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Original article

Efficacy of Ballast Water Treatment Systems Installed Onboard Ships Entering the Seaport of Novorossiysk, the Black Sea

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Abstract

The paper aims to assess the quality of ballast water purification of phyto- and microzooplankton using various ballast-water treatment systems. The analysis of treatment systems performance was based on the results of the study of phyto- and microzooplankton taxonomic composition and abundance in 19 samples of ballast water treatment after their treatment in the ships' systems. The samples were taken onboard 12 oil tankers and 7 bulk carriers originating from the ports representing the Mediterranean basin, tropical West Africa and the NW Indian Ocean. The vessels entered the seaport of Novorossiysk for cargo loading from October 2022 to March 2023. In 90% of all cases of the systems use, the ballast water purification of unicellular organisms met the Regulation D-2 Ballast Water Performance Standard of the International Convention for the Control and Management of Ships' Ballast Water and Sediments. The ballast of 10% of the vessels (from Turkish ports in the Marmara and Aegean Seas) equipped with DESMI CompactClean CC-500 (treatment by filtration + UV) and Pureballast 3.2 1500 EX (treatment by UV system) did not meet the cleaning quality standard: 1.19×10^6 and 1.21×10^4 cells/L, respectively, were detected after treatment. The ballast waters of vessels from the Gulf of Suez and Mauritania represented a moderate risk in terms of cell abundance (7.16×10^3 and 2.03×10^3 cells/L, respectively). In total, 20 microalgal species were found: diatoms (13), dinoflagellates (6), a silicoflagellate (1), several algal taxa not identified to species, as well as ciliates. *Proboscia alata* and *Prorocentrum micans* were the most frequent. No planktonic algae classified as invasive to the Black Sea were found.

Keywords: ballast water, marine ballast, seaport of Novorossiysk, ballast water systems, taxonomic composition, phytoplankton, Black Sea, anthropogenic pollution, biological invasion, invasive species

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Эффективность применения систем обработки балластных вод на судах, заходящих в морской порт Новороссийск, Черное море

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Аннотация

Цель работы – оценить качество очистки судового балласта от фито- и микрозоопланктона с помощью различных систем обработки балластных вод. В основу анализа эффективности систем очистки легли результаты исследования таксономического состава и численности фито- и микрозоопланктона в 19 пробах балластных вод после их обработки в судовых системах. Отбор проб морского балласта был осуществлен на борту 12 нефтяных танкеров и семи сухогрузов, прибывших из портов стран Средиземноморского бассейна, Тропической Западной Африки и северо-западной части Индийского океана и заходивших под погрузку в морской порт Новороссийск в октябре 2022 г. – марте 2023 г. Исследования показали, что в 90 % всех случаев использования установок результат очистки балластных вод от одноклеточных организмов удовлетворял стандарту *D*-2 Международной конвенции о контроле судовых балластных вод и осадков и управления ими. Балласт 10 % исследованных судов (из портов Турции в Мраморном и Эгейских морях), оснащенных системами *DESMI CompactClean CC*-500 (способ очистки: фильтрация + обработка ультрафиолетом) и *Pureballast* 3.2 1500 *EX* (способ очистки: обработка ультрафиолетом), не соответствовал стандарту качества очистки. После обработки численность одноклеточных водорослей в балласте составляла $1.19 \cdot 10^6$ и $1.21 \cdot 10^4$ кл./л соответственно. Балластные воды судов из Суэцкого залива и Мавритании представляли собой умеренную угрозу/опасность для окружающей среды: численность микроводорослей составляла $7.16 \cdot 10^3$ и $2.03 \cdot 10^3$ кл./л соответственно. Всего обнаружено 20 видов микроводорослей: 13 диатомовых, 6 динофлагеллят, 1 силикофлагеллят и несколько не идентифицированных до вида таксонов водорослей, а также инфузории. Наиболее часто встречались *Proboscia alata* и *Prorocentrum micans*. Видов планктонных водорослей, классифицируемых как вселенцы в Черное море, в балласте обнаружено не было.

Ключевые слова: балластные воды, морской балласт, порт Новороссийск, системы обработки балласта, таксономический состав, фитопланктон, Черное море, антропогенное загрязнение, биологические инвазии, виды-вселенцы

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Introduction

Biological pollution is one of the most important problems of anthropogenic influences on the ecosystems of the World Ocean. Every day, on a planetary scale, vessels carry from 3000 to 4000 species of organisms [1, 2]. The involuntary and uncontrolled transfer of microalgae and their cysts in ships' ballast water began in the 1870s. Due to the rapid development of metallurgy, wooden vessel hulls were replaced by metal ones, and instead of stones, gravel or sand, sea water began to be used as ballast [3].

The current composition of the Black Sea flora and fauna was formed under the influence of the fresh waters of the Sea of Azov and large European rivers on the one hand and the Mediterranean waters on the other. Therefore, it is of a mixed nature and includes both freshwater and marine species.

Natural migration of species from the Mediterranean Basin through the Bosphorus and Dardanelles straits to the Black Sea and their distribution in the sea under the influence of currents have always existed since the formation of the Bosphorus Strait (presumably, 8–10 thousand years ago [4]) and still exist today.

Despite the fact that the salinity does not exceed 18 in the surface layer, the sea has low "biological immunity" against invasive species due to a significant proportion of relict and endemic species [1)]. Over the last half century, more than 200 species of flora and fauna new to this region, arriving from other areas of

[1)] Zaitsev, Y.P., 2006. *An Introduction on the Black Sea Ecology*. Odessa: Even, 224 p. (in Russian).

the World Ocean, have been found in the Black Sea, while about 150 Mediterranean species have successfully adapted to new conditions [5, 6]. By the beginning of the 20th century, more than 40 invasive species had become common inhabitants of the Black Sea and the Sea of Azov [7]. It is predicted that the rate of invasion of new species into the Black Sea will increase (up to two species per year). This is generally caused by the increase in shipping intensity and disruption of ecosystem stability due to eutrophication [8, 9].

Not every invasion of an alien organism results in tangible environmental and economic consequences, but some cases have been recorded. Thus, the invasion of the North American ctenophore *Mnemiopsis leidyi* A. Agassiz (Ctenophora: Tentaculata: Bolinopsidae) into the Black Sea in the early 1980s led to a decrease in the numbers of the European anchovy *Engraulis encrasicolus* (L.) (Clupeiformes: Engraulidae) and other commercial fish species. Consequently, economic losses amounted to US$240 million per year [2)].

Most phytoplankton cells do not survive in dark ballast tanks. However, resting stages of planktonic diatom and dinoflagellate species were found to be viable even after being transported in sediments at the bottom of ballast tanks for six months at 4 °C [10]. A microalgal study of 343 vessels entering 18 Australian ports found that 65% of the vessels carried significant amounts of sediments in their tanks [11]. Dinoflagellates account for the vast majority of toxic species compared to other marine microalgae, and almost all toxic dinoflagellate species are capable of photosynthesis.

In 2004, to reduce the environmental, epidemiological and other stresses on the aquatic environment caused by untreated ballast water discharge, the International Maritime Organization (IMO) adopted the International Convention for the Control and Management of Ships' Ballast Water and Sediments [3)]. The Convention includes five standard ballast water treatment procedures. The first, most reliable method of preventing the introduction of unwanted invasive species is the complete exclusion of ballast water discharge in the port water area. The remaining four methods involve treating ballast water to minimize the risk of discharge of unwanted organisms. From practical experience, they are all far from perfect [4)] [8, 12–15]. The second method includes the reduction of the marine organism

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[2)] Zaitsev, Y. and Öztürk, B., 2001. *Exotic Species in the Aegean, Marmara, Black, Azov and Caspian Seas*. Istanbul: Turkish Marine Research Foundation, 267 p.

[3)] IMO, 2004. *2004 International Convention for the Control and Management of Ships' Ballast Water and Sediments*. London: International Maritime Organization, 28 p.

[4)] Kudyukin, A.A., 2003. [Ballast Water Treatment in Shipboard Conditions: World Experience, Technological Approaches. Expert Evaluation of Proposals of National Manufacturers. First Results, Conclusions]. In: Global Ballast Water Management Program, 2003. [*The 4th Scientific-Practical Seminar on the Problem of Ship Ballast Water Management (for Specialists of Scientific Institutions Related to the Problem of Shipping, Marine Biology, Ecology and Environmental Protection), Odessa, Ukraine, 26–27 August 2003: Workshop Report*]. Odessa, pp. 19–23 (in Russian).

concentrations in the ballast water loaded by the vessel, by limiting the amount of water, selecting receiving sites, etc. The third method is coastal ballast treatment. The fourth and most widely used method is ballast change in open sea or ocean water (regulation D-1). The fifth, most effective, method involves ballast water treatment onboard the vessel (regulation D-2). This is a ballast water quality standard that requires vessels to install a ballast water treatment system (BWTS) onboard. BWTSs must discharge into the marine environment fewer than 10 viable organisms ≥ 50 μm in length per cubic metre and fewer than 10 viable organisms 10–50 μm in length per milliliter. By 2010, about 60 BWTSs were known, and new ones appear every year [15].

IMO developed several technological methods for this process, which can be divided into four groups [5)] [16]: 1) physical (heating, ultrasonic and ultraviolet treatment, silver ionization, etc.); 2) mechanical (filtration); 3) chemical (ozonation, deoxygenation, chlorination, use of bioreagents, etc.); 4) biological (adding predatory or parasitic organisms to ballast water to destroy unwanted invasive species).

The results of the study of various ballast water treatment methods revealed almost no sufficiently effective and economical ones [17].

To minimize damage from biological pollution, IMO required all merchant vessels to comply with regulation D-1 (full ballast water exchange or three sequential pumpings of ballast water) in the area of the recipient water body. However, the Convention stipulates that vessels built in 2017 and later must comply with regulation D-2. According to the binding regulations for the seaport of Novorossiysk, discharge of ballast is allowed subject to compliance with regulations D-1 and D-2.

In 2008, IMO developed and published Guidelines for approval of ballast water management systems (MEPC 2008). These Guidelines define the minimum BWTS technical specifications and technical documentation requirements. Furthermore, they define a manner of testing and targeted results of analysis of ballast water samples. Special attention is paid to the size and concentration of living organisms, including some types of bacteria [6)].

Long-term (2004–2019) monitoring studies of the marine environment conducted in the water areas of the large Russian commercial ports and resort cities, as well as in the open areas of the northeastern Black Sea, showed that in recent decades new invasive species continued to appear there despite the application of regulations D-1 and D-2 [5, 18–20]. It should be noted that some caused significant economic damage, as was the case with the emergence of the ctenophore *Mnemiopsis leidyi.*

[5)] Tamelander, J., Riddering, L., Haag, F. and Matheickal, J., 2010. *Guidelines for Development of a National Ballast Water Management Strategy*. London; Gland: GEF-UNDP-IMO GloBallast, 43 p.

[6)] MEPC, 2008. *Resolution MEPC.174(58). Guidelines for Approval of Ballast Water Management Systems (G8)*. 28 p. MEPC 58/23, Annex 4.

The literature covers the results of studies of phyto- and zooplankton in ballast water for the regulation D-1 efficacy evaluation. At the same time, there are fewer publications on the results of applying regulation D-2 in practice, and they mainly concern microbiological studies [23]. No information was published on the efficacy of long-term practical use of ballast water treatment systems for minimizing the concentration of plant and animal planktonic organisms in them. The aim of this paper is to assess the quality of ballast water treatment of phyto- and microzooplankton of the BWTSs on the vessels that entered the seaport of Novorossiysk in 2022–2023.

Materials and methods

Nineteen ballast water samples that underwent the treatment procedures of BWTSs were taken by inspectors of the Federal State Budgetary Institution "Administration of Seaports of the Black Sea" using a ship's cylindrical metal 1 liter sampler through ballast holes onboard 19 vessels (12 oil tankers and 7 bulk carriers) that entered the seaport of Novorossiysk for cargo loading from October 2022 to March 2023 (Table 1). The vessels loaded ballast in the ports of the following countries (Fig. 1): Romania (the Black Sea, 1 vessel), Turkey (8 vessels), Greece (1 vessel), Italy (1) and Tunisia (1) (the Mediterranean countries), Mauritania (1) (tropical West Africa), Egypt (5 vessels) (the Gulf of Suez, the Red Sea, the Indian Ocean) and Iran (1 vessel) (the Persian Gulf, the Indian Ocean). Marine ballast samples were fixed with neutral formaldehyde to a final concentration of 1–2% [7)] and concentrated in a land-based laboratory by sedimentation in cylinders with a diameter of 5.3 cm and a height of 36 cm for 2–3 weeks. Cell counts of phytoplankton were carried out using a MIKMED-2 microscope (LOMO, St. Petersburg, Russia), applying the bright-field technique in transmitted light using the 10×/0.30 and 40×/0.65 achromatic objectives produced by LOMO (St. Petersburg, Russia) in a 0.05 mL Nageotte counting chamber. To count rare and large species of phytoplankton and microzooplankton, an aliquot of the concentrate (1/2–1/10) and the entire sample were examined in a 1 mL Sedgwick–Rafter chamber. The minimum size of the cells taken into account was 3–5 μm. Phytoplankton abundance was calculated in accordance with the following formula:

$$N = \frac{V_2}{V_1}\frac{n}{V_3},$$

where V_1 – filtered water volume, mL; V_2 – concentrate volume, mL; V_3 – counting chamber volume, mL; n – number of cells in the counting chamber. The taxonomic affiliation of organisms was determined according to generally

[7)] Makarevich, P.R. and Druzhkov, N.V., 1989. [*Guidelines for the Analysis of Quantitative and Functional Characteristics of Marine Biocenoses of the Northern Seas. Part 1. Phytoplankton. Zooplankton. Suspended Organic Matter*]. Apatity: KNTs RAN, MMBI, 50 p. (in Russian).

T a b l e 1. Characteristics of the surveyed vessels entering the seaport of Novorossiysk for loading in 2022-2023, the ballast water systems and the phyto- and zooplankton abundance after the ballast water treatment

Vessel number	Sampling date	Port of ballast water loading	Vessel name and type, flag	Type of BWTS	Ballast volume, m^3	BWTS treatment method	Total abundance of organisms, cells/L
1	19.10.2022	Suez, Egypt	BEKS FENIX, oil product carrier, Marshall Islands	HMT-1500-EX	17 152	Electrocatalysis	N/D
2	22.10.2022	Iskenderun, Turkey	MV POSEIDONS, bulk carrier, Liberia	HMT-800	12 714	Electrocatalysis	N/D
3	23.10.2022	Agioi Theodoroi, Greece	MT PHOENIX AN, oil tanker, Malta	Hiballast BWMS-HUB-1000-EX	14 025	Electrochlorination + Neutralization	N/D
4	28.10.2022	Damietta, Egypt	MV CLEAR SKY, bulk carrier, Panama	BalClor BC-1000	17 359	Electrolysis + Filtration	21
5	31.10.2022	Tuzla, Turkey	GEORGY MASLOV, crude oil tanker, Liberia	NK-03-Blue Ballast II Plus	37 998	Ozone Injection + Neutralization	4

Continued Table 1

Vessel number	Sampling date	Port of ballast water loading	Vessel name and type, flag	Type of BWTS	Ballast volume, m^3	BWTS treatment method	Total abundance of organisms, cells/L
6	31.10.2022	Suez, Egypt	CALIPSO, bulk carrier, Liberia	BalClor BC-2000	19 994	Electrolysis + Filtration	N/D
7	31.10.2022	Constanta, Romania	ELANDA OSPREY, oil tanker, Liberia	HiBallast TM System HIB-2000-EX	44 764	Electrolysis + Filtration	16
8	12.11.2022	Tutunciflik, Turkey	MARINER A, oil-chemical Tanker, Malta	HiBallast NF System	16 651	Electrolysis + Filtration	8
9	09.12.2022	Ain Sokhna, Egypt	IKARA, crude oil tanker, Panama	Ecochlor Series 200	46 801	Chlorine system + Filtration	N/D
10	11.12.2022	Искендерун, Турция / Iskenderun, Turkey	VIVA ECLIPSE, bulk carrier, Panama	Erma First FIT 800	13 973	Electrolysis+ Filtration	27
11	14.12.2022	La Skhirra, Tunisia	HISTRIA PERLA, oil-chemical tanker, Malta	Pure Ballast 3:2	16 773	Filter + UV treatment	6

Continued Table 1

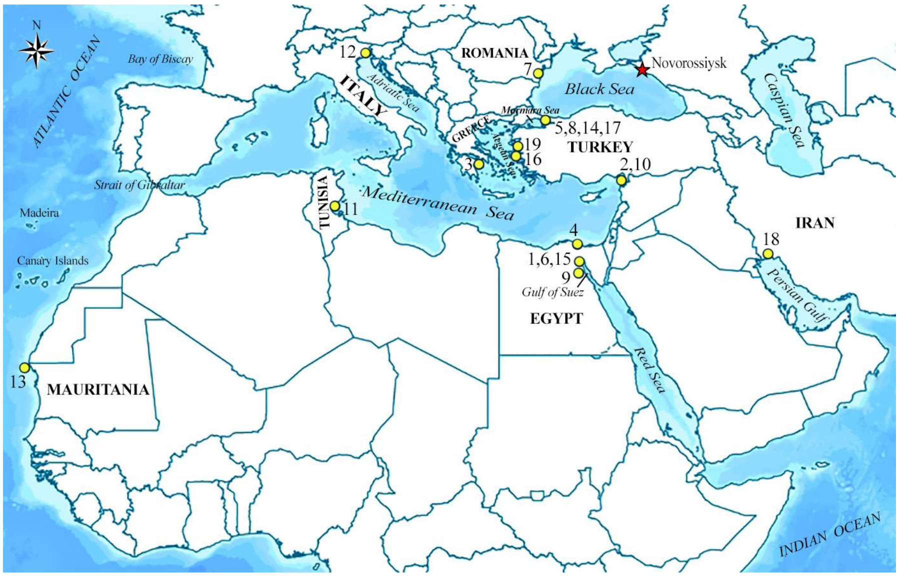
Vessel number	Sampling date	Port of ballast water loading	Vessel name and type, flag	Type of BWTS	Ballast volume, m^3	BWTS treatment method	Total abundance of organisms, cells/L
12	16.12.2022	Porto Monfal-cone, Italy	YASAR KEMAL, bulk carrier, Panama	Blue Ocean Shield BOS 300	11 390	Filter + UV treatment	6
13	15.01.2023	Nouadhibou, Mauritania	SEA HELIOS, oil tanker, Malta	Gloen-1200 Patrol	18 840	Filter + UV treatment	2034
14	26.02.2022	Tuzla, Turkey	NISSOS PAROS, oil tanker, Greece	Ex-Els-3000B 1:1	36 204	Electrolysis + electrochlorination	368
15	28.02.2023	Suez, Egypt	EUROSTRENGTH, oil tanker, Liberia	Erma First BWTS FIT-3000	34 400	Electrolysis + Filtration	7163
16	03.03.2023	Izmir, Turkey	SEA PEARL J, bulk carrier, Barbados	DESMI Compact Clean CC-500	11 332	Filtration + UV treatment	1 190 862

End of Table 1

Vessel number	Sampling date	Port of ballast water loading	Vessel name and type, flag	Type of BWTS	Ballast volume, m^3	BWTS treatment method	Total abundance of organisms, cells/L
17	14.03.2023	Tuzla, Turkey	MRC BELIZ, oil chemical tanker, Malta	Pureballast 3.2 1500 EX	23 202	UV System	12 057
18	27.03.2023	Port of BANDAR IMAM KHOMEINI (BIK), Iran	MV LEGENDI, балкер, Либерия / MV LEGENDI, bulk carrier, Liberia	Electro-Cleen System ECS-1350B	18 397	Electrolysis + Neutralization	9
19	31.03.2023	Aliaga, Turkey	TAHITI, oil carrier, Malta	Ecochlor Inc./Et-5000-4.0 Series 200	45 153	Chlorine system + Filtration	N/D

Note: Information obtained from the Ballast Water Reporting Form (Resolution A.868(20).

N/D – not determined.



F i g. 1. The routes of the ballast water transport in 2022–2023 onboard the surveyed ships from the ports of origin (yellow circles) to the port of destination (Novorossiysk, Russia, the Black Sea; marked with a red star). The examined vessels are indicated on the map by Arabic numerals at the location of their ports of origin (see Table 1)

accepted guidelines [8), 9)]. Intact algae cells with brightly colored chloroplasts were considered viable. Whole animal organisms that were accidentally included in the samples without visible destruction were also taken into account.

Results

Twenty species of planktonic algae belonging to four major taxonomic categories were found in the samples of the surveyed ships' ballast: Bacillariophyceae (diatoms), Dinoflagellata (dinoflagellates), Dictyochophyceae (silicoflagellates) and Euglenophyceae (euglenids) (Table 2, Fig. 2). Diatoms (13 species) and dinoflagellates (6 species) had the highest species richness. Silicoflagellates were represented by one species, *Dictyocha speculum*; in addition, the euglenid *Euglena* sp. was found in the ballast of some vessels. The total number of viable algae in each sample of the surveyed ballast varied from 0 to 1.19×10^6 cells/L.

[8)] Dodge J. D. *Marine Dinoflagellates of the British Isles.* London : Her Majesty's Stationary Office, 1982. 303 p.

[9)] Tomas, C., 1997. *Identifying Marine Phytoplankton.* San Diego: Academic Press, Inc., 821 p.

T a b l e 2. Taxonomic composition of unicellular planktonic organisms in the ballast water of the surveyed ships

Taxa	Number of the vessel (Table 1), in the ballast of which live cells of phyto- and microzooplankton were found
PHYTOPLANKTON	
BACILLARIOPHYCEAE	
Chaetoceros affinis Lauder (Fig. 2, *a*)	16
Chaetoceros danicus Cleve (Fig. 2, *b*)	16
Coscinodiscus sp. * (Fig. 2, *c*)	15, 16
Dactyliosolen fragilissimus (Bergon) Hasle	4
Ditylum brightwellii (T. West) Grunow *(Fig. 2, *d*)	14, 17
Melosira moniliformis (O.F. Müller) C. Agardh	17
Nitzschia tenuirostris Manguin	13, 15,
Proboscia alata (Brightw.) Sundström * (Fig. 2, *e*)	10, 14, 15, 16, 17
Pseudo-nitzschia delicatissima (Cleve) Heiden complex sp. (рис. 2, *f*)	7, 15, 16, 17
Pseudo-nitzschia seriata (Cleve) H. Perag. complex sp.	14, 15, 16, 17
Pseudo-nitzschia sp.	14
Pseudosolenia calcar-avis (Schultze) B.G. Sundström *	13, 14, 16, 17
Skeletonema costatum (Grev.) Cleve (Fig. 2, *g*)	15, 16, 17
Sundstroemia setigera (Brightw.) Medlin in Medlin et al. (=*Rhizosolenia setigera* Brightw.) ** (Fig. 2, *h*)	12, 17
Thalassionema nitzschioides (Grunow) Mereschk. (Fig. 2, *i*)	5, 14, 15, 16
Thalassiosira sp. (Fig. 2, *j*)	4, 7, 17
DINOFLAGELLATA	
Alexandrium sp.	14
Ensiculifera carinata Matsuoka, Kobayashi et Gains	16
Gonyaulax sp.	16
Prorocentrum compressum (J.W. Bailey) T.H. Abé ex J.D. Dodge (Fig. 2, *l*)	13
Prorocentrum micans Ehrenb. (Fig. 2, *m*)	10, 11, 13, 14, 16
Prorocentrum scutellum Schröd. (Fig. 2, *n*)	11, 14, 15, 17
Prorocentrum sp.	14
Protoperidinium sp. *	16
Scrippsiella acuminata (Ehrenb.) Kretschmann (Fig. 2, *o*)	16
Tripos furca (Ehrenb.) F. Gómez, 2013 * (Fig. 2, *k*)	16

Continued Table 2

Taxa	Number of the vessel (Table 1), in the ballast of which live cells of phyto- and microzooplankton were found
DICTYOCHOPHYCEAE	
Dictyocha speculum Ehrenb.	16
EUGLENOPHYCEAE	
Euglena sp.	8
MICROZOOPLANKTON	
PROTOZOA	
Amphorellopsis acuta (Schmidt, 1902)	10
Ciliophora gen. sp. (? *Euplotes* sp.)	13, 15, 18
Ciliophora gen. sp. (? *Vorticella* sp.) (Fig. 2, *p*)	18

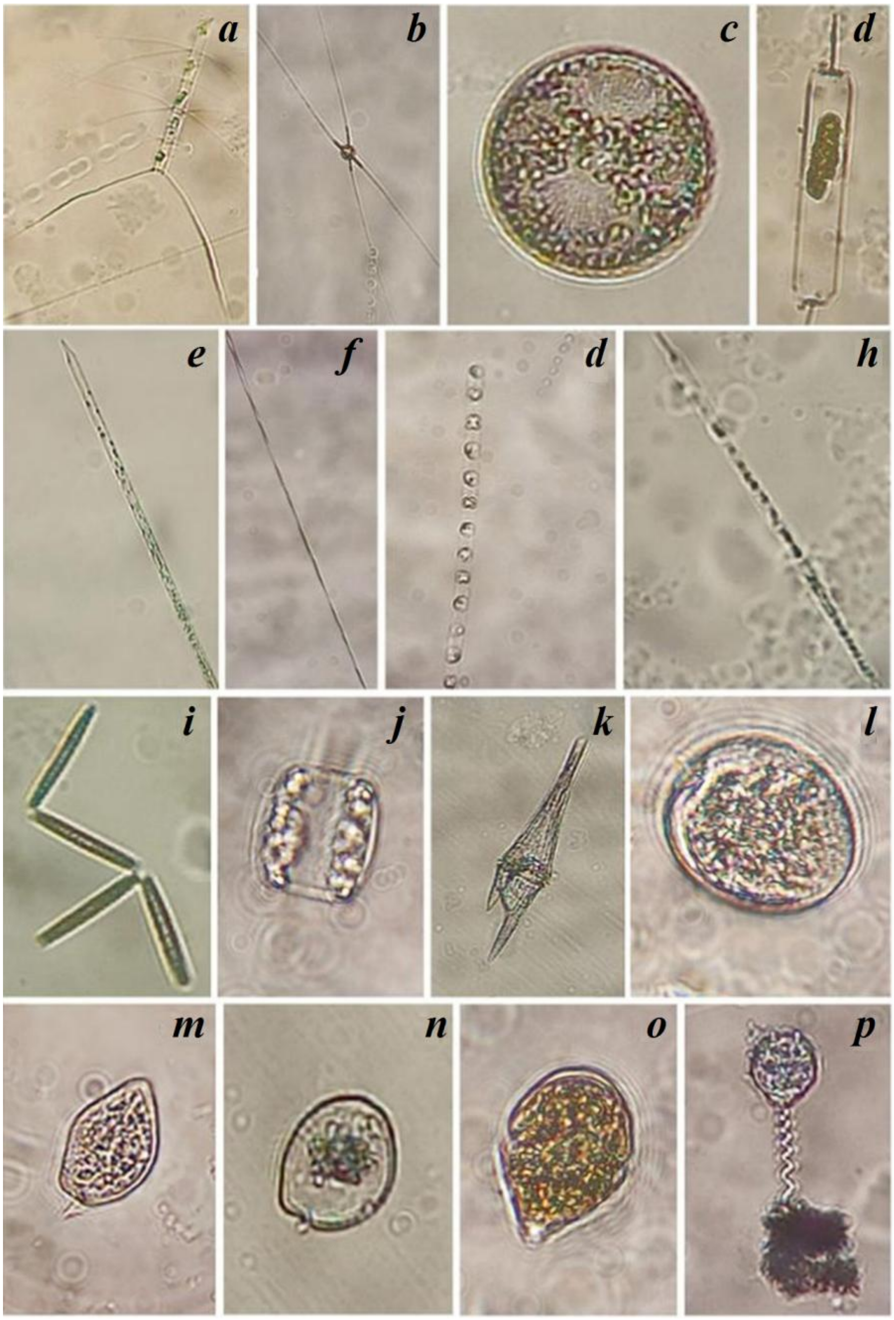
* The species with cells of > 50 μm long.

** Species not characteristic of the northeastern Black Sea.

The total number of living microzooplankton organisms (ciliates) ranged from 0 to 6.20×10^3 cells/L.

No living organisms were found in the ballast water of six vessels (1–3, 6, 9 and 19) out of 19 (32% of all cases) (100% ballast treatment). These vessels used the HMT-1500-EX, HMT-800, HiBallast BWMS-HUB-1000-EX, BalClor BC-2000, Ecochlor Series 200 or Ecochlor Inc./Et -5000-4.0 Series 200 BWTSs. The following treatment methods are used in these systems: electrocatalysis, electrolysis + filtration, chlorination + filtration, electrochlorination + neutralization.

DESMI CompactClean CC-500 (treatment method: filtration + UV) and Pureballast 3.2 1500 EX (treatment method: UV) systems used on vessels 16 and 17 (10% of all cases) failed to treat marine ballast. The number of unicellular algae (1.21×10^4 and 1.19×10^6 cells/L) in their ballast exceeded the permissible concentrations of living organisms from 10 μm to 50 μm long ($< 1.00 \times 10^4$ cells/L) established by regulation D-2. In the case of vessel 17 (ballast water loading region: the Marmara Sea, the port of Tuzla, Turkey), this excess was insignificant – by 1.2 times, but the number of phytoplankton cells in the ballast water of vessel 16 (ballast water loading region: the Aegean Sea, the port of Izmir, Turkey) exceeded the maximum permissible concentration of regulation D-2 by 119 times. The unsatisfactory degree of ballast water treatment on these vessels could be associated with improper operation or ineffective ballast systems operation.



F i g. 2. Phyto- and zooplankton found in the ballast water of the surveyed vessels (light microscope): *a – Chaetoceros affinis*; *b – Chaetoceros danicus*; *c – Coscinodiscus* sp.; *d – Ditylum brightwellii*; *e – Proboscia alata*; *f – Pseudo-nitzschia* sp.; *g – Skeletonema costatum*; *h – Sundstroemia setigera*; *i – Thalassionema nitzschioides*; *j – Thalassiosira* sp.; *k – Tripos furca*; *l – Prorocentrum compressum*; *m – Prorocentrum micans*; *n – Prorocentrum scutellum*; *o – Scrippsiella acuminata*; *p – Ciliophora* gen. sp. (?*Vorticella* sp.)

Onboard the other eleven vessels (No. 4, 5, 7, 8, 10–15, and 18 – 58% of cases) the following BWTS systems of classes NK-03-Blue-Ballast II Plus, HiBallast TM System HIB-2000-EX, HiBallast NF System, Erma First FIT-800, Pure Ballast 3:2, Blue Ocean Shield BOS 300, Gloen-1200 Patrol, Ex-Els-3000B 1:1, Erma First BWTS FIT-3000 or Electro-Cleen System ECS-1350B were used. Their procedures were based on the following treatment methods: electrolysis + neutralization, electrolysis + filtration, electrolysis + electrochlorination, UV treatment + filtration, ozonation + neutralization. These systems coped with the ballast water disposal: the content of live phytoplankton cells in ballast water ranged from 4 to 963 cells/L, microzooplankton (ciliates – Ciliophora) did not exceed 6.20×10^3 cells/L, which met regulation D-2: the discharge of less than 10 viable organisms that are from 10 μm to 50 μm long, per milliliter, that is, no more than 1.00×10^4 cells/L. It should be noted that the concentration of large-celled (more than 50 μm in length) phytoplankton species (mainly the diatoms *Proboscia alata, Pseudosolenia calcar-avis* and *Ditylum brightwellii*) found in the ballast of vessels No. 10, 12–17 (37% of cases) ranged from 2 to 312 cells/L (i.e. from 2 to 3.1×10^5 cells/m^3) and exceeded the requirements of regulation D-2: discharge of fewer than 10 viable organisms ≥ 50 μm in length, per cubic metre. Since the width of the cells of these algal types did not exceed 30 μm, the ballast of the vessels in which they were found can be considered conditionally clean.

Discussion

In the published literature containing the results of the analysis of ballast water and sediment samples, most of the studies were carried out on bulk carriers [24]. Our study is based on phytoplankton samples collected from the ballast tanks of 12 oil tankers and 7 bulk carriers.

All species of unicellular [10)] algae found in ballast water were previously found in the Black Sea [25]. However, the diatom *Sundstroemia setigera*, which lives in the southern Black Sea, is not characteristic of the northeastern part [11)]. Although this species is not toxic, it can be classified as potentially harmful. With its long and stiff setae located at both ends of the cell, it can injure the gill apparatus of anchovies (anchovies *Engraulis encrasicolus*) and small herring fish species: sprat *Sprattus sprattus* (L.) (Clupeiformes: Clupeidae) and kilka – *Clupeonella cultriventris* (von Nordmann) (Clupeiformes: Ehiravidae). Similarly, the diatoms [12)] *Chaetoceros convolutus* Castracane and *C. concavicornis* L.A. Mangin injure the gill apparatus of other fish species [26–29].

Unspecified taxa from two *Pseudo-nitzschia* complexes (Table 1) arguably pose the greatest threat to ecosystems and human health. They can cause amnesic

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[10)] UP-GRADE BS-SCENE project, 2010. *Phytoplankton Check List.* Seventh Framework Programme. Work Package 9. Deliverable D 9-1-3 Annex A. Grant agreement No. 226592. 66 p.

[11)] Boicenco, L., 2014. *Black Sea Phytoplankton Checklist.*

[12)] Hasle G. R., Fryxell G. A. Taxonomy of Diatoms. In: IOC, 1995. *Manual on Harmful Marine Microalgae. IOC Manual and Guides No. 33*. Paris: UNESCO, pp. 339–364.

shellfish poisoning. In addition, some potentially toxic organisms are capable of producing domoic acid. *P. delicatissima* and *P. prolongatoides* (Hasle) Hasle from the *Pseudo-nitzschia delicatissima* complex, *P. inflatula* (Hasle) Hasle from the *P. pseudodelicatissima* complex and *P. seriata,* and *P. pungens* from the *Pseudo-nitzschia seriata* complex were found in the Black Sea [10)]. Of these taxa, *P. delicatissima, P. pseudodelicatissima, P. pungens* and *P. seriata* are potentially toxic.

Species of the genus *Alexandrium* Halim produce neurotoxins and toxins that cause paralytic shellfish poisoning. In some cases, they cause fish death [30].

Ciliates, apparently, should be considered one of the most common zooflagellates transported with ballast waters [2]. For example, during a microscopic examination of marine ballast brought from Japan to the State of Washington (the Pacific coast of the USA), living ciliates 5–30 μm long were found in half of the tank sediment samples. The euglenid *Eutreptiella* sp. was also cultivated from sediments [31]. In general, protozoa are the dominant component of ballast water biota [32].

Thus, our research showed that in not all cases of using different BWTS types onboard vessels that discharged ballast in the seaport of Novorossiysk was 100% elimination of living organisms from ballast water achieved. The use of a number of ballast systems in 32% of the surveyed vessels showed excellent results (100% ballast treatment). Treatment results that met regulation D-2 were observed in 58% of vessels: their BWTSs did not completely cope with the ballast water disposal, but did significantly reduce the number of viable organisms in the ballast. In 10% of all studied cases, the result of ballast water treatment was unsatisfactory (a high number of living organisms remained in ballast water).

The Black Sea is a part of the Mediterranean Basin, and it has been intensively exchanging waters with the Mediterranean Sea over the past 8–10 thousand years. Therefore, the taxonomic compositions of the marine flora and fauna of these two water bodies have significant similarities [4]. The process of mediterranization of the Black Sea has accelerated significantly over the past half century. The mediterranization of fauna means the acquisition of a Mediterranean appearance by the fauna of the Black Sea and the Sea of Azov as a result of constant penetration of the Mediterranean animal species into these seas. In the biogeographical context, the term was introduced by I. I. Puzanov in 1960 [13)]. Over the period from 1960 to 2010, more than 100 new records of plants and animals of the Mediterranean origin were reported in the northern and western Black Sea. Forty-three species had successfully adapted to new conditions [5].

Whereas the majority of the surveyed vessels (12 out of 19) loaded ballast water exclusively in the Mediterranean Basin (Fig. 1), a relatively low-risk scenario can be assumed. However, the significant proportion of vessels arriving

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[13)] Puzanov, I.I., 1960. [*Over Untraversed Crimea*]. Moscow: Geografgiz, 286 p. (in Russian).

from the ports of the Gulf of Suez (the Red Sea), the coast of tropical West Africa and the Persian Gulf (the Indian Ocean) should be taken into account. It is expected that the likelihood of harmful effects from living organisms of Mediterranean origin penetrating the Black Sea will be less than from species coming from other regions of the World Ocean. Hence, elevated concentrations of phytoplankton transported in ballast water to the seaport of Novorossiysk from the Gulf of Suez (7.16×10^3 cells/L; vessel 15) and Mauritania (2.03×10^3 cells/L; vessel 13) can pose a moderate risk. However, without more detailed studies of the species composition and cell viability, it is still impossible to assess the real risk.

In general, it is assumed that among cargo ships, it is bulk carriers from the countries exporting raw materials (timber, grain, sugar, coal, iron ore) that pose the greatest risk because this category of vessels spends 50% of the time at sea with ballast water, and after delivery of cargo it needs full ballast water exchange [31]. Previously, a detailed study was carried out on phytoplankton collected using a 10-liter water bottle from the ballast waters of 9 vessels in the State of North Carolina (the Atlantic coast of the USA), followed by filtration through a set of sieves (333, 62 and 33 μm) and cultivation. As a result of this study, 342 species of microalgae (mainly blue-greens, dinoflagellates, diatoms and greens) were found in marine ballast [33]. This number greatly exceeds the number of species found by other authors, suggesting that ships carry thousands of phytoplankton species across the planet at any given time. Thus, most published results of studies of ballast water phytoplankton do not provide a true picture of the risk associated with the penetration of invasive microalgae into new regions. Moreover, we should remember the role of intraregional maritime transport in the distribution of invasive species [34].

Green and blue-green algae were also common biota components in ships' ballast water in the European Region [2], although they were not found in our samples. This fact is probably associated with the complete or almost complete absence of large rivers in the areas where the marine ballast was taken. It should be noted that these two taxonomic groups are most characteristic of freshwater bodies.

We believe that continued monitoring of the biological diversity of ballast water to assess the efficacy of using various types of BWTSs for the ballast water disposal is one of the priority areas in the field of applied scientific research of the Russian Academy of Sciences and Ministry of Transport of the Russian Federation. However, without knowledge of local biodiversity, which is an area of fundamental research, it is impossible to separate invasive species from native inhabitants.

Conclusions

Biological pollution is one of the most important problems of anthropogenic influences on the ecosystems of the World Ocean. To reduce environmental, epidemiological and other stresses on the aquatic environment caused by untreated ballast water discharge, the International Maritime Organization has required all merchant vessels to follow regulation D-1 in the area of the recipient water body since 2004, and since 2017, all new vessels must comply with regulation D-2, which requires vessels to have a ballast water treatment system (BWTS) onboard. According to the binding regulations for the seaport of Novorossiysk, it is allowed to discharge ballast that complies with regulations D-1 and D-2. For the first time concerning Russian waters, this paper presents the results of a study of the quality of ballast water treatment from unicellular planktonic organisms using BWTSs on vessels that entered the seaport of Novorossiysk.

Ballast water studies were carried out on 19 vessels (12 oil tankers and 7 bulk carriers) that entered the seaport of Novorossiysk for cargo loading from October 2022 to March 2023. The vessels loaded ballast in the ports of the following countries: Romania (the Black Sea, 1 vessel), Turkey (8 vessels), Greece (1 vessel), Italy (1) and Tunisia (1) (the Mediterranean countries), Mauritania (1) (tropical West Africa), Egypt (5 vessels) (the Gulf of Suez, the Red Sea, the Indian Ocean) and Iran (1 vessel) (the Persian Gulf, the Indian Ocean). In our opinion, the greatest risk of introducing harmful organisms into the Black Sea ecosystem with ballast water is represented by the vessels arriving from more distant ports with the warmest waters, i. e. from the Red Sea, the coast of tropical West Africa and the Indian Ocean.

Twenty species of planktonic algae were found in the samples of the surveyed ships' ballast. Diatoms (13 species) and dinoflagellates (6 species) had the highest species richness. Moreover, ciliates *Amphorellopsis acuta*, *Euplotes* sp. and *Vorticella* sp. were found. All species of unicellular organisms found in the ballast water are common in the Black Sea. Potentially dangerous representatives of diatoms and dinoflagellates were also found among them. The total number of viable algae in each sample of the surveyed ballast varied from 0 to 1.19×10^6 cells/L. The total number of living microzooplankton organisms (ciliates) ranged from 0 to 6.20×10^3 cells/L.

No living organisms were found in the ballast water of six vessels (32% of all cases) (100% ballast treatment). These vessels used the HMT-1500-EX, HMT-800, HiBallast BWMS-HUB-1000-EX, BalClor BC-2000, Ecochlor Series 200, Ecochlor Inc./Et-5000-4.0 Series 200BWTSs. The following treatment methods are used in these systems: electrocatalysis, electrolysis + filtration, chlorination + filtration, electrochlorination + neutralization.

DESMI CompactClean CC-500 (treatment method: filtration + UV) and Pureballast 3.2 1500 EX (treatment method: UV treatment) systems used on two vessels (10% of all cases) arriving from the Marmara (the port of Tuzla, Turkey) and the Aegean Sea (the port of Izmir, Turkey) failed to treat marine ballast. The number of unicellular algae (1.21×10^4 and 1.19×10^6 cells/L) in their ballast exceeded the permissible concentrations of living organisms established by regulation D-2.

The systems of 11 out of 19 ships coped with the ballast water disposal: the content of live phyto- and microzooplankton cells in their ballast water met regulation D-2. These were BWTS systems of classes NK-03-Blue-Ballast II Plus, HiBallast TM System HIB-2000-EX, HiBallast NF System, Erma First FIT-800, Pure Ballast 3:2, Blue Ocean Shield BOS 300, Gloen-1200 Patrol, Ex-Els-3000B 1:1, Erma First BWTS FIT-3000, Electro-Cleen System ECS-1350B. These systems use the following treatment methods: electrolysis + neutralization, electrolysis + filtration, electrolysis + electrochlorination, UV treatment + filtration, ozonation + neutralization.

Thus, the studies have shown that the use of different BWTS types onboard vessels does not always provide 100% clearance of living organisms from ballast water. Therefore, continued research and biological control of ballast water to assess the efficacy of using various types of BWTSs for ballast disposal, as well as monitoring of local biodiversity, are key tasks for minimizing possible biological pollution of the Black Sea.

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Yuri B. Okolodkov – analysis of the results and their interpretation, preparation of graphic materials, editing of the manuscript

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