaete worms, etc.).

2.2 Plankton sampling
The experience has shown that the sampling ships’ ballast water is challenging. On most ships with integral sampling devices, sampling should be done in an engine room with the limited space. To ensure a representative sample of organisms in ballast water, samples should be taken during the ballast water draining from different ballast tanks. The organisms in the ballast tank can be unevenly distributed and the concentration of organisms in the discharged ballast water can vary greatly. Therefore, in each ballast tank, samples of nano- and microplankton should be collected from two horizons (surface, bottom) in triplicate. When pumps are used, the suction pipes of the pump must be placed to different depths of the ballast tank, and the volume of water samples taken should be measured using flow meters installed in the hose. For adequate accounting, microplankton can be concentrated using a filter cup or plankton net with a mesh size of 40–55 μm. To avoid biological pollution of the coastal waters of the recipient port, the filtered non-native ballast water should be pumped back into the ballast tank. If the BWTS performance check is carried out on native seawater (immediately after the ship is built or at the construction site), the ballast water from the bottom layers can be taken from the vessel’s drainage hole at a low rate of water discharge into the sea.

Mesoplankton samples are collected by vertical total catches with a small-sized Juday net (inlet diameter is 12 cm, mesh size is 40–55 μm) at the maximum accessible depth. If, when taking samples from ballast tanks, ladders and platforms prevent the lowering of fishing gear to the entire depth of the tank, ballast water samples are taken by means of pumps with flow meters and a filter cup (mesh size 40–55 μm). For the sampling of mesoplankton, the sampling device developed by the Hydrobios
Company has proven itself well in practice [4]. This device consists of a flexible cone (bag) (prevents the destruction of organisms) with an integrated flow meter and a screw-down filter can. The device passes up to 2.5 tons of ballast water in 30 minutes and provides a representative sample of a higher density of aquatic organisms when unloading ballast water from a ship. For adequate accounting of phyto- and zooplankton organisms, according to the IMO convention, at least 1000 liters of ballast water should be taken (filtered) on board the ship.

3. Conclusion

Nowadays there is not a single perfect method for the qualitative and quantitative analysis of all groups of organisms when assessing the performance of BWTS, considered by the IMO ballast water quality standard.

For the express analysis of phyto- and zooplankton in the ballast water of merchant ships, carried out by the port state control officers on board, there is no need for a detailed assessment of the taxonomic composition, determination of morphometry, size and counting of the number of aquatic organisms of different taxonomic groups, as required by the IMO convention. Therefore, the IMO documents [1] should include accurate definitions of the number, size and taxa of viable phyto- and zooplankton organisms. In disinfected ballast water there should be 100% mortality of aquatic organisms. Standard D-2 of the Convention should include marine organisms ranging in size from 2 to 10 microns, among which potentially dangerous species may be found. For effective decontamination of ballast water using UV irradiation, it is necessary to additionally install the BWTS on the ballast water flow from the vessel.

All efforts of hydrobiological researchers should be aimed at solving the issues of adequate sampling with a representative sample and rapid determination of the viability of plankton organisms in ballast water. On board the ship for the express analysis of phyto- and zooplankton samples from the ballast water (microscopy), it is recommended to use closed Sorokin-type chambers of different sizes – 1–5 ml (nanoplankton and protozoa), 10–20 ml (protozoan microplankton) and 30 ml (metazoic plankton). It is necessary to develop instructions for carrying out the procedure for taking ballast water samples for different types of ships and analyzing them for facilities under the technical supervision of the Russian Maritime Register of Shipping.

Acknowledgments

The reported study was supported by the Russian Academy of Sciences (No. AAAA-A18-118021490093-4 A.O. Kovalevsky Institute of Biology of the Southern Seas of Russian Academy of Sciences on the topic Functional, Metabolic and Toxicological Aspects of Hydrobionts Existence and their Populations in Biotopes with Different Physical and Chemical Condition) and according to the research project of Murmansk Marine Biological Institute, Russian Academy of Science.

References

[1] IMO 2004 International Convention for the Control and Management of Ships’ Ballast Water and SedimentsBWM/CONF/36 IMO pp 1–120
[2] IMO 2016 Guidelines for approval of ballast water management systems (G8) MEPC 279(70) (London) pp 1–40
[3] RS 2017 Guidance on the application of the requirements of the 2004 International Convention for the Control and Management of Ships’ Ballast Water and Sediments. Russian Maritime Register of Shipping (SPb) pp 1–61
[4] Gollasch S 2006 A new ballast water sampling device for sampling organisms above 50 micron Aquatic Invasions 1(1) 46–50
[5] Peperzaka L, Zetschea E-M, Gollasch S, Artigas L F, Bonato S et al 2020 Comparing flow cytometry and microscopy in the quantification of vital aquatic organisms in ballast water J. Mar. Eng. & Technology 19(2) 68–77
[6] Zetsche E, Meysman F J R 2012 Dead or alive? Viability assessment of micro- and
mesoplankton *J. Plankton Res.* **34**(6) 493–509

[7] Tang Y Z, Dobbs F C 2007 Green autofluorescence in dinoflagellates, diatoms, and other microalgae and its implications for vital staining and morphological studies *Appl. Env. Microbiol.* 732306–2313

[8] Fokanov V P, Gavrilova O V, Shallar A V 2017 Study of the effectiveness of UV irradiation of unicellular organisms carried with the ballast water of ships *Rus. J. Biol. Invasions* **3** 113–121

[9] Sorokin Yu I 2002 *The Black Sea. Ecology and Oceanography* (Backhuys Leiden) pp 391–542

[10] Orlova T Yu 2005 Red tides and toxic microalgae in the Far Eastern seas of Russia *Bull. of Far Eastern Branch of RAS* **1** 27–31

[11] Silkin V A, Pautova L A, Fedorov A V, Shitikov E I, Drozdov V V, Lukasheva T A, Zasko D A 2018 Formation of artificial communities for testing ballast water management systems in accordance with the requirements of the International Maritime Organization *Rus. J. Biol. Invasions* **11**(1) 114–129

[12] Selifonova Zh P 2009 Marine bioinvasions in the waters of the Novorossiysk port of the Black Sea *Rus. J. Marine Biology* **35**(3) 212–219

[13] Zvyagintsev A Yu, Selifonova Zh P 2010 Hydrobiological studies of ballast water of commercial ships in the ports of Novorossiysk and Vladivostok *Oceanology* **50**(6) 925–933

[14] Sorokin Yu I 1999 *Aquatic microbial ecology* (Leiden Backhuys) pp 210–211

[15] Sorokin Y I 1980 Camera for quantitative registration of protozoa and nanoplanckton organisms in the field *Hydrobiol. J.* **16**(6) 84–86