МОРСКОЙ БИОЛОГИЧЕСКИЙ ЖУРНАЛ

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МОРСКОЙ БИОЛОГИЧЕСКИЙ ЖУРНАЛ

MARINE BIOLOGICAL JOURNAL

2020 Vol. 5 no. 1

Established in February 2016 SCIENTIFIC JOURNAL *4 issues per year*

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ИнБЮМ – IBSS

Морской биологический журнал, 2020, том 5, № 1, с. 3–11
Marine Biological Journal, 2020, vol. 5, no. 1, pp. 3–11
https://mbj.marine-research.org; doi: 10.21072/mbj.2020.05.1.01
ISSN 2499-9768 print / ISSN 2499-9776 online

SCIENTIFIC COMMUNICATION

UDC 574.64:595.384.2(262.5)

TOXIC METALS IN THE WARTY CRAB IN THE SOUTHERN BLACK SEA: ASSESSMENT OF HUMAN HEALTH RISK

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Received by the Editor 07.11.2019; after revision 05.03.2020;
accepted for publication 27.03.2020; published online 31.03.2020.

The present study was performed to assess Cd, Pb, and Hg contaminations and human health risk in the warty crab *Eriphia verrucosa* (Forskål, 1775) in Akliman shores of Sinop Peninsula of the Black Sea. Heavy metals analysis was performed by inductively coupled plasma mass spectrometry. Among studied toxic metals, Pb had the highest mean concentration in *E. verrucosa*. The highest mean concentration of Pb (0.2 mg per kg of wet weight) was observed in male samples of the warty crab. However, higher concentrations of Cd and Hg (0.11 and 0.019 mg per kg of wet weight, respectively) were observed in females of *E. verrucosa*. The mean Cd values found in the warty crabs were higher in May and June than those in July and August. On the other hand, Pb values were recorded in July and August. The mean Hg values were not different between months except July and August for male samples of *E. verrucosa*. Foraging seasons of these crabs are different, which can lead to differences in prey size and ultimately metals intake. However, the results show that a toxic heavy metal concentration in edible tissues of crab from the southern Black Sea was within the permissible limits given by national and international food codices. Target hazard quotient (THQ) for each metal and hazard index (HI) were calculated to evaluate non-carcinogenic human health risks. Estimated THQs of Cd, Pb, and Hg suggest that these metals in the warty crab do not pose any apparent threat to humans, when the HI value is below the value of 1. The result of the analysis has shown that the warty crab *E. verrucosa* can be used as bioindicator as it contains variable levels of the metals observed. Since consumption is the main source of heavy metal intake by humans, monitoring studies are needed to protect public health and take preventive measures.

Keywords: heavy metal, Black Sea, *Eriphia verrucosa*, hazard index, target hazard quotient

The Black Sea takes up a variety of contaminants from agricultural, mining, touristic, domestic, and other anthropogenic activities via direct dumping from major rivers and in other ways [1]. Toxic metals such as mercury, cadmium, and lead, among others, are of great importance, since their anthropogenic contribution outweighs the one which is provided through life span and likewise because they show much toxic properties along the food chain. Toxic metals eventually enter seafood, and their bioaccumulation and magnification can cause physiological and morphological alterations not only in marine coastal animals but in people as well [2]. With the ever-increasing contamination of the coastal ecosystem, risk of toxic metal contamination of seafood is increasing day by day.

Many crustacean species were used as organisms for biomonitoring toxic metals in contaminated marine coastal ecosystems due to a number of suitable characteristics such as their convenient size, ease of sampling, abundance, ease of handling in the laboratory, and ability to accumulate metals. Therefore, it is of great interest to carry out investigations on metal concentrations in crustaceans [3].

People in Sinop city of the southern Black Sea consume a considerable amount of seafood. Since seafood is an important diet for humans, its quality and safety aspects are of particular interest. Over the past many decades, the amounts of heavy metals in seafood have been studied off the coasts of the Black Sea. Heavy metals are known to accumulate in benthic organisms and increase in the food chain. Since consumption is the main source of heavy metal intake by humans, the major interest is in the edible commercial species like the warty crab. It is necessary to have data on the levels of heavy metals in the warty crab in order to assess whether there is a health hazard.

The aim of this study is to evaluate the levels of Cd, Pb, and Hg in the warty crab *Eriphia verrucosa* (Forskål, 1775) in Akliman shores of Sinop Peninsula of the Black Sea.

MATERIAL AND METHODS

E. verrucosa is a type of crab which is locally called "küflü" and consumed extensively by people in spring and summer. All the warty crab samples were captured in 2017. This crab usually lives in shallow water under rocks and between seagrasses. *E. verrucosa*, which is a characteristic form for the hard substratum of the upper-infralittoral at depths of 1–8 m, has an average length of (10 ± 1.5) cm (Fig. 1).

Fig. 1. *Eriphia verrucosa* in Akliman shores of Sinop Peninsula of the Black Sea

The meat was removed from the shell and washed with double-distilled water. Then the samples were frozen at the temperature of −21 °C and stored in polyethylene bags until analysis. Water temperatures at the time of sampling in May, June, July, and August were of +15.8, +21.5, +25.6, and +26.2 °C, respectively. A total of 40 warty crabs, 20 males and 20 females, were used for the heavy metal analysis. Ten individuals were analyzed in each sampling month.

Warty crabs is active at dawn and at night. Diet of *E. verrucosa* consists of molluscs, crustaceans, and worms. Chelipeds (claws) are not equal to each other and are strong. Warty crab catches prey with its claws and cuts it into suitable pieces with cutter plates in its mouth. It should be noted that the size of the claws has an influence on the prey: with increasing size, crab can hunt bigger organisms.

Location of sampling areas is given in Fig. 2. Akliman is located on the outer harbor side of Sinop Peninsula. The distance to the city center is of 11 km. The coast length is of approximately four thousand meters. The slope of the beach is quite low. Sampling covered areas of direct or indirect influence of urban releases and touristic and fishing activities, those located near the mouths of Karasu and Sirakaraagaçlar streams which carry domestic and agricultural discharges to the Akliman coasts of Sinop province as well as a locality not under the influence of industrial releases. However, under the influence of the prevailing winds, pollutants reach the shore by the discharge. The sampling station was chosen to reflect progression of contamination, ecological particularity, and human activities in the area. Tourist activities are very intense in Akliman area, especially during summer months.

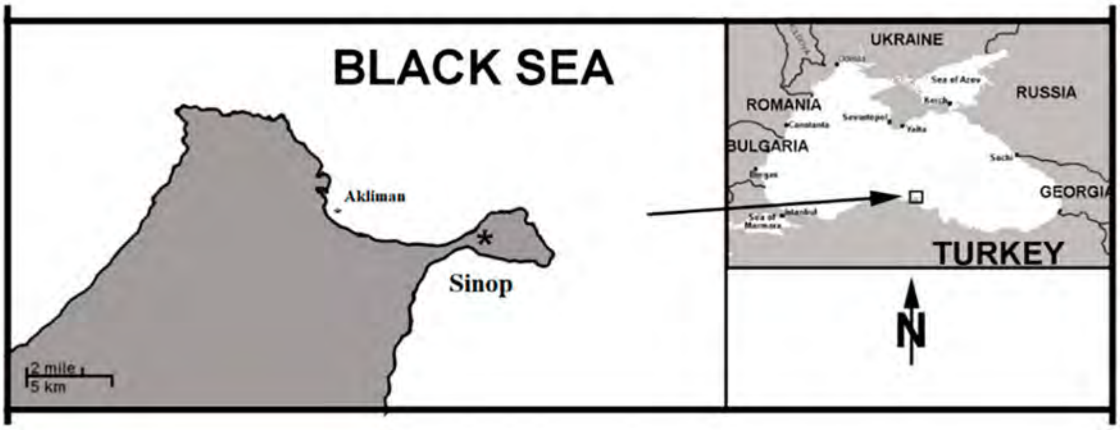

Fig. 2. Sampling area

About 1 g of edible tissues was wet digested with Suprapur® HNO_3 (nitric acid) using a microwave digestion system (Start D 260, Milestone Systems) for analysis and evaluations of concentrations of three non-essential heavy metals. Cd, Pb, and Hg were determined using inductively coupled plasma mass spectrometry (7700x ICP-MS, Agilent).

The element standard solutions used for calibration were prepared by diluting a stock solution of 1000 mg per L (Cd, Hg, and Pb) supplied by Merck (Germany). Standard reference material of lobster hepatopancreas TORT-3 for the metals was used to validate the analysis. Recovery percentages results ranged 95 to 102 %, indicating the accuracy of the results (Table 1).

Table 1. Reference concentration values of standard reference material TORT-3 (mg per kg)

	Cd	Hg	Pb
Certified	42.3	0.137	0.225
Found	41.47	0.140	0.237

Toxic metals in warty crab tissues were expressed as milligram of metal per kilogram of wet weight sample. The sensitivity of the method was determined according to the detection limits established for the spectrometer, which were of < 0.001 μg per L for Pb and Cd and of < 0.01 μg per L for Hg. The operating conditions of ICP-MS set for the analysis of the metals are shown in Table 2.

Assessments of hazard index of toxic metals in the warty crab. The estimated daily intake (hereinafter EDI) depends on both the metal level and the quantity of seafood consumption. The EDI of toxic metals was computed using Equation (1) below modified from [15]:

Table 2. ICP-MS operating conditions for the metal analysis

Operating conditions	Values
Plasma mode	normal, robust
RF power (W)	1550
Sampling depth (mm)	8
Nebulizer (ml per min)	0.2
Spray chamber temperature (°C)	+2
Carrier gas flow (L per min)	0.95
Dilution gas flow (L per min)	0.15
Extraction lens 1 (V)	0
Kinetic energy discrimination (V)	4
Cell gas (He) flow (ml per min)	4
Background on-mass (cps)	< 2
Integration time (μs)	100

$$EDI = \frac{F_{ir} \times C_m}{W_{ab}} \times 10^{-3}\ , \tag{1}$$

where F_{ir} is the seafood ingestion rate (g·person^{-1}·day^{-1}), which was considered to be of 15 g·person^{-1}·day^{-1} in Turkey [17], and this value was calculated as 105 g a week;

C_m is the level of toxic metal in the warty crab (mg per kg of wet weight);

W_{ab} is the mean adult body weight (70 kg).

The estimated weekly intake (hereinafter EWI) values were calculated from EDI values. Intake estimates were determined as per unit body weight (mg·week^{-1}·kg^{-1} of body weight).

Target hazard quotient (hereinafter THQ) [15], which is a proportion of the estimated exposure (EDI) to the oral reference dose (hereinafter R_fD), is used to evaluate the potential non-carcinogenic risk to humans from the sensed contaminated crab, and is shown by Equation (2):

$$TQH = \frac{EDI}{R_f D}\ , \tag{2}$$

where the R_fD values for Hg and Cd are of 0.0003 and 0.001 mg·kg^{-1}·day^{-1}, respectively [20].

R_fD is not available for Pb [5]. The U. S. Department of Health and Human Services Public Health Service [16] pointed out that it would be unsuitable to develop R_fD for inorganic Pb and its compounds, because some of the sanitary impacts related to the exposure to Pb occur with blood Pb levels as low as to be essentially without a threshold [21]. Therefore, the R_fD value for Pb in this study was of 0.0035 mg·day^{-1}·kg^{-1} of body weight as used by many researchers [5, 10, 11].

Exposure to more than one contaminant may induce contribution and/or interactive impacts; hereby, accumulative health effect from plural contaminants' exposure was determined by summing THQ value of individual contaminant and clarified as hazard index (hereinafter HI) as shown in Equation (3):

$$HI = THQ_{Cd} + THQ_{Pb} + THQ_{Hg}\ . \tag{3}$$

HI value of > 1 shows the possibility of reverse health effects and commits the necessity for bearing a further appraisal and likely remedial action. However, HI of < 1 shows no feasible health consequence from exposure of the examined contaminants at existing consumption rate.

Statistical analysis. Statistical analysis was carried out using IBM SPSS Statistics V21. One-way ANOVA and Duncan multiple range test were used to calculate a significant difference in the concentration of different studied metals with respect to different sexes and months. The significance was set at 5 % confidence level.

RESULTS

Mean values of toxic metal concentrations (mg per kg ± SD wet weight) found in the warty crab *E. verrucosa* in Akliman shores of Sinop Peninsula of the Black Sea are presented in Fig. 3.

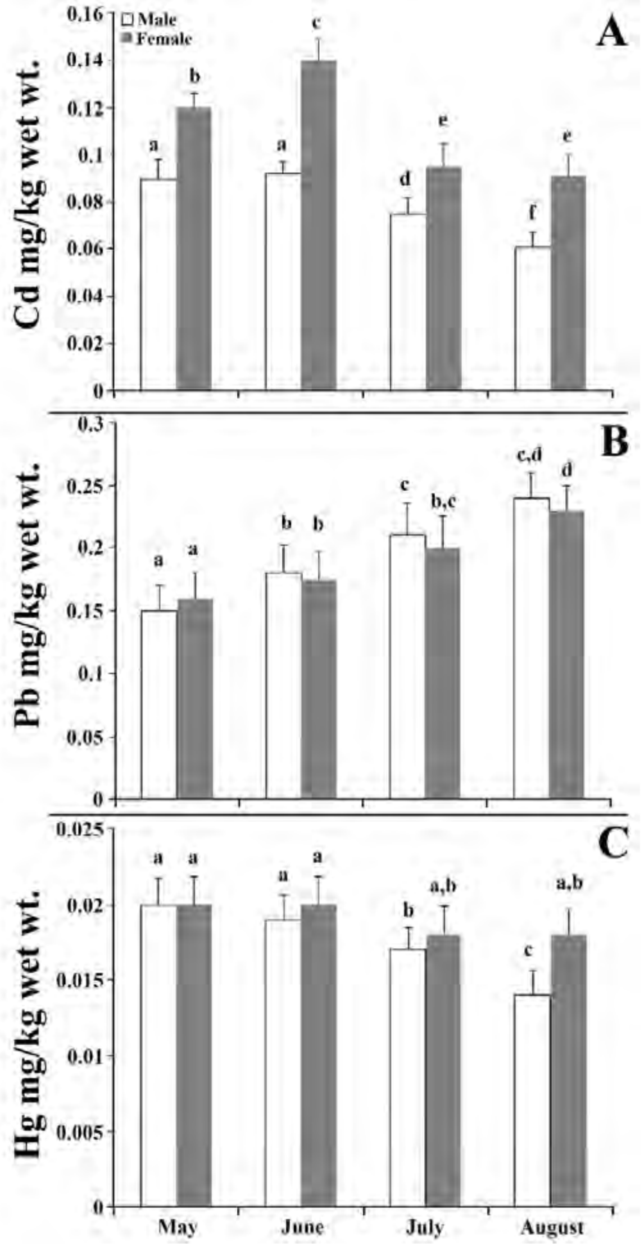

Fig. 3. Mean (± SD) Cd (A), Pb (B), and Hg (C) levels in *E. verrucosa* (N = 40) among sexes and months. Different letters beside vertical bars indicate that the values are significantly different ($p < 0.05$)

The risk to human health as a result of consuming the warty crab was evaluated by calculating EDI, EWI, provisional tolerable weekly/daily intake (hereinafter PTWI and PTDI, respectively), THQ, and HI. The maximum toxic metal values in the edible tissues of *E. verrucosa* were used to evaluate the human health risk from the consumption. EDI, EWI, PTWI, PTDI, R_fD, THQ, and HI of these metals are shown in Table 3.

Table 3. Human health risk parameters in the warty crab collected from the Black Sea

Health risks parameters	Toxic heavy metals		
	Cd	Pb	Hg
Estimated daily intake, EDI, mg·day^{-1}·kg^{-1} of body weight	2.39×10^{-5}	4.18×10^{-5}	4.07×10^{-6}
Estimated weekly intake, EWI, mg·week^{-1}·kg^{-1} of body weight	1.67×10^{-4}	2.93×10^{-4}	2.85×10^{-5}
Provisional tolerable weekly intake, PTWI, mg·week^{-1}·kg^{-1} of body weight	7×10^{-3}	2.5×10^{-2}	4×10^{-3}
Provisional tolerable weekly intake, PTWI, mg per week per 70 kg of body weight	0.49	1.75	0.28
Provisional tolerable daily intake, PTDI, mg per day per 70 kg of body weight	0.07	0.25	0.04
Oral reference dose, R_fD, mg·day^{-1}·kg^{-1} of body weight	1×10^{-3}	3.5×10^{-3}	3×10^{-4}
Target hazard quotient, THQ	2.39×10^{-2}	1.19×10^{-2}	1.36×10^{-2}
Hazard index, HI	4.94×10^{-2}		

DISCUSSION

The non-essential metals such as Cd, Pb, and Hg are highly toxic contaminants, and their uptake and bioaccumulation in coastal ecosystems may cause serious effects straight on food chain as well as on human beings. Among toxic metals studied, Pb had the highest mean concentration in *E. verrucosa*. Higher mean concentration of Pb (0.2 mg per kg of wet weight) was observed in male samples of the warty crab (Fig. 3B). However, the highest concentrations of Cd and Hg (0.14 and 0.019 mg per kg of wet weight, respectively) were observed in females of *E. verrucosa* (Figs 3A and 3C). The mean Cd values found in the warty crabs were higher in May and June than those in July and August ($p < 0.05$). On the other hand, Pb values were recorded in July and August ($p < 0.05$). The mean Hg values were not different between months ($p > 0.05$) except July and August for male samples of *E. verrucosa*. High metal levels may change within individuals depending on feeding habits of crabs, age, size, and length as well as on their habitats and metabolisms. *E. verrucosa* is carnivore and mainly feed on benthic organisms such as mussels, and this can be one of the reasons for their metal bioaccumulation. In this study, female individuals with eggs were found in May and June. The warty crabs were collected from the cliffs and habitats where the sea meadows were abundant. Individuals collected from rocks can take up metals from food and water, while those from seagrass habitat can also accumulate metals from sediment. As known, sediments are the final destination where heavy metals sink [6]. The increased amounts of toxic metals in sediments are the result of higher levels of these metals in surrounding water, which is easily adsorbed on the surface of the sediment and poses an ecological risk, especially to benthic species. In summer, increase in tourist activities and excess of domestic wastes pollute the coastal areas. Although the central population of Sinop is 65 thousand, it reaches 400 thousand, especially in July and August. Akliman coast is also used extensively as a picnic area. During these periods, this coast is exposed to contamination.

Although heavy metal studies related to *E. verrucosa* were conducted in the early 1990s, very little literature is available. These data were recently reviewed by Bat and Arıcı [3]. According to these studies, Cd, Pb, and Hg values in *E. verrucosa* were determined as 0.06–5.04, 0.06–0.8, and 0.02–0.022 mg per kg of wet weight, respectively [4, 7, 12, 14]. Data obtained in the current study were compared with the literature data, and Hg values were similar while Cd and Pb values were very low.

Durmus et al. [9] studied the edible tissues of *E. verrucosa* recorded on the Ordu coasts in the Black Sea with regard to toxic heavy metals Cd and Pb. Their values were of 0.17–0.32 and 0.13–0.36 mg per kg of wet weight, respectively. In our study, when the maximum values were compared, the values on Sinop coasts were lower than those on Ordu coasts. It is concluded that this was due to the difference between the regions [9]. This may also be caused by the contaminants carried by the streams of various sizes on the southeastern coast of the Black Sea [1].

In the present study, Cd, Pb, and Hg concentrations in *E. verrucosa* edible tissues were found to be below the permissible limit which was of 0.5 mg per kg of wet weight for these metals studied in Crustaceans [8, 18, 19]. Our research also aimed to assess whether there is an impact on the health of people consuming these warty crabs. In this sense, the current study has been carried out in accordance with the Marine Environment Policy Marine Strategy Framework Directive [13]. Marine Strategy Framework Directive Descriptor 8 "Concentrations of contaminants are at levels not giving rise to pollution effects" and Descriptor 9 "Contaminants in seafood for people consumption do not exceed amounts established by Community legislation or other relevant standards" are aiming the subject of marine contamination. For this purpose, EDI, EWI, PTWI, PTDI, THQ, and HI calculations were performed. The amount of fish consumed in Turkey is much higher compared to amount of other seafood. Crustacean species are mostly consumed in the coastal cities, and Sinop is one of them. Cd, Pb, and Hg are non-essential toxic metals in organisms; the presence of these metals in seafood may cause risk to human health via consumption. Values given in the present study are significantly lower than the recommended values of Turkish Food Codex [18, 19]. Estimated THQs of Cd, Pb, and Hg suggest that these metals in the warty crab do not pose any apparent threat to humans, where the HI value was of < 1 (U. S. Environmental Protection Agency) as shown in Table 3.

Conclusion. The toxic and non-essential metals in warty crab samples have been analyzed. According to the results obtained, Cd, Pb, and Hg are present in *E. verrucosa*, but in low concentrations. The concentrations of Cd, Pb, and Hg in *E. verrucosa* samples from Akliman shores of Sinop of the Black Sea did not exceed the permissible limits set for metals by EU Commission Regulation and Turkish Food Codex. EDI, EWI, PTWI, PTDI, THQ, and HI of these metals were estimated taking into account the mean concentration value of metal in all the warty crab individuals and the average consumption of them per day for adults. Since HI is of < 1 described by U. S. Environmental Protection Agency, *E. verrucosa* is considered safe for consumption. It is suggested that further investigation should be carried out of the parameters in coastal waters as well as sediment analysis which may help find the cause of each metal bioaccumulation in these species. This kind of biomonitoring studies is necessary to assess the risk to human health due to dynamic character of the marine ecosystems and their constant exposure to toxic metals as mentioned in Marine Strategy Framework Directive.

REFERENCES

1. Bat L., Öztekin A., Şahin F., Arıcı E., Özsandıkçı U. An overview of the Black Sea pollution in Turkey. *Mediterranean Fisheries and Aquaculture Research*, 2018, vol. 1, no. 2, pp. 67–86.
2. Bat L. Pollution and fishing. In: *Black Sea*

and Fishing Workshop Book / M. Sezgin, F. Şahin, U. Özsandıkçı (Eds). Sinop : University of Sinop, 2017, pp. 17–54. (in Turkish).

3. Bat L., Arıcı E. Heavy metal levels in fish, molluscs, and crustacea from Turkish seas and potential risk of human health. In: *Handbook of Food Bioengineering. Vol. 13. Food Quality: Balancing Health and Disease* / A. M. Holban, A. M. Grumezescu (Eds). [London] : Elsevier ; Academic Press, 2018, chap. 5, pp. 159–196. https://doi.org/10.1016/B978-0-12-811442-1.00005-5
4. Bat L., Öztekin H. C. Heavy metals in *Mytilus galloprovincialis*, *Rapana venosa* and *Eriphia verrucosa* from the Black Sea coasts of Turkey as bioindicators of pollution. *Walailak Journal of Science and Technology*, 2016, vol. 13, no. 9, pp. 715–728.
5. Bat L., Şahin F., Öztekin A., Arici E., Yardim O. Assessment of Cd, Hg, Pb, Cu, and Zn amounts in muscles of *Cyprinus carpio* from Karasu Stream, Sinop. *Current Agriculture Research Journal*, 2019, vol. 7, no. 2, pp. 171–180. http://dx.doi.org/10.12944/CARJ.7.2.05
6. Bat L., Özkan E. Y. Heavy metal levels in sediment of the Turkish Black Sea coast. In: *Oceanography and Coastal Informatics: Breakthroughs in Research and Practice*, [S. l.] : [S. n.], 2019, chap. 4, pp. 86–107. https://doi.org/10.4018/978-1-5225-7308-1.ch004
7. Bat L. *A Study on Trace Element Levels in Some Organisms Collected From Upper-infralittoral of Sinop Peninsula*. MSc thesis. Sinop : Ondokuz Mayıs University, 1992, 108 p. (in Turkish).
8. *Commission Regulation (EC) No. 1881/2006 setting maximum levels for certain contaminants in foodstuffs.* 19 Dec., 2006. URL: https://openlaws.com/detail/b61d36b4-4f02-4383-b65f-d0438b9ddd96/en/SINGLE (accessed 25.10.2019).
9. Durmus M., Ayas D., Aydin M., Kosker A. R., Ucar Y., Ozogul Y. The effects of sex and seasonality on the metal levels of warty crab (*Eriphia verrucosa*) in the Black Sea. *Journal of Aquatic Food Product Technology*, 2018, vol. 27, iss. 6, pp. 749–758. https://doi.org/10.1080/10498850.2018.1485196
10. Harmanescu M., Alda L. M., Bordean D. M., Gogoasa I., Gergen I. Heavy metals health risk assessment for population via consumption of vegetables grown in old mining area; a case study: Banat County, Romania. *Chemistry Central Journal*, 2011, vol. 5, article no. 64 (10 p.). https://doi.org/10.1186/1752-153X-5-64
11. Khan S., Cao Q., Zheng Y. M., Huang Y. Z., Zhu Y. G. Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environmental Pollution*, 2008, vol. 152, iss. 3, pp. 686–692. https://doi.org/10.1016/j.envpol.2007.06.056
12. Mulayim A., Balkis H. Toxic metal (Pb, Cd, Cr, and Hg) levels in *Rapana venosa* (Valenciennes, 1846), *Eriphia verrucosa* (Forskal, 1775), and sediment samples from the Black Sea littoral (Thrace, Turkey). *Marine Pollution Bulletin*, 2015, vol. 95, iss. 1, pp. 215–222. https://doi.org/10.1016/j.marpolbul.2015.04.016
13. Directive 2008/56/Ec of the European Parliament and of the Council of 17 June, 2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive). *Official Journal of the European Union*, 2008, L 164, pp. 19–40.
14. Öztürk M., Bat L. Heavy metal levels in some edible organisms from Sinop coast of the Black Sea. *EÜ Fen Fakültesi Dergisi*, 1994, vol. 16, no. 1, pp. 177–186. (in Turkish).
15. *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* : Risk assessment forum technical panel / U. S. Environmental Protection Agency. Washington, DC : National Center for Environmental Assessment, 2000, [209 p.]. (EPA/630/R00/002).
16. *Toxicological Profile for Lead : Draft for Public Comment. May, 2019* / U. S. Department of Health and Human Services Public Health Service. Agency for Toxic Substances and Disease Registry. URL: https://www.atsdr.cdc.gov/toxprofiles/tp13.pdf (accessed 25.10.2019).
17. *TUIK, Turkish Fishery Statistics*, 2019 : [site]. URL: http://www.tuik.gov.tr/ (accessed 25.10.2019).
18. *Turkish Food Codex. Official Gazette of Republic of Turkey.* Notifications about determination of the maximum levels for certain contaminants in foodstuffs of Turkish Food Codex, 2002. (Notification no.: 2002/63), iss. 24885. (in Turkish).

19. *Turkish Food Codex. Official Gazette of Republic of Turkey.* Notifications changes to the maximum levels for certain contaminants in foodstuffs, 2009. (Notification no.: 2009/22), iss. 27143 (in Turkish).
20. *U. S. Environmental Protection Agency, Integrated Risk Information System.* 2018 : [site]. URL: https://www.epa.gov/risk/regional-screening-levels-rsls-generic-tables (accessed 25.10.2019).
21. *U. S. Environmental Protection Agency IRIS, Lead.* Integrated Risk Information System. Washington, DC, 2005 : [site]. URL: http://www.epa.gov/iris/ (accessed 25.10.2019).

ТОКСИЧНЫЕ МЕТАЛЛЫ В КАМЕННЫХ КРАБАХ ЮЖНОЙ ЧАСТИ ЧЁРНОГО МОРЯ: ОЦЕНКА РИСКА ДЛЯ ЗДОРОВЬЯ ЧЕЛОВЕКА

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Проведена оценка загрязнения тяжёлыми металлами — Cd, Pb и Hg — каменных крабов *Eriphia verrucosa* (Forskål, 1775), обитающих на Аклиманском побережье Синопского полуострова Чёрного моря, и потенциальных рисков для здоровья человека. Анализ содержания тяжёлых металлов выполнен методом масс-спектрометрии с индуктивно-связанной плазмой. Среди изученных токсичных металлов свинец имел самую высокую среднюю концентрацию в тканях *E. verrucosa*. При этом максимальные концентрации Pb отмечены в тканях самцов краба (0,2 мг·кг$^{-1}$ сырой массы), тогда как у самок зарегистрированы более высокие, чем у самцов, концентрации Cd и Hg (0,11 и 0,019 мг·кг$^{-1}$ сырой массы соответственно). Средние значения содержания Cd в каменных крабах были выше в мае и июне, чем в июле и августе. Pb отмечен только в июле и августе. Средние значения содержания Hg в образцах самцов *E. verrucosa* были примерно одинаковыми в разные месяцы, за исключением июля и августа. Сезоны активного питания самцов и самок каменных крабов отличаются, что может приводить к различиям в размере пищевых объектов и в итоге — в уровнях накопления металлов. В целом концентрация токсичных тяжёлых металлов в съедобных тканях каменных крабов из южной части Чёрного моря находилась в допустимых пределах, установленных национальным и международным стандартами для пищевых продуктов. Для оценки рисков неканцерогенной природы для здоровья человека определены целевой коэффициент опасности (target hazard quotient) для каждого металла и индекс опасности (hazard index). По рассчитанным целевым коэффициентам опасности Cd, Pb и Hg можно заключить, что эти металлы в каменном крабе не представляют явной угрозы для людей, если, по результатам измерений, значение индекса опасности ниже 1. Результаты анализа показали, что *E. verrucosa* могут быть использованы в качестве биоиндикатора, так как рассмотренные металлы содержатся в этих каменных крабах в разных количествах. Поскольку пищевой путь поступления тяжёлых металлов является для человека основным, необходимо проводить дальнейшие мониторинговые исследования, чтобы защитить здоровье населения.

Ключевые слова: тяжёлый металл, Чёрное море, *Eriphia verrucosa*, индекс опасности, целевой коэффициент опасности

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ИнБЮМ – IBSS

Морской биологический журнал, 2020, том 5, № 1, с. 12–19
Marine Biological Journal, 2020, vol. 5, no. 1, pp. 12–19
https://mbj.marine-research.org; doi: 10.21072/mbj.2020.05.1.02
ISSN 2499-9768 print / ISSN 2499-9776 online

UDC 582.261.1:551.464.6

THE CARBON FIXATION EFFICIENCY IN BIOMASS OF *CYLINDROTHECA CLOSTERIUM* (EHRENBERG) REIMANN & J. C. LEWIN (BACILLARIOPHYCEAE) UNDER THE CONDITIONS OF CUMULATIVE CULTIVATION

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Received by the Editor 24.10.2019; after revision 28.01.2020;
accepted for publication 27.03.2020; published online 31.03.2020.

The carbon utilization efficiency is an important characteristic of the cultivated object. Diatom *Cylindrotheca closterium* (Ehrenberg) Reimann & J. C. Lewin is known to use carbon from aquatic environment quite effectively, as it has many unique carbonic anhydrases and carbon transporters. However, the carbon fixation efficiency for many types of diatoms in culture is still unknown. When calculating the carbon fixation efficiency, researchers use different terminology and methods, and it leads to significant difficulties when comparing the carbon fixation efficiency in the biomass of various types of microalgae. The aims of this study are: 1) to update terms and definitions used in literature on the basis of modern concepts of carbon fixation in microalgae biomass, as well as absorption of inorganic carbon by microalgae culture; 2) to evaluate the carbon fixation efficiency in the biomass of *C. closterium* diatom under conditions of cumulative cultivation. *C. closterium* was grown at a temperature of +20 °C on a nutrient medium RS. During the cultivation, the culture was bubbled with air (1.1 L of air per 1 L of culture per minute). The air temperature at the outlet of the suspension was of +19 °C; the maximum productivity of the culture was of 1.254 $g \cdot L^{-1} \cdot day^{-1}$. According to the results of the CHN analysis, the proportion of carbon in *C. closterium* dry biomass was of 23 %. Under the conditions of cumulative cultivation in *C. closterium*, the carbon fixation efficiency in biomass was of 90 %. Compared with other algae species, *C. closterium* is characterized by a rather high CO_2 fixation efficiency. For example, in green microalga *Chlorella protothecoides* and *Ch. vulgaris*, the CO_2 fixation efficiency was of 20 % and 55.3 %, respectively; in cyanobacteria *Spirulina* sp. – of 38 %; in red microalgae *Porphyridium purpureum* – of 69 %. It was observed that to ensure an increase of 1 g of *C. closterium* dry biomass per day at a temperature of +19 °C, a minimum of 0.46 L of CO_2, or 1132 L of air, should be consumed. Possibly, it is high carbon fixation efficiency, as well as low carbon fraction in *C. closterium* biomass, that explains the high production indices of this species. Under equal conditions of cultivation in terms of light and carbon availability, the productivity of *C. closterium* can exceed the productivity of other types of microalgae by 5–10 times. So, while *Spirulina* sp. productivity reaches 0.2 $g \cdot L^{-1} \cdot day^{-1}$, *C. closterium* productivity is of 1.254 $g \cdot L^{-1} \cdot day^{-1}$.

Keywords: diatom *Cylindrotheca closterium*, cultivation, carbon fixation efficiency

Marine microalgae are widely used in modern biotechnology as producers of valuable biologically active compounds [11, 14, 20]. Many types of marine microalgae are capable of synthesizing unique pigments, fatty acids, carbohydrates, etc. [9, 14, 15]. Among producers of valuable substances on an industrial scale, benthic diatoms are of particular interest, since they are characterized by high efficiency of utilization of light energy. Besides that, due to high silicon content in the absence of mixing, they quickly settle to the bottom of the photobioreactor, which significantly reduces the cost and facilitates harvesting [2].

Diatom *Cylindrotheca closterium* (Ehrenberg) Reimann & J. C. Lewin, 1964 is one of the most promising cultivation objects for the production of valuable polyunsaturated fatty acids and fucoxanthin on an industrial scale. This is due to the fact that *C. closterium* has sufficiently high production indices [2, 4] and is capable of accumulating fucoxanthin to 2.3–2.6 % of dry weight [18, 21]. The diatom is also characterized by high (up to 10 % of dry weight) content of polyunsaturated fatty acids in biomass [17, 19].

Carbon, along with nitrogen, is the main component of the life of microalgae. The availability of carbon determines chemical composition of cells and rate of biosynthesis of waste products; therefore, the carbon utilization efficiency is an important characteristic of the cultivated object [13]. It is known that diatoms, in comparison with other microalgae, are able to use carbon from the aquatic environment quite effectively, since they have many carbon transporters [12]. However, the carbon fixation efficiency for many types of diatoms in culture is still unknown.

To calculate the carbon fixation efficiency, researchers use different terminology and methods [6, 7, 8], which leads to significant difficulties when comparing the carbon fixation efficiency in the biomass of various types of microalgae. Therefore, the aims of this study are: 1) to update terms and definitions used in literature on the basis of modern concepts of carbon fixation in microalgae biomass, as well as of absorption of inorganic carbon by microalgae culture; 2) to evaluate the carbon fixation efficiency in *C. closterium* diatom biomass under conditions of cumulative cultivation.

MATERIAL AND METHODS

We used the culture of *Cylindrotheca closterium* from IBSS RAS culture collection. *C. closterium* culture was grown on RS nutrient medium [16], all components of which were increased three times [5], at a constant suspension temperature of (20 ± 1) °C, in the accumulative cultivation mode in plane-parallel photobioreactors with a working volume of 2 L and a layer of 5 cm, at round-the-clock lighting with CE-PIL-1-LF 46W/54-765 fluorescent lamps. The average light intensity on the working surface of the photobioreactor was of 150 μmol quanta$\cdot$m$^{-2}\cdot$s^{-1} (33 W per m^2). During the cultivation, the culture was bubbled with air (1.1 L of air per 1 L of culture per minute) through a compressor unit. The temperature of the air flow at the outlet of the suspension was of (19 ± 0.5) °C. A dispersant nozzle was used to increase the solubility of atmospheric CO_2 in the culture medium. Culture density at the beginning of cumulative cultivation was of 0.1–0.2 g of dry matter per 1 L.

The culture density was determined by two methods: 1) by the method of iodide oxidation [1]; 2) by direct weighing of *C. closterium* wet weight in polypropylene tubes on an analytical balance with an error of 0.1 mg after cell precipitation by centrifugation (1600g for 2 minutes). To recalculate the obtained data on dry weight, we used the coupling coefficient between dry and wet weight ($k = 0.1$; $n = 20$). To determine the carbon fraction in biomass, a suspension of *C. closterium* cells was taken on the 6th day of the experiment, centrifuged for 1–2 minutes at 1600g, and washed twice with isotonic NaCl solution (9 g per L). The crude biomass was then dried at +105 °C for 24 hours to constant weight. The CHN analysis of dry biomass samples was performed using a Flash EA 1112 (Thermo Finnigan, Italy) at the centre of collective usage of the Russian Technological University (Moscow). Acetanilide was used as a standard.

RESULTS AND DISCUSSION

The maximum density of *C. closterium* culture ($B_{max} = 3$ g per L) was observed on the 6th day of the experiment (Fig. 1), and the maximum increase ($P_{max} = 1.254$ g$\cdot$L$^{-1}\cdot$day^{-1}) was observed on the 5th day of the experiment. According to the results of CHN analysis, the proportion of carbon in *C. closterium* dry biomass was of 23 %.

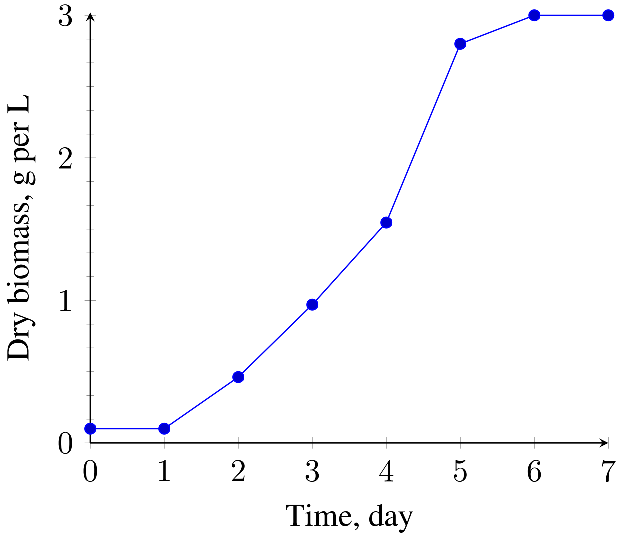

Fig. 1. Dynamics of density of *Cylindrotheca closterium* batch culture on a nutrient medium RS [16]

Extensive literature is concerned with the study of the carbon nutrition of microalgae cultures, and the researchers use different terminology in their publications, for example, "carbon binding", "carbon utilization", "usage" "assimilation", "adsorption", etc. [6, 7, 8, 12, 13]. In some cases, this leads to an obscure understanding of the processes studied; therefore, to avoid ambiguity, we give definitions of the terms used in this paper.

The inorganic carbon flow is the amount of inorganic carbon carried per unit of time. The units are mol per s, kg per s, and L per min.

The inorganic carbon flow density is the amount of carbon supplied to a suspension of microalgae (per unit of volume or unit of phase interface area) per unit of time. The units are mol·L^{-1}·day^{-1}, mol·m^{-2}·day^{-1}, g·L^{-1}·day^{-1}, and g·m^{-2}·day^{-1}.

In algological practice, a mixture of air and CO_2 is usually used to provide algae with carbon; therefore, *the carbon flow density* is expressed in the volume of the air-gas mixture supplied to the microalgae culture per unit of volume of suspension in one minute and is denoted as the ventilation coefficient of the culture, L·L^{-1}·min^{-1}. Given the molar volume of the gas at a given temperature, the carbon flow is easily expressed in mass units. For example, when a 2 % gas-air mixture is supplied (*i. e.* taking into account carbon in air of 2.04 % vol.) at the rate of 1 L per 1 L of suspension per minute at a temperature of +20 °C, the carbon flow density is of 10.2 mg per L of suspension in minute.

The assimilation (binding, utilization) of inorganic carbon by a microalgae culture is a set of biological processes in a suspension of microalgae, as a result of which inorganic carbon is converted into organic substances. At the same time, bound carbon is a part of organic substances, both biomass itself and exometabolites of microalgae (exopolysaccharides, proteins, etc.).

The fixation of inorganic carbon by microalgae culture is a combination of carbon assimilation and carbon-concentrating mechanism in microalgae cells. In addition to bound organic carbon, a certain amount of inorganic carbon is present in biomass. If carbon fixation is considered only in biomass (excluding exometabolites), then the carbon fixation rate (F_C^b) is determined by the expression:

$$F_C^b = c \times P\,, \tag{1}$$

where c is the carbon fraction in the biomass;

P is the growth rate of biomass (productivity), g·L^{-1}·day^{-1}.

The inorganic carbon absorption by a microalgae culture is a combination of the processes of fixation and physico-chemical absorption of inorganic carbon (solubility of CO_2 in the culture medium, formation of HCO_3^- and CO_3^{2-}).

The efficiency of carbon absorption by a microalgae culture is the ratio of the mass of inorganic carbon absorbed by the microalgae culture to the mass of carbon supplied to the microalgae suspension:

$$E_C = (F_0 - F)/F_0 \times 100\% \,, \tag{2}$$

where F_0 and F are the carbon flow density at the inlet for the cell suspension and at the outlet, respectively.

The efficiency of carbon fixation in the microalgae biomass is the ratio of the mass of carbon fixed in the biomass to the mass of carbon supplied to the suspension of microalgae:

$$E_C^b = F_C^b/F_0 \times 100\% \,, \tag{3}$$

where F_C^b is the rate of carbon fixation in the microalgae biomass;

F_0 is the carbon flow density at the inlet for the cell suspension.

When CO_2 is used to provide the culture with inorganic carbon, then, taking into account (1), the rate of carbon fixation in biomass ($F_{CO_2}^b$) can be calculated as follows:

$$F_{CO_2}^b = M(CO_2)/M(C) \times F_C^b = 44/12 \times c \times P \,, \tag{4}$$

where $M(CO_2)$ and M(C) are the molar mass of carbon dioxide and carbon, respectively, g per mol.

From formulas (1) and (4), the limiting ratio follows, indicating the minimum CO_2 flow density (g·L^{-1}·day^{-1}), which is necessary to ensure a given microalgae growth rate:

$$F_C^b = M(C)/M(CO_2) \times F_{CO_2}^b = c \times P \,,$$

i. e.

$$P = 0.273/c \times F_{CO_2}^b \,. \tag{5}$$

For those cases when a gas-air mixture with a given percentage of CO_2 is fed into the suspension, expression (5) is converted to the following:

$$P = \frac{0.273}{c} \frac{M(CO_2)}{V_{CO_2}(T)} \frac{\nu}{100} 1440 F_{\mathrm{GA}}^{\mathrm{min}} \,, \tag{5a}$$

where $V_{CO_2}(T)$ is the molar volume of gas at a given temperature, L per mol;

ν is the proportion of CO_2 in the gas-air mixture, % vol.;

$F_{\mathrm{GA}}^{\mathrm{min}}$ is the minimum gas-air mixture flow density necessary to ensure a given microalgae growth rate, L·L^{-1}·min^{-1}.

At a temperature different from +273 °K, the gas volume is equal to:

$$V_{CO_2}(T) = \frac{V_0}{T_0} T = 0.082T \,, \tag{6}$$

where V_0 is the molar volume of gas under normal conditions (V_0 = 22.4 L per mol at T_0 = +273 °K).

Using expressions (3), (5a), and (6), we can calculate *the efficiency of carbon fixation in the biomass* of any species of cultivated algae by the formula:

$$E_C^b = \frac{c \times P}{0.273 \frac{M(CO_2)}{0.082T} \frac{\nu}{100} 1440 F_{\mathrm{GA}}} 100\% \,, \tag{7}$$

where F_{GA} is the gas-air mixture flow density at the inlet for the cell suspension, $\mathrm{L \cdot L^{-1} \cdot min^{-1}}$.

Concerning our experiment with the cultivation of *C. closterium* under the conditions of cumulative cultivation, *the rate of carbon fixation* from the 4th to the 5th day of the experiment was the following: $F_C^b = 0.23 \times 1.254 = 0.29\ \mathrm{g \cdot L^{-1} \cdot day^{-1}}$.

The efficiency of carbon fixation in biomass was:

$$E_C^b = \frac{0.23 \times 1.254}{0.273 \frac{44}{0.082 \times (273+19)} \frac{0.0405}{100} 1440 \times 1.1} 100\% \approx 90\% \,.$$

For comparison: green microalga *Chlorella protothecoides* and *Ch. vulgaris* are characterized by the efficiency of CO_2 fixation in biomass of 20 % and 55.3 %, respectively; cyanobacteria *Spirulina* sp. – of 38 % [7, 10]; red microalga *Porphyridium purpureum* – of 69 % [6]. So, compared with other algae species, *C. closterium* is characterized by a rather high CO_2 fixation efficiency.

It is important to note that in calculating the carbon fixation efficiency by a microalgae culture, many researchers do not take into account increase in gas volume with increasing temperature in accordance with formula (6). Typically, the calculations use values for standard conditions, although the experimental conditions with a microalgae culture are not standard; it leads to erroneous results. Thus, if experiments with *C. closterium* culture are carried out at a temperature of +30 °C, and the calculation of the carbon fixation efficiency is made for standard conditions (0 °C), then the values obtained can be significantly underestimated. This can be easily verified by substituting the temperature values in formula (7). For the standard temperature (0 °C), the carbon fixation efficiency E_C^b is of 84 %, and for +30 °C it is of 93 %.

In practice of intensive microalgae cultivation, it is often necessary to calculate the cost of CO_2 for obtaining a unit of biomass. Assuming that all carbon entering the microalgae suspension is converted to organic matter, it is possible to estimate the cost of CO_2 using the limiting ratio (5a). It should be noted that the concentration of carbon dioxide in the atmosphere in 2018 according to the World Meteorological Organization reached 0.0405 % vol. [3].

Thus, substituting the data of our experiment in (5a), we see the following: to ensure an increase of 1 g of *C. closterium* dry biomass per day at a temperature of +19 °C, it is necessary to consume a minimum of 0.46 L of CO_2, or 1132 L of air.

Conclusion. Under equal cultivation conditions in terms of light and carbon availability, the productivity of *C. closterium* can exceed the productivity of other types of microalgae by 5–10 times. So, while *Spirulina* sp. productivity reaches $0.2\ \mathrm{g \cdot L^{-1} \cdot day^{-1}}$, *C. closterium* productivity is of $1.254\ \mathrm{g \cdot L^{-1} \cdot day^{-1}}$. Possibly, it is high carbon fixation efficiency, as well as low carbon fraction in *C. closterium* biomass that explains the high production indices of this species.

This work was carried out within the framework of government research assignment of IBSS RAS "Investigation of the mechanisms of controlling production processes in biotechnological complexes with the aim of developing the scientific foundations for the production of biologically active substances and technical products of marine genesis" (no. AAAA-A18-118021350003-6) with financial support of the RFBR grant no. 18-34-00672.

REFERENCES

1. Gevorgiz R. G., Zheleznova S. N., Nikonova L. L., Bobko N. I., Nekhoroshev M. V. *Otsenka plotnosti kul'tury fototrofnykh mikroorganizmov metodom iodatnoi okislyaemosti.* Sevastopol, 2015, 31 p. (Priprint / Kovalevsky Institute of Marine Biological Research of RAS). (in Russ.) http://repository.marine-research.org/handle/299011/43
2. Gevorgiz R. G., Zheleznova S. N., Zozulya Yu. V., Uvarov I. P., Repkov A. P., Lelekov A. S. Industrial production technology biomass marine diatoms *Cylindrotheca closterium* (Ehrenberg) Reimann & Lewin using gas vortex photobioreactor. *Aktual'nyye voprosy biologicheskoy fiziki i khimii*, 2016, no. 1–1, pp. 73–77. (in Russ.)
3. *Gosudarstvennyi doklad "O sostoyanii i ob okhrane okruzhayushchei sredy Rossiiskoi Federatsii v 2018 godu".* Moscow : Minprirody Rossii ; NPP "Kadastr", 2019, 844 p. (in Russ.)
4. Zheleznova S. N. Production characteristics of the diatom *Cylindrotheca closterium* (Ehrenb.) Reimann et Lewin grown in an intensive culture at various nitrogen sources in the medium. *Morskoj biologicheskij zhurnal*, 2019, vol. 4, iss. 1, pp. 33–44. (in Russ.) https://doi.org/10.21072/mbj.2019.04.1.04
5. Zheleznova S. N., Gevorgiz R. G., Bobko N. I., Lelekov A. S. The culture medium for the intensive culture of diatomic alga *Cylindrotheca closterium* (Ehrenb.) Reimann et Lewin – promising biotech facility. *Aktual'naya biotekhnologiya*, 2015, no. 3 (14), pp. 46–48. (in Russ.)
6. Lelekov A. S., Gudvilovich I. N., Gevorgiz R. G., Trenkenshu R. P., Badisova A. O. Evaluation of carbon absorption coefficient by *Porphyridium purpureum* (Bory) Ross. In: *Morskie biologicheskie issledovaniya: dostizheniya i perspektivy* : v 3-kh t. : sb. materialov Vseros. nauch.-prakt. konf. s mezhdunar. uchastiem, priuroch. k 145-letiyu Sevastopol'skoi biologicheskoi stantsii, Sevastopol, 19–24 Sept., 2016. Sevastopol, 2016, vol. 3, pp. 404–407. (in Russ.)
7. Klinthong W., Yang Y.-H., Huang C.-H., Tan C.-S. A review: Microalgae and their applications in CO_2 capture and renewable energy. *Aerosol and Air Quality Research*, 2015, vol. 15, iss. 2, pp. 712–742. https://doi.org/10.4209/aaqr.2014.11.0299
8. Kroth P. G., Chiovitti A., Gruber A., Martin-Jezequel V., Mock T., Parker M. S., Stanley M. S., Kaplan A., Caron L., Weber T., Maheswari U., Armbrust E. V., Bowler C. A. Model for carbohydrate metabolism in the diatom *Phaeodactylum tricornutum* deduced from comparative whole genome analysis. *PLoS One*, 2008, vol. 3, iss. 1, article e1426. https://doi.org/10.1371/journal.pone.0001426
9. Lauritano C., Ferrante M. I., Rogato A. Marine natural products from microalgae: An -omics overview. *Marine Drugs*, 2019, vol. 17, iss. 5, pp. 269 (18 p.). https://doi.org/10.3390/md17050269
10. Liu S., Elvira P., Wang Y., Wang W. Growth and nutrient utilization of green algae in batch and semicontinuous autotrophic cultivation under high CO_2 concentration. *Applied Biochemistry and Biotechnology*, 2019, vol. 188, iss. 3, pp. 836–853. https://doi.org/10.1007/s12010-018-02940-9
11. Martínez Andrade K. A., Lauritano C., Romano G., Ianora A. Marine microalgae with anti-cancer properties. *Marine Drugs*, 2018, vol. 16, iss. 5, pp. 165 (17 p.). https://doi.org/10.3390/md16050165
12. Matsuda Y., Hopkinson B. M., Nakajima K., Dupont C. L., Tsuji Y. Mechanisms of carbon dioxide acquisition and CO_2 sensing in marine diatoms: A gateway to carbon metabolism. *Philosophical Transactions of the Royal Society B*, 2017, vol. 372, pp. 1–12. https://doi.org/10.1098/rstb.2016.0403

13. Obata T., Fernie A. R., Nunes-Nesi A. The central carbon and energy metabolism of marine diatoms. *Metabolites*, 2013, vol. 3, iss. 2, pp. 325–346. https://doi.org/10.3390/metabo3020325
14. Priyadarshani I., Rath B. Bioactive compounds from microalgae and cyanobacteria: Utility and applications. *International Journal of Pharma Sciences and Research*, 2012, vol. 3, iss. 11, pp. 4123–4130. https://doi.org/10.13040/IJPSR.0975-8232.3(11).4123-30
15. Quigg A., Finkel Z. V., Irwin A. J., Rosenthal Y., Ho T.-Y., Reinfelder J. R., Schofield O., Morel F. M., Falkowski P. G. The evolutionary inheritance of elemental stoichiometry in marine phytoplankton. *Nature*, 2003, vol. 425, pp. 291–294. https://doi.org/10.1038/nature01953
16. Ryabushko V. I., Zheleznova S. N., Gevorgiz R. G., Bobko N. I., Lelekov A. S. The medium for intensive culture of the diatom *Cylindrotheca closterium* (Ehrenb.) Reimann et Lewin (Bacillariophyta). *International Journal on Algae*, 2016, vol. 18, iss. 3, pp. 279–286. https://doi.org/10.1615/InterJAlgae.v18.i3.70
17. Suman K., Kiran T., Devi U. K., Sarma N. S. Culture medium optimization and lipid of *Cylindrotheca*, a lipid- and polyunsaturated fatty acid-rich pennate diatom and potential source of eicosapentaenoic acid. *Botanica Marina*, 2012, vol. 55, iss. 3, pp. 289–299. https://doi.org/10.1515/bot-2011-0076
18. Wang S., Verma S. K., Said I. H., Thomsen L., Ullrich M. S., Kuhnert N. Changes in the fucoxanthin production and protein profiles in *Cylindrotheca closterium* in response to blue light-emitting diode light. *Microbial Cell Factories*, 2018, vol. 17, article no. 110 (13 p.). https://doi.org/10.1186/s12934-018-0957-0
19. Ying L., Kang-sen M., Shi-chun S. Effects of harvest stage on the total lipid and fatty acid composition of four *Cylindrotheca* strains. *Chinese Journal of Oceanology and Limnology*, 2002, vol. 20, iss. 2, pp. 157–161. https://doi.org/10.1007/BF02849653
20. Zhang H., Tang Y., Zhang Y., Zhang S., Qu J., Wang X., Kong R., Han C., Liu Z. Fucoxanthin: A promising medicinal and nutritional ingredient. *Evidence-Based Complementary and Alternative Medicine*, 2015, Article ID 723515 (10 p.) http://dx.doi.org/10.1155/62015/723515
21. Zheleznova S. N., Gevorgiz R. G., Nekhoroshev M. V. Conditions optimization of the *Cylindrotheca closterium* (Ehrenberg) Reimann et Lewin cultivation in order to obtain a high yield of fucoxanthin. In: *3rd Russian Conference on Medicinal Chemistry*, Kazan, 28 Sept. – 03 Oct., 2017 : Abstr. book. Kazan : Kazan Federal University, 2017, pp. 261.

ЭФФЕКТИВНОСТЬ ФИКСАЦИИ УГЛЕРОДА В БИОМАССЕ *CYLINDROTHECA CLOSTERIUM* (EHRENBERG) REIMANN & J. C. LEWIN (BACILLARIOPHYCEAE) В УСЛОВИЯХ НАКОПИТЕЛЬНОГО КУЛЬТИВИРОВАНИЯ

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Эффективность утилизации углерода является важной характеристикой объекта культивирования. Известно, что диатомовая водоросль *Cylindrotheca closterium* (Ehrenberg) Reimann & J. C. Lewin может эффективно использовать углерод из водной среды, так как имеет множество уникальных карбоноангидраз и углеродных транспортёров. Между тем эффективность фиксации углерода для многих видов диатомей в культуре по-прежнему неизвестна. Для её расчёта ряд авторов используют разную терминологию и способы, что приводит к значительным трудностям при сравнении эффективности фиксации углерода в биомассе различных видов микроводорослей. Цели работы: 1) на основе современных представлений о фиксации углерода в биомассе микроводорослей, а также о поглощении неорганического углерода культурой микроводорослей актуализировать используемые в литературе термины и определения; 2) оценить эффективность фиксации углерода в биомассе диатомеи *C. closterium* в условиях накопительного культивирования. Культуру *C. closterium* выращивали при температуре +20 °C в интенсивном режиме на питательной среде RS. В процессе выращивания культуру барботировали воздухом (1,1 л воздуха на 1 л культуры в минуту). Температура воздуха на выходе из суспензии составляла +19 °C, максимальная продуктивность культуры — 1,254 $г{\cdot}л^{-1}{\cdot}сут^{-1}$. По результатам CHN-анализа, доля углерода в сухой биомассе *C. closterium* составляла 23 %. В условиях накопительного культивирования у *C. closterium* эффективность фиксации углерода в биомассе достигла 90 %. По сравнению с другими видами водорослей *C. closterium* характеризуется достаточно высокой эффективностью фиксации CO_2. Так, у зелёных микроводорослей *Chlorella protothecoides* и *Ch. vulgaris* эффективность фиксации CO_2 составляет 20 % и 55,3 % соответственно, у цианобактерии *Spirulina* sp. — 38 %, у красной микроводоросли *Porphyridium purpureum* — 69 %. Отмечено, что для обеспечения прироста 1 г сухой биомассы *C. closterium* в сутки при температуре +19 °C необходимо затратить минимум 0,46 л CO_2, или 1132 л воздуха. Возможно, именно высокая эффективность фиксации углерода, а также низкая доля углерода в биомассе *C. closterium* позволяют объяснить высокие продукционные показатели этого вида. В равных условиях культивирования по свету и обеспеченности углеродом продуктивность *C. closterium* может превышать продуктивность других видов микроводорослей в 5–10 раз. Так, у *Spirulina* sp. продуктивность достигает 0,2 $г{\cdot}л^{-1}{\cdot}сут^{-1}$, у *C. closterium* — 1,254 $г{\cdot}л^{-1}{\cdot}сут^{-1}$.

Ключевые слова: диатомовая водоросль *Cylindrotheca closterium*, культивирование, эффективность фиксации углерода

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ИнБЮМ – IBSS

Морской биологический журнал, 2020, том 5, № 1, с. 20–28
Marine Biological Journal, 2020, vol. 5, no. 1, pp. 20–28
https://mbj.marine-research.org; doi: 10.21072/mbj.2020.05.1.03
ISSN 2499-9768 print / ISSN 2499-9776 online

UDC 582.261.1:581.16:551.465.62

SALINITY OPTIMA FOR VEGETATIVE GROWTH AND SEXUAL REPRODUCTION OF THE DIATOM *TOXARIUM UNDULATUM*

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Received by the Editor 03.09.2019; after revision 03.09.2019;
accepted for publication 27.03.2020; published online 31.03.2020.

Distribution of diatom algae is limited by their tolerance to environmental factors. Although a genus *Toxarium* has been evolving for more than 100 million years, it is represented by only two species. *Toxarium undulatum* is widely spread in tropical and subtropical seas, and it can be also found in the Black Sea, the salinity of which is twice lower than the oceanic one. Ecological and psychological characteristics research of this species is of great interest in terms of its relationship to salinity. *T. undulatum* clonal cultures were sampled in the Donuzlav Lake connected to the Black Sea (southwest of the Crimean Peninsula) and on Gran Canaria coast (Canary Islands archipelago). Experiments on the salinity tolerance limits showed, that the Black Sea clones were viable in a range of at least 30 ‰ (12 to 42 ‰). The same wide range of salinity tolerance with slightly higher values was observed among oceanic clones of this species. Optima of vegetative growth and sexual reproduction were determined. Optima of the Black Sea clones appeared to be 27.8 and 27.2 ‰, respectively, which was significantly higher than salinity observed in population habitat. Similar higher optima of vegetative growth and sexual reproduction, compared with those salinity values, at which natural population developed, were observed for a number of other Black Sea diatoms, which proved their oceanic (Mediterranean) origin. It was concluded that *T. undulatum*, along with other species, began to populate the Black Sea basin about seven thousand years ago after Mediterranean Sea water started to flow into the freshened Novoevksinsky Sea-Lake through the Bosporus Strait. However, the evolution rate did not allow bringing physiological and ecological characteristics of the species studied into full agreement with environmental conditions. Oceanic origin is evidently seen in its physiological reactions to salinity. Possibility of speciation due to settlement of the Black Sea with oceanic species is discussed.

Keywords: diatom, *Toxarium undulatum*, evolution, salinity, tolerance limits

One of the largest diatoms, *Toxarium undulatum* Bailey, 1854, can be found in the Black Sea. This species is common for tropical and subtropical seas [22] characterized by a higher salinity than the Black Sea. It has previously been shown that a number of diatom species found in the Black Sea have salinity optima for vegetative growth and sexual reproduction higher than in their populations habitats [2, 3]. The most reasonable explanation seems to be possibility of Black Sea basin being populated with originally oceanic species evolutionarily adapted to 35–36 ‰ salinity after Bosporus Strait formation, 7–8 thousand years ago, when the highly desalinated Novoevksinsky Sea-Lake began to fill up by waters coming from the Mediterranean Sea [5, 7]. Study of *T. undulatum* physiological reactions has provided us with new data on this cosmopolitan species, common in all coastal seas of subtropical zone, and its relation to lower (Black Sea) salinity.

MATERIAL AND METHODS

Periphyton samples were collected from a depth of about 40 cm near the eastern shore of the Donuzlav Lake (south-west of the Crimean Peninsula, point with coordinates 45°22′23″N, 33°05′40″E) and on the Gran Canaria Island (Canary Islands archipelago, 27°59′27″N, 15°22′06″W). Single cells given rise to clonal cultures (Fig. 1) were isolated from the samples using glass micropipettes and inverted microscopes Zeiss Primovert (Carl Zeiss AG, Germany) and Nib-100 (China).

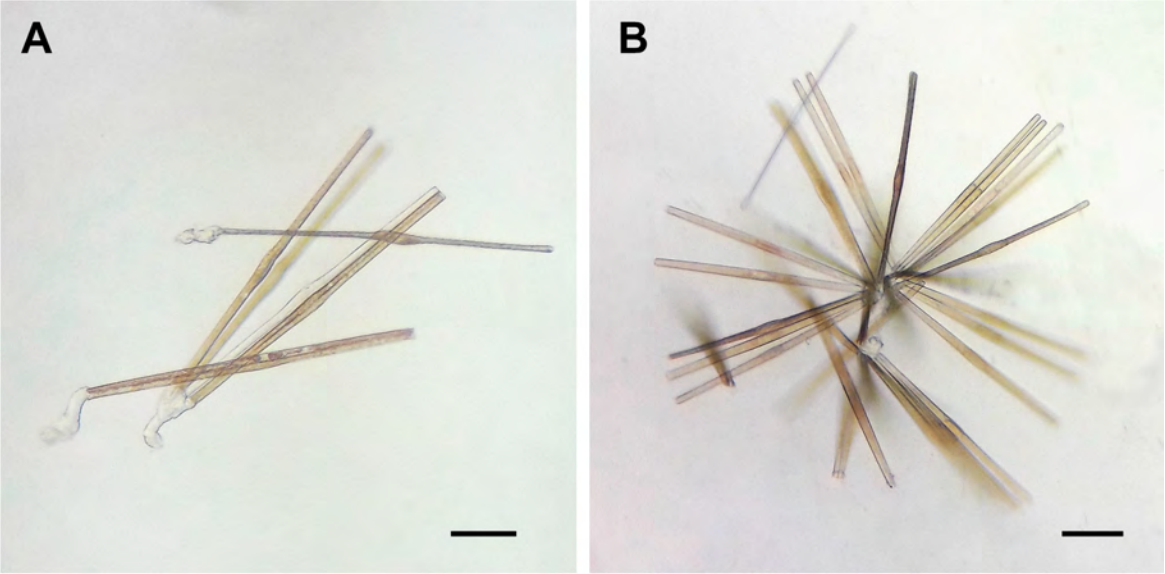

Fig. 1. *Toxarium undulatum*, attached to substrate with the help of mucilage secreted by one of the apical ends of the cell, forms tuft colonies in the process of division. A – clone 8.0827-Y, the Black Sea population; B – clone 9.0620-C, the Canary population. Scale bar is 100 μm

The cultures were maintained in 100-ml Erlenmeyer glass flasks in the modified ESAW medium [9], periodically (every 7–14 days) transferred in the fresh medium. Conditions of maintaining and the method of clones naming are described in [1]. The Black Sea clones were maintained at 20 ‰, The Canary ones – at 30 ‰. In the sampling points, salinity was 20 ‰ and 36 ‰, respectively. Salinity was measured with RHS-10ATC refractometer (China).

After we had succeeded in stimulating sexual reproduction in mixtures of clones from the Black Sea population, two pairs of clones, (8.0820-E + 8.0830-K) and (8.0827-Y + 8.0830-K), were chosen for further experiments. As a result of the last pair crossbreeding, initial cells were obtained. They were characterized by sizes close to a maximum value for the species. Several postinitial cells (average size of 1270 μm) were chosen for the culture and used later in experiments as cells being at the beginning of their life cycle. In addition, clones 8.0822-D, 8.0920-E, and 8.1227-A from the Black Sea population with average cell sizes of 642, 518, and 963 μm, respectively, were used in the experiments. In clones 9.0618-A, 9.0618-E, 9.0620-B, and 9.0621-DD from the Gran Canaria population, the average cells sizes at the time of the experiments were 680, 212, 284, and 334 μm, respectively.

Salinity factor effect on algae was estimated by mortality of cells while transferring them from the medium in which they had been maintained in the collection to the new medium with salinity gradation: 8, 12, 18, 24, 30, 36, 42, 48, 54 ‰. The required salinity was obtained by either diluting the modified ESAW medium (36 ‰) with distilled water, or adding sodium chloride to it. The cells were put into 50-mm diameter glass Petri dishes (bottom area of 494 mm^2). Mortality was determined as a ratio between a number of dead cells and a total number of cells. The cell was regarded as dead based on visual estimation of the inner content state, mainly chloroplasts (their color, location, or complete absence). The number of cells was counted in ten Nib-100 microscope fields of view (field of view of 0.88 mm^2) at 20× lens and 10× eyepiece magnification within five days: on the first, second and fifth days of the experiment – for the Black Sea clones, on the first, second, fourth and fifth days – for the Canary clones. The average death rate for each clone was calculated for all days of the experiment.

To determine the optima, we used a second-order equation approximating the data obtained. Coefficients of the equation were calculated by the least squares method [6]. The value of the argument of the first derivative of this equation, equaled to zero, determined the position of the optimum.

RESULTS

Both the Black Sea and oceanic *T. undulatum* populations demonstrated wide salinity tolerance, with tolerance range of at least 30 ‰ (Fig. 2).

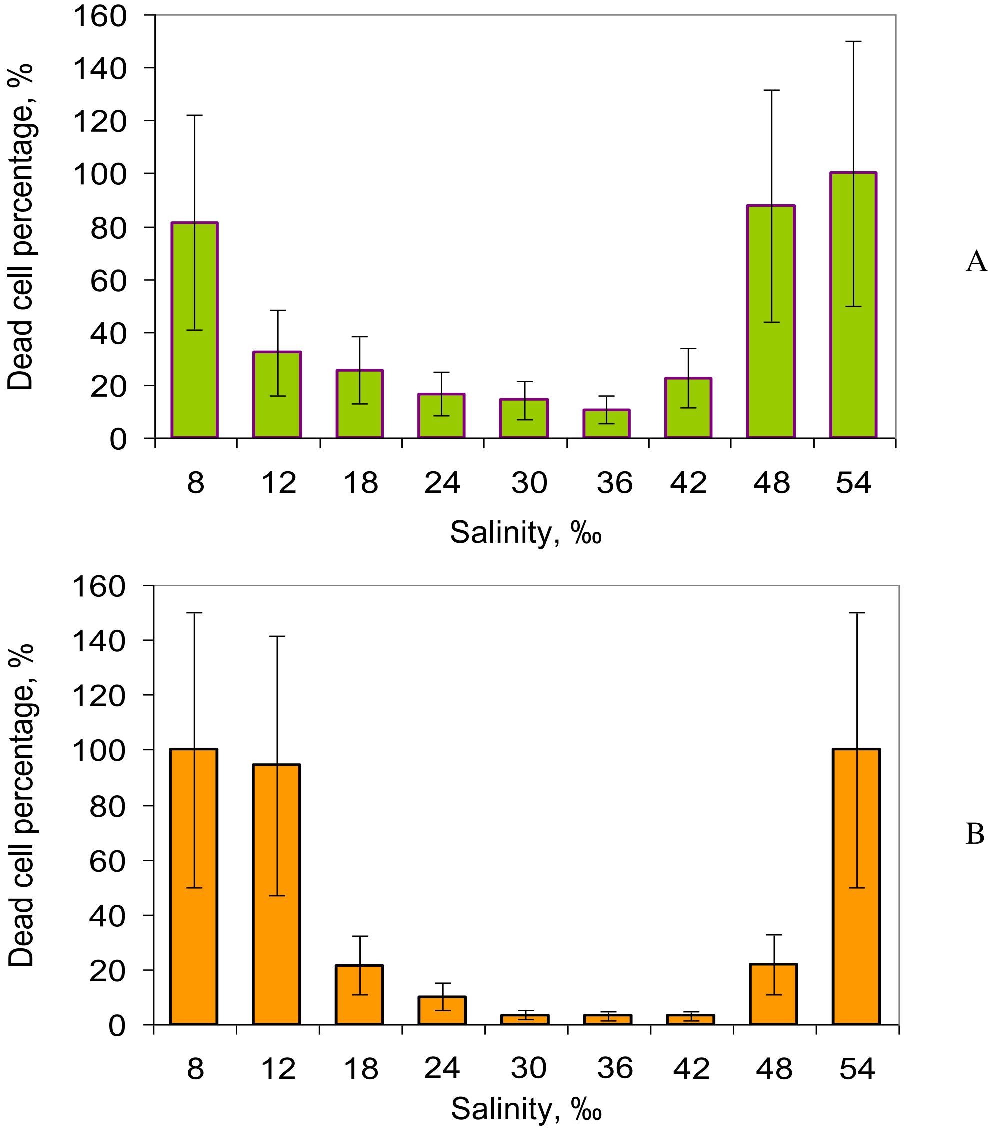

Fig. 2. Relative number of dead *Toxarium undulatum* cells when transferred from medium with salinity of 20 ‰ (A, the Black Sea population) and 30 ‰ (B, the Canary population) to the new medium with different salinity levels (on average for four clones for five days of growth in the new medium)

The Black Sea clones were viable in a range 12 to 42 ‰, possibly slightly wider, taking into account 6 ‰ gradation step set in the experiments. The Canary clones tolerated salinity 18 to 48 ‰. A shift towards higher salinity, which was observed in the oceanic clones compared with the Black Sea ones, could be explained by the fact that before experiments, they had been grown at different salinity levels (30 and 20 ‰, respectively). However, some authors believe that preliminary adaptation of diatoms to salinity levels extreme for these species cannot result in a noticeable increase of tolerance limits [8].

For the Black Sea clones, a relationship was found between the cells size and the number of dead cells, when transferred into the new medium, on average for five days of growth at nine experimental salinity levels (Fig. 3). The largest cells, arose as a result of the reproductive process and being at the beginning of the life cycle, turned out to be the most sensitive to changes in growth conditions. This dependence was not observed among the Canary clones. It should be noted, however, that their sexual reproduction has not been studied, and the largest cells at the beginning of the life cycle have not been obtained.

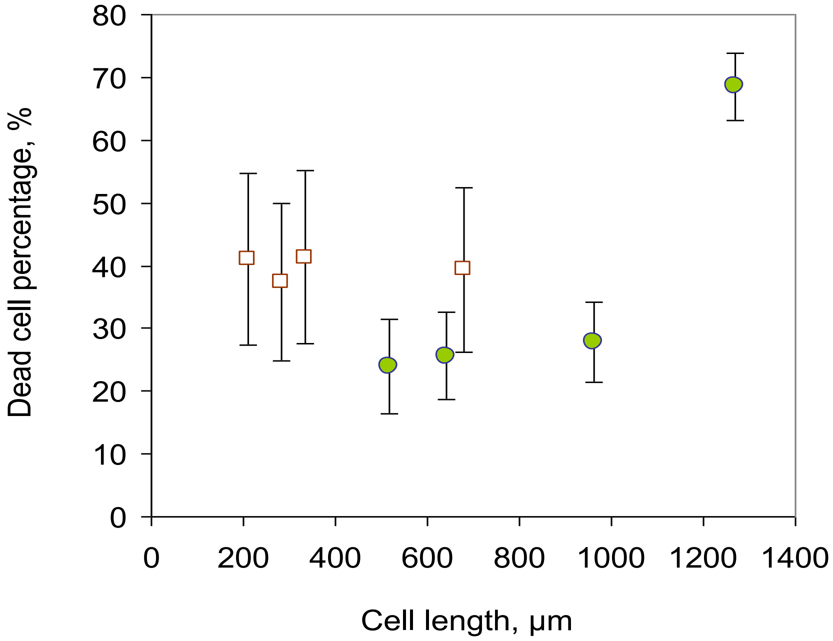

Fig. 3. Mortality of *Toxarium undulatum* cells depending on their apical length (when transferred to a new culture medium, on average for nine salinity levels); ☐ are the Canary clones; ● are the Black Sea clones

The salinity optimum for vegetative growth of the Black Sea *T. undulatum* population was of 27.8 ‰, while for vegetative growth of the Canary clones – of 32.4 ‰. The optimal salinity for sexual reproduction of the Black Sea clones was of 27.2 ‰ (Fig. 4).

DISCUSSION

Morphology and way of life of four genera – *Ardissonea* De Notaris, *Climacosphenia* Ehrenberg, *Synedrosphenia* (H. Peragallo) Azpeitia Moros, and *Toxarium* J. W. Bailey (family Toxariales Round), named also toxariids, – are in evident disagreement with data on genosystematics, which places them among polar centric diatoms [19, 20, 21, 24, and others]. Distinction and evolutionary uniqueness of this group have recently been confirmed by results of study of *Ardissonea crystallina* (C. Agardh) Grunow reproductive biology. The mode of reproduction of this alga was not oogamous, which is typical for all centric diatoms studied by far, but was similar to that of pennates [13]. Judging by molecular phylogeny [21],

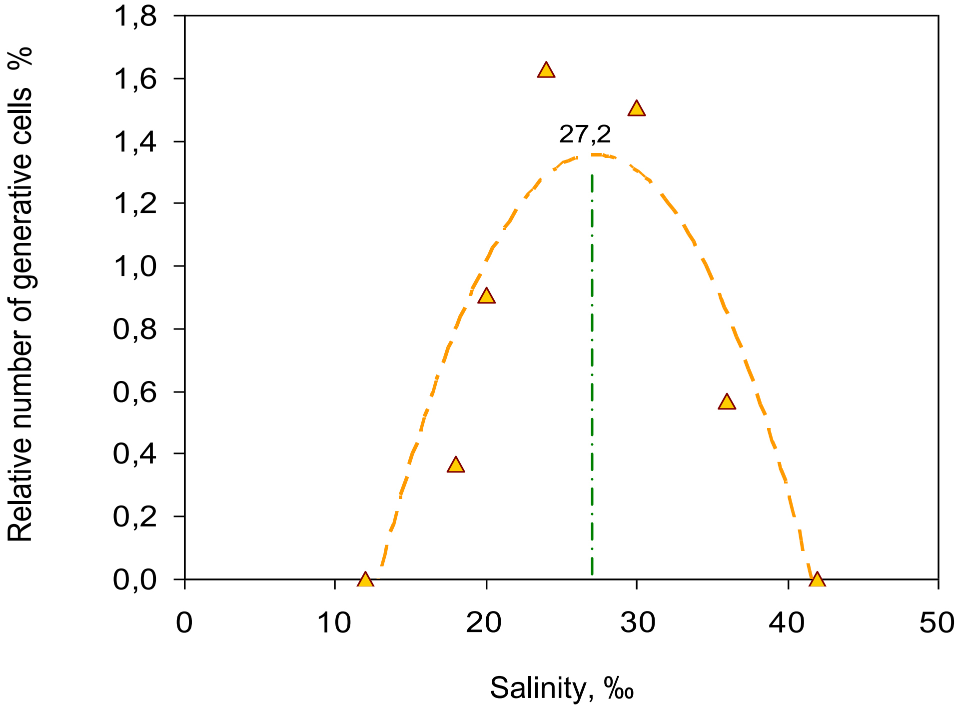

Fig. 4. Relative number of generative cells in mixtures of reproductively compatible *Toxarium undulatum* clones depending on a salinity level of a medium. Approximation was performed by a second-degree polynomial. A dash-dotted line indicates an optimum position

it can be concluded, that evolutionary line of toxariids separated from its ancestors at the turn of the Cretaceous and Jurassic periods about 150 million years ago; 35 million years later, *Toxarium* separated from *Ardissonea* and *Climacosphenia*. It should be noted, that there are only two species in the genus *Toxarium* [22]. This testifies a significant conservativeness of its genetic composition: for more than 100 million years, it has changed a little. At least, those changes have not resulted in occurring new, morphologically distinguishable species. Therefore, it can be assumed that inhabiting new places that differ in salinity level, *Toxarium* retains its features developed by millions of years of evolution, which at the physiological level are shown in certain physiological optima. With evolutionary adaptation to new salinity, a shift in tolerance limits and physiological (ecological) optima is inevitable; the question is the tempo of such changes.

Are 7–8 thousand years a sufficient period for evolutionary changes that may lead not only to emergence of new properties but also to emergence of new diatom species? The answer can be positive, if we take a complex of cryptic species of the genus *Haslea* as an example [17, 18]. *H. karadagensis* recently described by us [16] is a Black Sea endemic [18]. Moreover, this species has tolerance limits and physiological optima proving its oceanic origin: for its growth, 25–30 ‰ were optimal, and sexual reproduction did not occur in the media with salinity below 17 ‰ [14].

T. undulatum is widely tolerant in relation to salinity. The cells remained viable (in varying degrees) in salinity range 12 to 42 ‰; sexual reproduction occurred in a narrower range. Such a wide range with an optimum of about 30 ‰ proves an assumption that it was the relative euryhalinity that allowed the species to inhabit new place after appearance of straits connecting the Black and the Mediterranean seas. It is unlikely that the ancestral Mediterranean (oceanic) population was stenohaline, and the tolerance range of the Black Sea population has expanded significantly due to evolutionary changes over a relatively short period (7–8 thousand years). This can also be confirmed by results obtained while studying the position of physiological optima and tolerance limits of the oceanic clones sampled by us from Gran Canaria coast and determined morphologically as *T. undulatum*. Unlike the salinity of the Black Sea, the salinity of this part of the World Ocean has been unchanged for many millions of years; however, the selected clones had similar wide range of tolerance as the Black Sea ones, and their optima for growth has not differed significantly.

Any conclusions regarding possible reproductive barriers between the Black Sea population of *T. undulatum* and populations from other parts of the World Ocean are too premature before crossing experiments are performed.

The size of cells is known to determine a phase of their life cycle [22]. *A priori*, before position of the upper limit of size range of auxospore formation is identified, one can claim, based on the general principles [12], that cells of clones 8.0822-D and 8.0920-E were in the generative phase, while cells of clone 8.1227-A did not reach the size that allows entering sexual process. Correlation between resistance to a change of the medium salinity and cell size was observed in four studied clones from the Black Sea: small cells turned out to be more resistant to abrupt salinity changes (Fig. 2). However, final conclusion about the dependence of salinity tolerance on a life cycle phase is premature, first of all, due to small amount of data obtained, and, in addition, due to a possible clone-specific response. In many experiments, we faced clone specificity; this concerned a wide range of characteristics, from an ability to synthesize and accumulate certain substances (for example, pigments in species of the genus *Haslea*) to an ability to enter sexual process and an intensity of auxosporulation. It should be noted, that in the closely related species, *A. crystallina*, on the contrary, larger cells adapted more quickly to a transfer from a medium with salinity of 20 ‰ to a medium with salinity of 12 ‰ [15].

With these differences, it is important to note the following general pattern: for all Black Sea diatoms investigated to date, salinity optima for vegetative growth and sexual reproduction were higher than salinity of 17–18 ‰ typical for the Black Sea near the Crimean coast (Table 1). An excess was of 8–12 ‰ or more. An evidence, that Black Sea diatoms divide faster at salinity levels higher than Black Sea one, is not new (for example, [4, 8, 10]). Such a discrepancy between physiological (ecological) optimal values and conditions, typical for population habitats of the species under study (however, see different opinion [10]), clearly shows that these species entered the Black Sea but an evolution rate has not allowed them to bring their physiological and ecological characteristics into full agreement with environmental conditions.

Table 1. Salinity levels, favorable for vegetative growth and sexual reproduction of some Black Sea diatoms

Species	Optima for vegetative growth (tolerance limits), ‰	Optima for sexual reproduction, ‰	Source of data
Ardissonea crystallina	29 (9–45)	30	[15]
Climaconeis scalaris	30 (8–48)	30	[3]
Haslea karadagensis	25–30 (8–45*)	25–30	[14]
Nitzschia longissima	27 (8–45)	30	[23]
Tabularia tabulata	30 (2,25–49*)	30	[2]
Toxarium undulatum	28 (8–48)	27	present paper

Note: * – higher salinity values were not checked in experiments.

The compliance with these tolerance range conditions, related not only to vegetative growth, but also to sexual reproduction, is extremely important for the species to exist under certain salinity conditions. Sexual reproduction for the overwhelming majority of diatoms is known to be the essential stage in their life cycle [11, 22]. The next generation may not appear due to inappropriate environmental conditions. *T. undulatum* demonstrated an ability to reproduce sexually in sufficiently wide ranges of salinity. However, in comparison with vegetative growth range, a salinity tolerance range in case of sexual reproduction was narrower. The optimum is much higher than the salinity of the Black Sea and the Donuzlav Lake (the sampling point).

Thus, the data obtained testifies, that *T. undulatum*, along with many other species, populated the Black Sea basin in the post-novoevksinsky period. In its physiological reactions with respect to salinity of the environment, an oceanic origin is seen. An objective of subsequent research will be to study the reproductive relations of the Black Sea and oceanic populations and to determine presence or absence of the reproductive barriers between them.

This work was supported by a grant of the Russian Foundation for Basic Research "Study of the species-specificity of the evolutionary transition from oogamy to neoogamous sexual reproduction in polar centric diatoms (Mediophyceae)" (no. 19-04-00070_a).

REFERENCES

1. Davidovich N. A., Davidovich O. I., Podunai Yu. A. Diatom culture collection of the Karadag scientific station (Crimea). *Morskoj biologicheskij zhurnal*, 2017, vol. 2, no. 1, pp. 18–28. (in Russ.) https://doi.org/10.21072/mbj.2017.02.1.03
2. Davidovich O. I. Vliyanie solenosti na vegetativnyi rost i polovoe vosproizvedenie diatomovoi vodorosli *Tabularia tabulata* (C. A. Agardh) Snoeijs. In: *Aktual'nye problemy bioraznoobraziya i prirodopol'zovaniya* : materialy Vserossiiskoi nauchno-prakticheskoi konferentsii, Kerch, 26 Sept. – 1 Oct., 2017. Simferopol : IT "ARIAL", 2017, pp. 38–43. (in Russ.)
3. Davidovich O. I., Davidovich N. A. Vliyanie solenosti sredy na vegetativnyi rost i polovoe vosproizvedenie dvukh vodoroslei iz roda *Climaconeis* (Bacillariophyta). In: *Morskie biologicheskie issledovaniya: dostizheniya i perspektivy* : v 3-kh t. : sbornik materialov Vserossiiskoi nauchno-prakticheskoi konferentsii s mezhdunarodnym uchastiem, priurochennoi k 145-letiyu Sevastopol'skoi biologicheskoi stantsii, Sevastopol, 19–24 Sept., 2016 / A. V. Gaevskaya (Ed.). Sevastopol : EKOSI-Gidrofizika, 2016, vol. 1, pp. 166–169. (in Russ.)
4. Dzhafarova S. K. Growth of polygalobe diatoms clone cultures in case of ionic medium composition variation. *Al'gologiya*, 1992. vol. 2, no. 2, pp. 20–24. (in Russ.)
5. Dimitrov P. S., Dimitrov D. P., Solakov D. P., Peichev V. D. Noveishaya geologicheskaya istoriya Chernogo morya i problema potopa. *Geologiya i poleznye iskopaemye Mirovogo okeana*, 2005, vol. 1, pp. 102–111. (in Russ.)
6. Zaitsev G. N. *Matematicheskaya statistika v eksperimental'noi botanike.* Moscow : Nauka, 1984, 424 p. (in Russ.)
7. Zaitsev Yu. P. *Eto udivitel'noe more.* Odessa : Izd-vo "Mayak", 1978, 159 p. (in Russ.)
8. Karaeva N. I., Dzhafarova S. K. Experimental investigations of polygalobic Bacillariophyta in connection with medium salinity. *Al'gologiya*, 1993, vol. 3, no. 2, pp. 97–105. (in Russ.)
9. Polyakova S. L., Davidovich O. I., Podunai Yu. A., Davidovich N. A. Modification of the ESAW culture medium used for cultivation of marine diatoms. *Morskoj biologicheskij zhurnal*, 2018, vol. 3, no. 2, pp. 73–78. (in Russ.) https://doi.org/10.21072/mbj.2018.03.2.06
10. Finenko Z. Z., Lanskaya L. A. Rost i skorost' deleniya vodoroslei v limitirovannykh ob"emakh vody. In: *Ekologicheskaya fiziologiya morskikh planktonnykh vodoroslei (v usloviyakh kul'tur)* / K. M. Khailov (Ed.). Kiev : Naukova dumka, 1971, pp. 22–50. (in Russ.)
11. Chepurnov V. A., Mann D. G., Sabbe K., Vyverman W. Experimental studies on sexual reproduction in diatoms. *International Review of Cytology*, 2004, vol. 237, pp. 91–154. https://doi.org/10.1016/S0074-7696(04)37003-8

12. Davidovich N. A. Species-specific sizes and size range of sexual reproduction in diatoms. In: *Proceedings of the 16th International Diatom Symposium*, Athens & Aegean Islands, 25 Aug. – 1 Sept., 2000 / A. Economou-Amilli (Ed.), 601 p. Athens : University of Athens, Faculty of Biology, 2001, pp. 191–196.
13. Davidovich N. A., Davidovich O. I., Podunay Y. A., Gastineau R., Kaczmarska I., Poulíčková A., Witkowski A. *Ardissonea crystallina* has a type of sexual reproduction that is unusual for centric diatoms. *Scientific Reports*, 2017, vol. 7, no. 1467, pp. 1–16. https://doi.org/10.1038/s41598-017-15301-z
14. Davidovich N. A., Gastineau R., Gaudin P., Davidovich O. I., Mouget J.-L. Sexual reproduction in the newly-described blue diatom, *Haslea karadagensis*. *Fottea*, 2012, vol. 12, iss. 2, pp. 219–229. https://doi.org/10.5507/fot.2012.016
15. Davidovich O. I., Davidovich N. A., Podunay Yu. A., Shorenko K. I., Witkowski A. Effect of salinity on vegetative growth and sexual reproduction of algae from the genus *Ardissonea* de Notaris (Bacillariophyta). *Russian Journal of Plant Physiology*, 2016, vol. 63, no. 6, pp. 776–782. https://doi.org/10.1134/S1021443716060030
16. Gastineau R., Davidovich N. A., Bardeau J.-F., Caruso A., Leignel V., Hardivillier Y., Jacquette B., Davidovich O. I., Rincé Y., Gaudin P., Cox E. J., Mouget J.-L. *Haslea karadagensis* (Bacillariophyta): A second blue diatom, recorded from the Black Sea and producing a novel blue pigment. *European Journal of Phycology*, 2012, vol. 47, iss. 4, pp. 469–479. https://doi.org/10.1080/09670262.2012.741713
17. Gastineau R., Davidovich N., Hansen G., Rines J., Wulff A., Kaczmarska I., Ehrman J., Hermann D., Maumus F., Hardivillier Y., Leignel V., Jacquette B., Meleder V., Hallegraeff G., Yallop M., Perkins R., Cadoret J.-P., Saint-Jean B., Carrier G., Mouget J.-L. *Haslea ostrearia*-like diatoms: Biodiversity out of the blue. In: *Advances in Botanical Research: Sea Plants* / N. Bourgougnon (Ed.). United Kingdom : Academic Press, 2014, vol. 71, pp. 441–465. https://doi.org/10.1016/B978-0-12-408062-1.00015-9
18. Gastineau R., Prasetiya F. S., Falaise C., Cognie B., Decottignies P., Morançais M., Méléder V., Davidovich N., Turcotte F., Tremblay R., Pasetto P., Dittmer J., Bardeau J.-F., Pouvreau J.-B., Mouget J.-L. Marennine-like pigments: Blue diatom or green oyster cult? In: Blue Biotechnology: Production and Use of Marine Molecules. Vol. 1 / S. La Barre, S. S. Bates (Eds). Weinheim : Wiley-VCH Verlag GmbH & Co. KGaA, 2018, chap. 16, pp. 529–551. https://doi.org/10.1002/9783527801718.ch16
19. Kooistra W. H. C. F., De Stefano M., Mann D. G., Salma N., Medlin L. K. Phylogenetic position of *Toxarium*, a pennate-like lineage within centric diatoms (Bacillariophyceae). *Journal of Phycology*, 2003, vol. 39, iss. 1, pp. 185–197. https://doi.org/10.1046/J.1529-8817.2003.02083.X
20. Medlin L. K., Sato S., Mann D. G., Kooistra W. H. C. F. Molecular evidence confirms sister relationship of *Ardissonea*, *Climacosphenia*, and *Toxarium* within the bipolar centric diatoms (Bacillariophyta, Mediophyceae), and cladistic analyses confirm that extremely elongated shape has arisen twice in the diatoms. *Journal of Phycology*, 2008, vol. 44, iss. 5, pp. 1340–1348. https://doi.org/10.1111/J.1529-8817.2008.00560.X
21. Nakov T., Beaulieu J. M., Alverson A. J. Accelerated diversification is related to life history and locomotion in a hyperdiverse lineage of microbial eukaryotes. *New Phytologist*, 2018, vol. 219, iss. 1, pp. 462–473. https://doi.org/10.1111/NPH.15137
22. Round F. E., Crawford R. M., Mann D. G. *The Diatoms. Biology and Morphology of the Genera*. Cambridge : Cambridge University Press, 1990, 747 p.
23. Shorenko K. I., Davidovich O. I., Davidovich N. A. On the taxonomy, reproduction, and distribution of the diatom *Nitzschia longissima* (Brèb.) Grunow (Bacillariophyta). *International Journal on Algae*, 2014, vol. 16, no. 1, pp. 5–28. https://doi.org/10.1615/INTERJALGAE.V16.I1.10
24. Theriot E. C., Ashworth M., Ruck E., Nakov T., Jansen R. K. A preliminary multigene phylogeny of the diatoms (Bacillariophyta): Challenges for future research. *Plant Ecology and Evolution*, 2010, vol. 143, no. 3, pp. 278–296. https://doi.org/10.5091/plecevo.2010.418

ОПТИМУМЫ СОЛЁНОСТИ ДЛЯ ВЕГЕТАТИВНОГО РАЗМНОЖЕНИЯ И ПОЛОВОГО ВОСПРОИЗВЕДЕНИЯ ДИАТОМОВОЙ ВОДОРОСЛИ *TOXARIUM UNDULATUM*

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Распространение диатомовых водорослей ограничивается пределами их толерантности по отношению к факторам среды. Род *Toxarium* представлен всего двумя видами, хотя его эволюция продолжается более 100 млн лет. *Toxarium undulatum* широко распространён в тропических и субтропических морях; он встречается и в Чёрном море, солёность которого вдвое ниже океанической. Представляет интерес изучение эколого-физиологических характеристик этого вида с точки зрения его отношения к солёности. Клоновые культуры *T. undulatum* выделены из проб, собранных в озере Донузлав, сообщающемся с Чёрным морем (юго-запад Крымского полуострова), и в прибрежье острова Гран-Канария (архипелаг Канарских островов). Эксперименты по изучению пределов толерантности по отношению к солёности показали, что черноморские клоны жизнеспособны в диапазоне, охватывающем как минимум 30 ‰ (от 12 до 42 ‰). Такой же широкий диапазон солёностной толерантности, слегка сдвинутый в сторону бо́льших значений, наблюдали у океанических клонов этого вида. Установлены оптимумы для вегетативного роста и полового воспроизведения. У черноморских клонов они оказались равными 27,8 и 27,2 ‰ соответственно, что заметно выше солёности, которая наблюдается в месте обитания популяции. Аналогичное превышение положения оптимумов в отношении вегетативного размножения и полового воспроизведения по сравнению с теми уровнями солёности, в которых находятся природные популяции, было отмечено для ряда других черноморских диатомовых, что свидетельствует об их океаническом (средиземноморском) прошлом. Сделан вывод о том, что *T. undulatum*, наряду с другими видами, начал заселять черноморский бассейн ориентировочно семь тысяч лет назад, после того как в распреснённое Новоэвксинское море-озеро через образовавшийся пролив Босфор стала поступать вода из Средиземного моря. Между тем темпы эволюции не позволили привести физиологические и экологические характеристики изучаемого вида в полное соответствие с условиями окружающей среды. В его физиологических реакциях по отношению к солёности прослеживается океаническое происхождение. Обсуждается возможность видообразования в связи с заселением Чёрного моря океаническими видами.

Ключевые слова: диатомовые, *Toxarium undulatum*, эволюция, солёность, пределы толерантности

Морской биологический журнал, 2020, том 5, № 1, с. 29–42
Marine Biological Journal, 2020, vol. 5, no. 1, pp. 29–42
https://mbj.marine-research.org; doi: 10.21072/mbj.2020.05.1.04
ISSN 2499-9768 print / ISSN 2499-9776 online

UDC 574.64:597.556.35(26)

ORGANOCHLORINE COMPOUNDS IN FLOUNDERS OF GENUS *HIPPOGLOSSOIDES* GOTTSCHE, 1835 FROM THE FAR EASTERN SEAS OF RUSSIA

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Received by the Editor 30.09.2019; after revision 20.11.2019;
accepted for publication 27.03.2020; published online 31.03.2020.

Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) are global superecotoxicants belonging to a group of persistent organic pollutants (POPs). Fish and seafood are an important source of high-grade protein and polyunsaturated fatty acids, especially for residents of coastal areas. Up to 90 % of all pollutants enter the human body through food. Final depot of POPs in environment is marine ecosystems; therefore, POPs can accumulate in various objects of marine fisheries. The paper presents information on the concentrations of OCPs [HCH isomers (α-, β-, γ-), as well as DDT and its metabolites (DDD and DDE)], and polychlorinated biphenyls (PCBs) in muscles of flounders of genus *Hippoglossoides* Gottsche, 1835 from the Far Eastern seas of Russia (the Sea of Okhotsk, the Tatar Strait, and the Sea of Japan). Lipids were extracted from fish tissue samples with a mixture of hexane and acetone, followed by destruction of fatty components by concentrated sulfuric acid. OCPs and PCBs were separated by column chromatography with polar and non-polar solvents. Xenobiotics were quantified by gas chromatography – mass spectrometry. To assess quality of this methodology, a standard addition method was used. The average reproducibility of analyte concentrations varied 94.6 to 103.7 %, and it indicates reliability of the data obtained as well as effectiveness of methods applied. Average concentrations of $\sum$DDT, $\sum$HCH, $\sum$OCP ($\sum$DDT + $\sum$HCH), and $\sum$PCB were: (62 ± 89), (50 ± 52), (100 ± 125), and (92 ± 45) $ng \cdot g^{-1}$ of lipids in the samples from the eastern part of the Sea of Okhotsk; (20 ± 17), (36 ± 37), (54 ± 41), and (99 ± 43) $ng \cdot g^{-1}$ of lipids from the southern part of the Sea of Okhotsk; (40 ± 29), (62 ± 36), (102 ± 50), and (1616 ± 1177) $ng \cdot g^{-1}$ of lipids from the Sea of Japan, respectively. In the samples from the Tatar Strait, the average levels of $\sum$HCH, $\sum$OCP, and $\sum$PCB were (221 ± 182), (224 ± 180), and (455 ± 317) $ng \cdot g^{-1}$ of lipids, respectively. DDT was detected in three samples. In the flounders from the eastern part of the Sea of Okhotsk, the highest concentrations of DDT and average concentrations of HCH were recorded, which may be due to the location of a "repository" of pesticides on the Kamchatka Peninsula, where OCPs are buried. The entrance of PCBs into the waters of the southern part of the Sea of Okhotsk can be associated both with intensive shipping and effluents from landfills that carry residual amounts of PCBs into the ecosystem. The southern part of the Sea of Okhotsk is the cleanest of the areas studied and is characterized by the lowest content of DDT, HCH, and PCB in organisms. DDT was practically absent in the flounders from the Nevelsky Bay (the Tatar Strait). At the same time, they showed the highest level of HCH, represented only by β-isomer, which indicates a prolonged circulation of the toxicant in the ecosystem. According to the decree of the Government of the Sakhalin Region, on the territory of Sakhalin there are landfills for out-of-use or banned pesticides; storage of them was performed (at the time of the decree entering the force) with violations that could lead to serious environmental pollution. Most likely, they became the source of pollution of the Tatar Strait. Another source of HCH pollution is currents that carry the waters of the Sea of Japan through the Nevelsky Bay into the Sea of Okhotsk. High levels of PCBs in the waters of the bay may result from intensive shipping and possible impact of household waste dumps on the Sakhalin Island. Flounders from the Sea of Japan are characterized by the highest POPs pollution. The entrance of OCPs into the sea may be due to surface runoffs, river flows, storage leaks of pesticides banned for use, and atmospheric transport from Asian countries where the use of some OCPs is still permitted. The determined levels of PCBs are an order of magnitude higher than those in the flounders from the Sea of Okhotsk and the Tatar Strait, which may be due to active shipping in Sea of Japan waters, influence of operating oil and coal ports in the city of Nakhodka, as well as local pollution of the coastal zone (so called wild beaches). Thus, we have studied the accumulation of organochlorine pesticides (HCH and DDT) and polychlorinated biphenyls in the muscles of flounders from the Far Eastern seas of Russia. With the existing global background of POPs formed on the planet, the levels of these compounds in the flounders of the southern part of the Sea of Okhotsk can be taken as background ones. The Sea of Japan is subject to the greatest anthropogenic pressure, and PCB concentrations are significantly higher in this area than in the Far Eastern seas of Russia and in the compared regions of the world as a whole.

Keywords: DDT, HCH, PCB, flounder, genus *Hippoglossoides*, Far Eastern seas of Russia

Organochlorine pesticides (hereinafter OCPs) and polychlorinated biphenyls (hereinafter PCBs) belong to a group of persistent organic pollutants (hereinafter POPs) and are global superecotoxicants [1]. Distribution of these compounds is due to transport by air and water, as well as bioaccumulation and biomagnification (an increase in concentration of toxicants in organisms with transition to higher levels of the food chain) [10, 11, 22, 26]. OCPs enter the environment through atmospheric transport from Asian countries where the use of DDT (for controlling disease carrier insects) and HCH (as a remedy for lice and scabies) is still permitted [21]. OCPs sources are landfills and pesticides burials, from which they are washed away by atmospheric precipitation and groundwater, entering marine ecosystems through river flows and surface runoffs. OCPs are known to get into freshwater bodies, including spawning grounds, through biotransport by migrating organisms [18]. PCBs enter ecosystems mainly during incineration of domestic and industrial waste, ignition of old transformers, evaporation from plasticizers, as well as leaks with other industrial wastes and leaks from PCBs-containing oils [13, 19, 20].

Fish and seafood are an important source of high-grade protein and polyunsaturated fatty acids, especially for residents of coastal areas. Up to 90 % of all pollutants enter the human body with food. The final depot of POPs in the environment is marine ecosystems; therefore, these substances can accumulate in various objects of marine fisheries [17, 22, 26].

The Far Eastern seas (the Sea of Japan, the Sea of Okhotsk, and the Bering Sea) are the main fishing zones of the Russian Federation. Flounders caught in the Far East are among the most important objects for fishing accounting for 9.5 % of the total fish catch in the region [2]. Catch volume, species diversity, and low market price predetermine their special significance in nutrition structure of local population. One of the most important types of flounders is halibut flounder of genus *Hippoglossoides* Gottsche, 1835 which is widespread in the Sea of Okhotsk, the Sea of Japan, and the Tatar Strait. We carried out preliminary monitoring of POPs content in flounders of the Sea of Okhotsk and made an assumption about possible use of the obtained concentrations of POPs as background ones for the Far Eastern seas [17].

The aim of the work is to assess levels of accumulation and biotransformation of OCPs and PCBs in halibut flounder from various regions of Far Eastern seas of Russia (the Sea of Okhotsk, the Tatar Strait, and the Sea of Japan).

MATERIAL AND METHODS

Flounders of genus *Hippoglossoides* (Gottsche, 1835) were caught in the eastern (off the coast of Kamchatka) and southern (off the coast of the Kuril Islands) parts of the Sea of Okhotsk, in the Nevelsky

Bay off the southwest coast of Sakhalin Island (the Tatar Strait), and in the Rifovaya Bay of Peter the Great Bay (the Sea of Japan) in summer (2016–2018) (Table 1, Fig. 1). Age of the fish ranged 0.6 to 1 year. Muscle (fillet) of fish was analyzed for the content of OCPs and PCBs. Frozen (−20 °C) tissue samples were delivered to the laboratory and homogenized before chemical analysis.

Table 1. Characteristics of samples studied

Sampling area	Sampling year	Number of samples	Weight, g*	Lipids, %*
Southern part of the Sea of Okhotsk	2016	10	219–402 294 ± 52	0.03–2.07 0.72 ± 0.64
Eastern part of the Sea of Okhotsk	2016	10	160–415 230 ± 82	0.06–0.47 0.20 ± 0.13
The Tatar Strait	2017	15	289–510 368 ± 72	0.03–0.6 0.17 ± 0.16
The Sea of Japan	2018	9	122–250 195 ± 45	0.04–1.06 0.62 ± 0.31

Note: * – range, min–max (above the bar); mean ± standard deviation (under the bar).

Lipids were extracted from muscle homogenates of individual specimens (10–20 g) using a mixture of *n*-hexane and acetone, followed by destruction of fatty components by concentrated sulfuric acid [23]. Next, the extract obtained was separated by nonpolar (for PCB) and polar (for OCP) solvents by column chromatography with Florisil® sorbent.

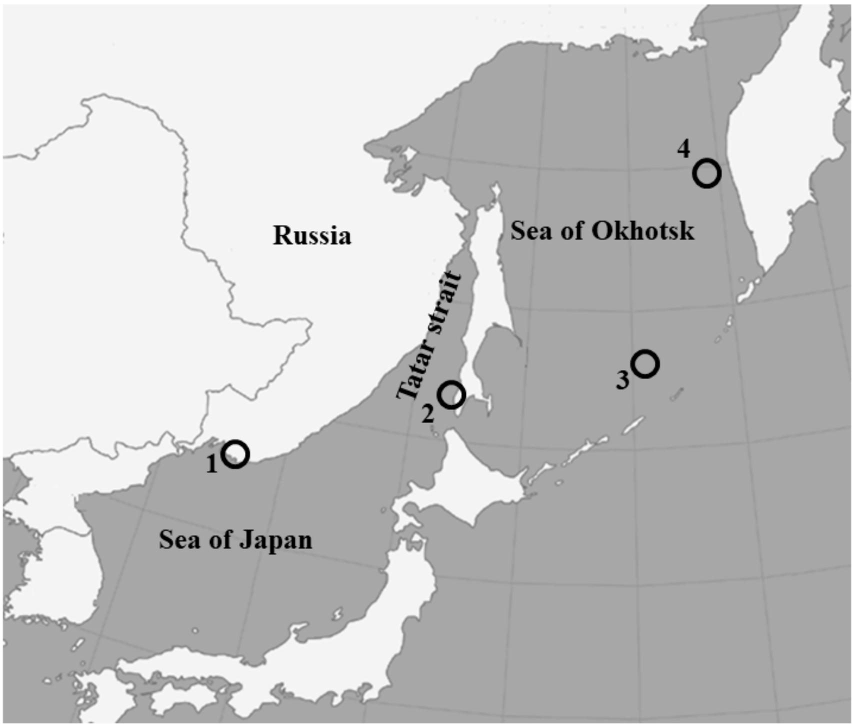

Fig. 1. Map of sampling sites of flounders: 1 – the Rifovaya Bay, Peter the Great Bay, the Sea of Japan; 2 – the Nevelsky Bay, the Tatar Strait; 3 – southern part of the Sea of Okhotsk (off the coast of the Kuril Islands); 4 – eastern part of the Sea of Okhotsk (off the coast of Kamchatka)

To prepare standard solutions of OCPs and PCBs, standard samples (Dr. Ehrenstorfer and Accu-Standard) of α-HCH, β-HCH, γ-HCH, *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDD, *o,p'*-DDD, *p,p'*-DDE, and *o,p'*-DDE, as well as mixture of PCB congeners 28, 52, 155, 101, 118, 143, 153, 138, 180, and 207 with established metrological characteristics (content of main substances of 99.4–99.6 %, determination error of 0.4 %) were used. To calibrate the chromato-mass spectrometer, working standard solutions of OCP and PCB with a concentration of 10 $ng \cdot ml^{-1}$ were used prepared by diluting standard solutions with an appropriate volume of *n*-hexane. Pesticides Library was also used. The main mass content of organochlorine compounds in the biomaterial was determined with Shimadzu GC MS-QP 2010 Ultra gas chromato-mass spectrometer equipped with AOC-5000 autosampler (detailed characteristics were indicated previously [27]). A SLB-5 capillary column was used for the study, as well as helium as a gas carrier (flow rate of 1 ml per minute). Temperatures of injector and detector were of +250 °C and +150 °C, respectively. The heating program was the following: increase in temperature to +100 °C for 4 minutes, heating to +310 °C at the rate of 7 °C per minute, and maintaining the final temperature for 6 minutes. Test mixture in a volume of 2 μl was added without separation, followed by opening the separation port after 1 minute. The ionization of substances in the gas phase was carried out in the electronic ionization mode (EI mode). Selected ion monitoring (SIM) was designed according to settings and detection limits of the device. Two ions (M+ and [M+2]+) were monitored for each chlorination level. To identify the test compound as supporting criteria, the exit time, mass, and relative content of the supporting ion were used. Measurement uncertainty of less than ±20 % was considered as acceptable. Peak areas were measured using GCMS Postrun Analysis.

To assess the quality of the methodology applied, the method of standard additives was used. Known amounts of test compounds were added to the muscle tissue of 10 flounder samples. Sample preparation and study of mixed samples were carried out using the method described above. The results showed that average reproducibility of analyte concentrations varied 94.6 to 103.7 %, which indicates reliability of the data obtained as well as efficiency of methods applied. Detection limits were calculated as 3 standard deviations of 10 samples in samples mixed with the standards. For analytes that were not identified in mixed samples, the detection limits were determined as an amount of analyte in the sample in relation to minimum concentration of calibration standard. For the OCPs investigated, the detection limits were: α-HCH – 0.2–0.3; β-HCH – 0.1–0.2; γ-HCH – 0.3–0.5; *p,p'*-DDT – 0.6–0.7; *o,p'*-DDT – 0.2–0.6; *p,p'*-DDD – 0–0.1; *o,p'*-DDD – 0.1–0.2; *p,p'*-DDE – 0.1–0.2; *o,p'*-DDE – 0.1–0.4 $ng \cdot g^{-1}$. For PCB congeners, the detection limits were: 28 – 0.5–0.6; 52 – 0.4–0.7; 155 – 0.1–0.5; 101 – 0.6–0.8; 118 – 0.7–0.8; 143 – 0.2–0.7; 153 – 0–0.1; 138 – 0.2–0.3; 180 – 0.5–0.6; 207 – 0.7–0.8 $ng \cdot g^{-1}$.

Statistical analysis of the results was made using IBM SPSS Statistics software. The reliability of the data was assessed using two-sided Kruskal – Wallis test with a significance level of $p \leq 0.05$. The results are presented as follows: concentration range, mean value ± standard deviation.

RESULTS

Eastern and southern parts of the Sea of Okhotsk. A concentration range of ∑OCP (∑DDT + ∑HCH) in the flounders from the eastern part of the Sea of Okhotsk varied widely 14 to 434 $ng \cdot g^{-1}$ of lipids with an average concentration of (100 ± 125) $ng \cdot g^{-1}$ of lipids. Total levels of HCH and DDT ranged 14 to 158 [average of (50 ± 52)] and 0.6 to 276 [average of (62 ± 89)] $ng \cdot g^{-1}$ of lipids, respectively. A range of OCP concentrations in fish from the southern part of the Sea of Okhotsk was 11–141 $ng \cdot g^{-1}$ of lipids with an average concentration of (54 ± 41) $ng \cdot g^{-1}$ of lipids, which was lower than in the waters off the coast of Kamchatka. Total levels of HCH and DDT in fish varied 3 to 103 [average of (36 ± 37)] and 1 to 45 [average of (20 ± 17)] ng·g^{-1} of lipids, respectively, i. e. they were also lower than off the coast of Kamchatka.

Of the HCH isomers in fish from the eastern part of the Sea of Okhotsk, the β-isomer with a concentration of 14–158 [average of (49 ± 51)] ng·g^{-1} of lipids was the most frequently recorded one (Fig. 2). Meanwhile, α-HCH was detected in only one sample with a concentration of 8 ng·g^{-1} of lipids. Concentrations of γ-HCH were below the detection limits in all samples.

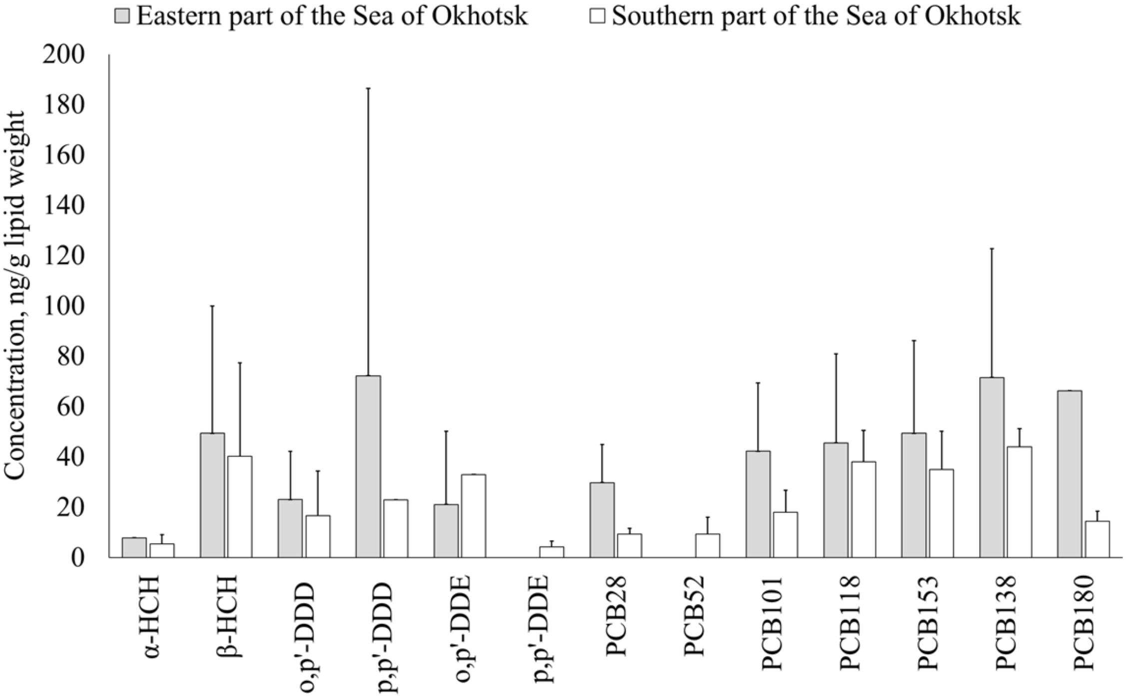

Fig. 2. Average concentration of pollutants in the flounders from the eastern and southern parts of the Sea of Okhotsk (error bars represent standard deviation value)

In the eastern part of the Sea of Okhotsk, of DDT and its metabolites concentrations of *o,p'*-DDT, *p,p'*-DDT, and *p,p'*-DDE were below the detection limits in all samples. The levels of *o,p'*-DDD and *p,p'*-DDD ranged 6 to 45 [average of (23 ± 19)] and 13 to 276 [average of (72 ± 114)] ng·g^{-1} of lipids (Fig. 2), respectively. Meanwhile, *o,p'*-DDE was identified in two samples at concentrations of 0.55 and 41.68 ng·g^{-1} of lipids.

In fish from the southern part of the Sea of Okhotsk, HCH was represented by α- and β-isomers with concentrations of 2–12 [average of (5 ± 4)] and 1–96 [average of (40 ± 37)] ng·g^{-1} of lipids, respectively (Fig. 2). The levels of γ-HCH were below the detection limits in all samples. Of DDT and its metabolites, concentrations of *o,p'*-DDT were below the detection limits in all the fish studied. Meanwhile, *p,p'*-DDT, *p,p'*-DDD, and *o,p'*-DDE were determined singly at concentrations of 7, 23, and 33 ng·g^{-1} of lipids, respectively. The content of *o,p'*-DDD ranged 2 to 45 ng·g^{-1} of lipids, with an average concentration of (17 ± 18) ng·g^{-1} of lipids. Concentrations of *p,p'*-DDE varied in the range of 1–7 [average of (4 ± 2)] ng·g^{-1} of lipids.

The sum of PCB concentrations in the flounders from the eastern part of the Sea of Okhotsk varied 24 to 279 [average of (125 ± 91)] ng·g^{-1} of lipids. PCBs were mainly represented by 101 and 153 congeners; 28, 52, 155, 118, 138, and 180 congeners were identified fragmentarily (in one or two samples) at the following concentrations: 28 – of 41 and 19; 52 – of 33; 155 – of 71; 118 – of 21 and 71; 138 – of 108 and 35;

180 – of 66 ng·g^{-1} of lipids (Fig. 2). Concentrations of PCB 207 were below the detection limits in all samples studied. The concentrations of PCB 101 and PCB 153 varied 11 to 81 [average of (42 ± 27)] and 49 to 117 [average of (49 ± 37)] ng·g^{-1} of lipids, respectively.

The sum of PCB levels in the samples ranged 25 to 150 [average of (99 ± 43)] ng·g^{-1} of lipids. PCBs were represented by 28, 101, 118, 153, and 138 congeners at concentrations of 6–12, 5–30, 15–50, 18–55, and 34––51 ng·g^{-1} of lipids, respectively. The average levels were (9 ± 2), (18 ± 9), (38 ± 13), (35 ± 15), and (44 ± 7) ng·g^{-1} of lipids (Fig. 2). PCBs 52, 155 and 180 were found fragmentary at following concentrations: 52 – of 14 and 5; 155 – of 17; 180 – of 17 and 12 ng·g^{-1} of lipids. The amounts of PCB 143 and PCB 207 were below the detection limits in all samples.

Nevelsky Bay, the Tatar Strait. In fish from the Tatar Strait, of OCPs mainly β-HCH was detected within a wide range of concentrations of 37–555 ng·g^{-1} of lipids (average of (224 ± 180) ng·g^{-1} of lipids). DDT and its metabolites were found in three samples and represented by *p,p'*-DDD (of 15 ng·g^{-1} of lipids) and *p,p'*-DDE (of 6 and 19 ng·g^{-1} of lipids) (Fig. 3). The sum of PCB concentrations in the flounders was within the range of 193–1384 ng·g^{-1} of lipids (with an average value of (455 ± 317) ng·g^{-1} of lipids). PCB congeners 28, 52, 155, 101, 118, 143, 153, 138, and 180 were found in the flounders from the Tatar Strait. PCB 207 concentrations were below the detection limits in all samples. Levels of PCB congeners were found in ranges: for 28 – 4–61; 52 – 3–287; 155 – 3–78; 101 – 23–108; 118 – 20–326; 143 – 25–56; 153 – 38–291; 138 – 8–423; 180 – 28––106 ng·g^{-1} of lipids. The average concentrations were (29 ± 21), (80 ± 83), (30 ± 28), (45 ± 23), (85 ± 80), (44 ± 13), (118 ± 72), (121 ± 109), and (59 ± 29) ng·g^{-1} of lipids. PCB 101 was detected in all the samples studied.

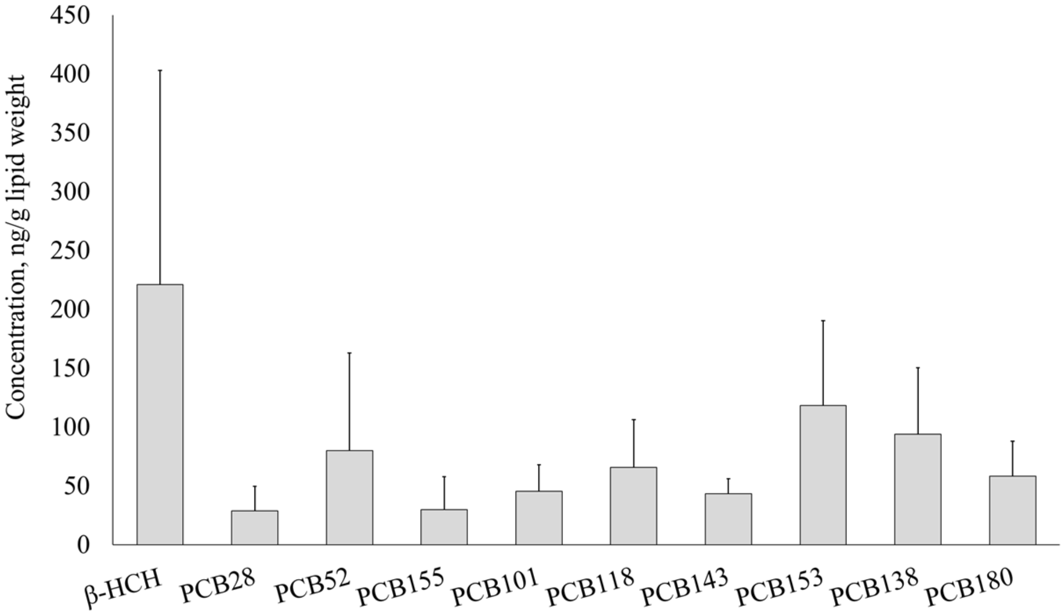

Fig. 3. Average concentration of pollutants in the flounders from the Tatar Strait (error bars represent standard deviation value)

Rifovaya Bay, Peter the Great Bay, Sea of Japan. Concentrations of OCP (∑HCH + ∑DDT) in the flounders from the Rifovaya Bay ranged 38 to 193 ng·g^{-1} of lipids with an average value of (102 ± 50) ng·g^{-1} of lipids. HCH and DDT isomers, as well as its metabolites, were found in all the samples studied. The levels of ∑HCH and ∑DDT varied 29 to 134 and 9 to 88 ng·g^{-1} of lipids, respectively; average concentrations were (62 ± 36) and (40 ± 29) ng·g^{-1} of lipids. In the flounders from the Sea of Japan, all HCH isomers were found. Levels of α-, β-, and γ-isomers were within the ranges of 0.4–5, 27–127, and 0.9–6 ng·g^{-1} of lipids, respectively. Average concentrations were: for α-HCH – of (2 ± 1); β-HCH – (59 ± 35); γ-HCH – (2 ± 2) ng·g^{-1} of lipids (Fig. 4). The β-isomer was found in all the samples studied.

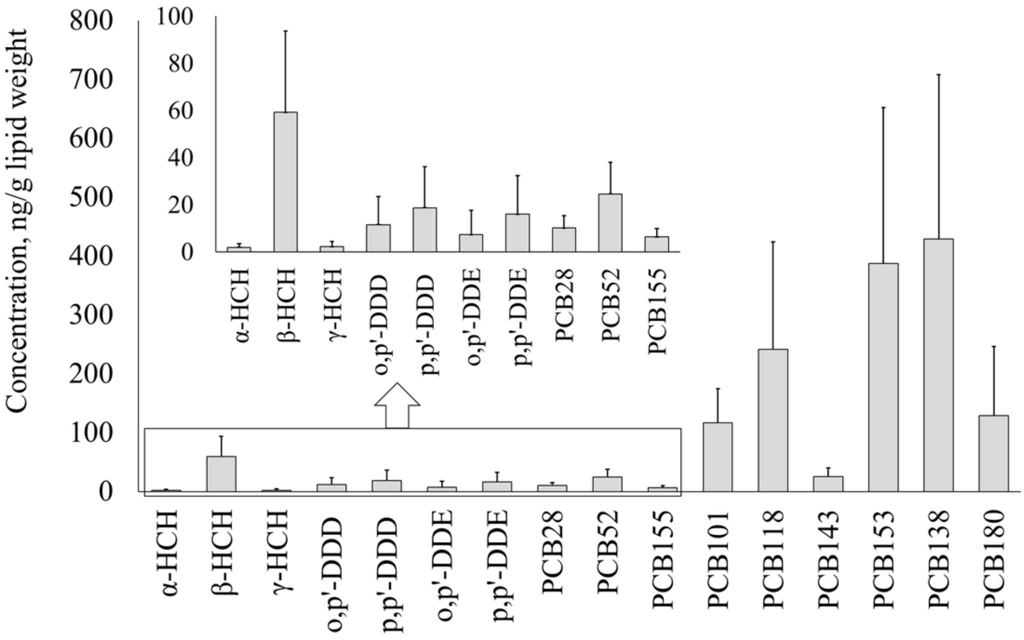

Fig. 4. Average concentration of pollutants in the flounders from the Sea of Japan (error bars represent standard deviation value)

Of DDT and its metabolites in the flounders, *o,p'*-DDT was not detected, while *p,p'*-DDT was found in only one sample (6 ng·g^{-1} of lipids). The concentration ranges of *o,p'*-DDD, *p,p'*-DDD, *o,p'*-DDE, and *p,p'*-DDE were 1–38, 6–52, 1–34, and 4–47 ng·g^{-1} of lipids, respectively. Average levels were (12 ± 12), (19 ± 18), (7 ± 10), and (16 ± 16) ng·g^{-1} of lipids (Fig. 4). The concentration of PCBs ranged 421 to 3716 ng·g^{-1} of lipids; average concentration was of (1616 ± 1177) ng·g^{-1} of lipids. PCBs were represented by 28, 52, 155, 101, 118, 143, 153, 138, and 180 congeners. PCB 207 concentrations were below the detection limits in all the samples studied. Congener levels varied as follows: 28 – 3–405; 52 – 7–287; 155 – 3–11; 101 – 40–207; 118 – 53–581; 143 – 11–46; 153 – 126–849; 138 – 126–936; 180 – 28–1835 ng·g^{-1} of lipids. Average concentrations were (54 ± 132), (54 ± 88), (6 ± 3), (117 ± 58), (241 ± 183), (26 ± 15), (387 ± 265), (429 ± 279), and (318 ± 579) ng·g^{-1} of lipids, respectively.

DISCUSSION

In the Far East, monitoring of POPs content in marine and onshore facilities was not carried out regularly until 2012. Currently, monitoring is carried out regularly throughout the Far Eastern seas. Data on the content of OCPs and PCBs in Pacific salmon, birds, and mammals were accumulated [16, 17, 18, 22, 24, 25, 26, 27, 28].

Flounders (Pleuronectidae family) are among the most common representatives of the bottom ichthyofauna that inhabit the entire shelf and continental slope of the seas. The main feature of flounders' biology is their lifestyle: they lie on the ground or swim in the bottom layer, remaining within their area and migrating seasonally to deeper regions [8]. Thus, they can be bioindicators of local pollution.

Flounders of genus *Hippoglossoides* belong to a group of flounders with mixed type of nutrition: both typical benthic (shrimps, bivalves, etc.) and plankton animals (hyperiids, sagittae, etc.) can be found in their food. Also, juveniles of smelt, herring, and other small fish species often become the food of flounders. The food composition is strongly dependent on the area. In warmer areas of the Far Eastern seas (the Sea of Japan, the southern part of the Tatar Strait), mainly flathead flounder (*Hippoglossoides dubious* Schmidt, 1904) is found, while in colder waters (the Sea of Okhotsk, the Bering Sea) it is Bering flounder (*Hippoglossoides robustus* Gill & Townsend, 1897). In the southeast of Sakhalin and in the Sea of Japan, molluscs dominate the diet; in the southeastern part of the Bering Sea, flounders feed mainly on echinoderms and chillums in the lower shelf, as well as plankton in shallow water [6, 8]. Both species belong to the same genus, are similar in environmental and biological characteristics, and can be used as bioindicators. Differences in the accumulation of POPs in flounders from different regions may be due to anthropogenic pressure on the habitat area or bioaccumulation of organisms included in the diet.

In the flounders in the eastern part of the Sea of Okhotsk, the highest concentrations of DDT and moderate concentrations of HCH were detected. This may be due to location of a "repository" of pesticides on the Kamchatka Peninsula, where aldrin, dildrin, hexachlorobenzene, and other OCPs are buried [3, 5]. DDD was the most common metabolite of DDT; HCH was represented by the most stable β-isomer. It indicates a prolonged circulation of both toxicants in the ecosystem and decomposition of initial compounds to more stable forms. Leakage from the reservoirs buried and evaporation of toxicants with subsequent atmospheric transport are probably the main reasons of environment and biota pollution in this area, since agriculture on the western side of the Kamchatka Peninsula is poorly developed. Currently, as there are no garbage processing plants in Kamchatka, garbage is buried at special landfills [3, 9], and leakages are quite possible. It is known that due to their chemical stability, insulating properties, and thermal stability, PCBs were used in various industries for production of thermal insulation, rubber, plastic, as well as dyes, pigments, and carbonless copy paper [19]. Entrance of polychlorinated biphenyls into Sea of Okhotsk waters off Kamchatka can result from both intensive shipping and effluents from the landfills that carry residual amounts of PCBs into the ecosystem.

The southern part of the Sea of Okhotsk is the cleanest of the areas studied and is characterized by the lowest content of DDT, HCH, and PCB in organisms. The south of the Sea of Okhotsk is located far from all surface sources of pollution. In addition, the region is characterized by active hydrodynamics and water exchange with the Pacific Ocean through the Kuril straits, which can contribute to the redistribution of POPs in the waters. The concentrations of all POPs found in the flounders from the southern part of the Sea of Okhotsk are the lowest, compared with those of the Sea of Japan, the Tatar Strait, and the eastern part of the Sea of Okhotsk. Thus, the hypothesis that the levels of pollutants in the muscles of flounders from the south of the Sea of Okhotsk are background ones for the Far Eastern seas of Russia [17] has been proved.

Interesting scientific information is provided by the data on OCP content in the muscles of flounders from the Nevelsky Bay (the Tatar Strait). DDT was hardly identified in the samples studied: only one sample contained *p,p'*-DDD, while two samples contained *p,p'*-DDE. This suggests that the area is not seriously polluted with the pesticide. At the same time, the highest level of HCH was detected in the muscles of flounders from the Tatar Strait (Fig. 5); HCH was represented only by β-isomer, which indicates the long-term circulation of this toxicant in the ecosystem.

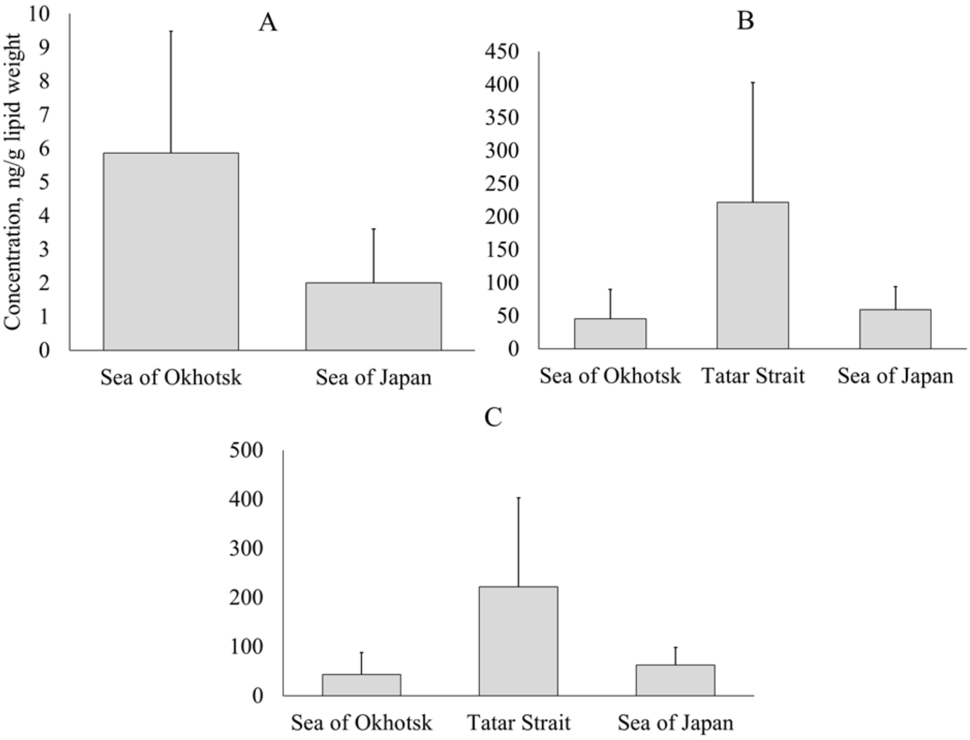

Fig. 5. Concentrations of α-HCH (A), β-HCH (B), and ∑HCH (C) in the muscles of the flounders from the areas studied (error bars represent standard deviation value)

The Nevelsky Bay is located far from large agricultural enterprises from the side both of Sakhalin Island and the mainland. However, according to the decree of the Government of the Sakhalin Region dated September 22, 2008, there are landfills for out-of-use or prohibited pesticides on the island, the storage of which was performed (at the time of the decree entering the force) with violations that could lead to serious environmental pollution [4, 7]. Most likely, it was the landfills that became the source of pollution of the Tatar Strait, and it led to the release of HCH into the ecosystem of the strait. This is also indicated by the detection of β-isomer of HCH, which is considered to be the most stable one. Another source of HCH pollution is currents that carry the waters of the Sea of Japan through the Nevelsky Strait into the Sea of Okhotsk.

PCB levels in fish from the Nevelsky Bay are several times higher than those from the Sea of Okhotsk, but noticeably lower than those of Sea of Japan flounders (Fig. 6). High concentrations of polychlorinated biphenyls can be related to intensive shipping and possible impact of landfills on Sakhalin Island (according to 2007 data, there were 54 authorized landfills and 37 unauthorized ones in the region [4]). The Tatar Strait is an economically important area with active fishing and variety of cargo transportation. Influence of warm currents of the Sea of Japan and cold currents of the Sea of Okhotsk, as well as limited water exchange with the open sea and with the ocean due to the “tightness” of the strait water body between the mainland and the island, are the factors contributing to the accumulation of pollutants in this region. Thus, the main source of PCBs in the waters of the Tatar Strait may be intensive activity of fishing and transport vessels.

In the flounders of the Sea of Japan, DDD and DDE were found of all DDT metabolites, and α-, β- and γ-isomers were found of HCH isomers. Primorye is an agriculturally developed region; in the middle of the XX century, pesticides including organochlorines were widely used there. Being chemically and biologically stable, these compounds could partially or completely remain in the soil, and now they are carried into Sea of Japan waters by surface runoff and river flow. In addition, there are pesticide

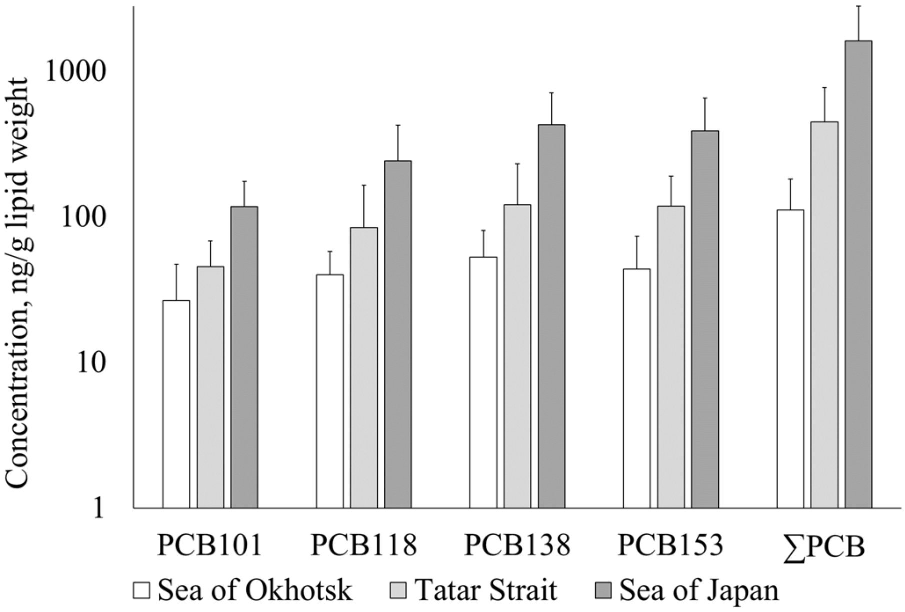

Fig. 6. Concentrations of highly chlorinated PCBs and ∑PCB in the muscles of the flounders from the Far Eastern seas of Russia (error bars represent standard deviation value)

burial sites on the territory of the region, and they can also be a source of toxicants to the environment. According to the Stockholm Convention, developing countries in Asia may use DDT (to protect population from malaria vectors) and HCH (as a remedy for lice and scabies) [21]. The Sea of Japan washes the shores of North and South Korea, and through the Korea Strait it is connected to the East China Sea washing the shores of China and the western shores of the Korean Peninsula. Pesticides can enter marine ecosystems through atmospheric transport, river flows, and currents from the East China Sea, carrying pesticide residues from agricultural land, as well as through industrial effluents. The most significant source of DDT and HCH seems to be China as one of the largest producers and consumers of pesticides in the world [12]. In the muscles of flounders, DDT is represented mainly by its metabolites DDD and DDE, which indicates that contamination occurred long time ago and initial compound decayed.

Data on the concentration of PCBs in the muscles of fish from the Sea of Japan are very important. The identified levels of PCBs are an order of magnitude higher than those in the flounders from the Sea of Okhotsk and the Tatar Strait (Fig. 6). The Rifovaya Bay is located within the boundaries of the Livadia village, where there are many recreation centers as well as so-called wild beaches. Every year, a huge number of tourists come to the bay coast from both the Far East and other regions of Russia. There are a lot of people at wild beaches, where garbage and waste products are neither cleaned nor taken away. In addition, the city of Nakhodka is located not far from the village, with its operating oil and coal ports, the impact of which can also affect coastal ecosystems. Moreover, the Sea of Japan is an area of intensive navigation and commercial fishing, which can result in PCBs entering the environment and organisms.

Comparison of the average concentrations of OCPs in the flounders studied by us with data for different regions of the World Ocean showed that DDT levels in all regions of the Far Eastern seas of Russia are significantly lower than those in the Atlantic Ocean (141 $ng \cdot g^{-1}$ of lipids), the Baltic Sea (579 $ng \cdot g^{-1}$ of lipids in the Gdansk Bay; 732 $ng \cdot g^{-1}$ of lipids at the mouth of the Visla River), and the Yellow Sea (122 $ng \cdot g^{-1}$ of lipids) [10, 15, 29], but an order of magnitude higher than in the Bering Sea (5 $ng \cdot g^{-1}$ of lipids) [14]. The average levels of α- and γ-HCH in fish are comparable with the concentrations given in the publications mentioned (1 to 6 $ng \cdot g^{-1}$ of lipids). In the data studied, the comparison was made with [10, 14], β-HCH concentrations were not shown. In [29], the amount of β-isomer was below the detection limits (< 0.002 ng·g^{-1} of wet weight); in [15], the toxicant levels were not given (since it was identified in 24 % of samples). Nevertheless, β-HCH was the dominant isomer in the flounders from all regions of the Far Eastern seas and exceeded the sum of HCH concentrations in the flounder muscles from the Yellow Sea (13 ng·g^{-1} of lipids) [10].

PCB levels in the fish of the Sea of Okhotsk did not exceed the concentrations found in flounders of the Atlantic Ocean (518 ng·g^{-1} of lipids) and the Baltic Sea (259 ng·g^{-1} of lipids in the Gdansk Bay; 373 ng·g^{-1} of lipids in the mouth of the Visla River) [15, 29], but significantly exceeded those in flounders from the Yellow (8 ng·g^{-1} of lipids) and Bering seas (15 ng·g^{-1} of lipids) [10, 14]. The total level of PCBs in the flounders of the Tatar Strait was between the values for fish from the Gdansk Bay and the mouth of the Visla River (the Baltic Sea). In the Sea of Japan, maximum PCB levels in fish were significantly higher than in the Baltic, Bering, and Yellow seas, as well as in the Atlantic Ocean. Such a big difference in average concentrations of POPs in flounders between the regions indicates a serious anthropogenic pressure on ecosystem of the Rifovaya Bay and the entire area.

Conclusions:

1. The accumulation of organochlorine pesticides and polychlorinated biphenyls in the muscles of flounders from the Tatar Strait, the Sea of Japan, and the Sea of Okhotsk of the Far East of Russia was studied. The average concentrations of ∑DDT, ∑HCH, ∑OCP (∑DDT + ∑HCH), and ∑PCB in the muscles of the flounders studied were: in the eastern part of the Sea of Okhotsk – (62 ± 89), (50 ± 52), (100 ± 125), and (92 ± 45) ng·g^{-1} of lipids; in the southern part of the Sea of Okhotsk – (20 ± 17), (36 ± 37), (54 ± 41), and (99 ± 43) ng·g^{-1} of lipids; in the Sea of Japan – (40 ± 29), (62 ± 36), (102 ± 50), and (1616 ± 1177) ng·g^{-1} of lipids, respectively. In the Tatar Strait, the average levels of β-HCH, ∑OCP (∑DDT + β-HCH) and ∑PCB were (221 ± 182), (224 ± 180), and (455 ± 317) ng·g^{-1} of lipids, respectively.
2. Xenobiotics should not have background concentrations in the environment; however, with the existing global background of POPs, the levels of these compounds in the flounders of the southern part of the Sea of Okhotsk (off the coast of the Kuril Islands), which is characterized by the absence of direct pollution sources and by active hydrodynamics, can be taken as background ones.
3. The Sea of Japan is subject to the greatest anthropogenic pressure, and its PCB concentrations are significantly higher than those in the Far Eastern seas of Russia and in the compared regions of the world as a whole.

This work was financially supported by the Russian Science Foundation (agreement no. 18-14-00120).

REFERENCES

1. Amirova Z. K., Speranskaya O. A. *Novye stoikie organicheskie superekotoksikanty i ikh vliyanie na zdorov'e cheloveka* / O. Yu. Tsittser (Ed.). Moscow : Eko-Soglasie, 2016. 170 p. (in Russ.)
2. Vorozhbit O. Yu., Danilovskikh I. A., Kuzmicheva N. Yu., Titova N. Yu., Shashlo N. V. *Rybnaya promyshlennost' Dal'nego Vostoka Rossii: sovremennoe sostoyanie, problemy i perspektivy konkurentosposobnosti* / O. Yu. Vorozhbit (Ed.). Vladivostok : Izd-vo VGUES, 2016, 156 p. (in Russ.)
3. *Doklad o sostoyanii okruzhayushchei sredy v Kamchatskom krae v 2018 godu.* Petropavlovsk-Kamchatskii : Ministerstvo prirodnykh resursov i ekologii Kamchatskogo kraya, 2019, 395 p. (in Russ.)

4. Zubtsova I. L. Razmeshchenie otkhodov na territorii Sakhalinskoi oblasti. *Vologdinskie chteniya*, 2008, no. S, pp. 109–118. (in Russ.)
5. Informatsionnyi otchet po ob"ektu "*Monitoring Kozel'skogo poligona zakhoroneniya yadokhimikatov i pestitsidov"*. Elizovo : OOO "Nedra", 2018, 49 p. (in Russ.)
6. Napazakov V. V., Chuchukalo V. I. Pitanie i pishchevye otnosheniya kambal v zapadnoi chasti Beringova morya v letne-osennii period. *Izvestiya TINRO*, 2002, vol. 130, pp. 595–617. (in Russ.)
7. Postanovlenie Pravitel'stva Sakhalinskoi oblasti. *O dolgosrochnoi oblastnoi tselevoi programme "Otkhody proizvodstva i potrebleniya Sakhalinskoi oblasti (2009–2015)"*. [Electronic resource]. URL: http://docs.cntd.ru/document/819065590 [accessed: 20.07.2019]. (in Russ.)
8. Fadeev N. S. *Spravochnik po biologii i promyslu ryb severnoi chasti Tikhogo okeana*. Vladivostok : TINRO-tsentr, 2005, 366 p. (in Russ.)
9. Khristoforova N. K. *Dal'nii Vostok Rossii: prirodnye usloviya, resursy, ekologicheskie problemy*. Moscow : Magistr, 2018, 830 p. (in Russ.)
10. Byun G. H., Moon H. B., Choi J. H., Hwang J., Kang C. K. Biomagnification of persistent chlorinated and brominated contaminants in food web components of the Yellow Sea. *Marine Pollution Bulletin*, 2013, vol. 73, iss. 1, pp. 210–219. https://doi.org/10.1016/j.marpolbul.2013.05.017
11. Drouillard K. G. Biomagnification. In: *Encyclopedia of Ecology* : 2nd edition. Vol. 1 / B. Fath (Ed.). [London] : Elsevier, 2008, pp. 353–358. https://doi.org/10.1016/B978-0-444-63768-0.00377-2
12. Grung M., Lin Y., Zhang H., Steen A. O., Huang J., Zhang G., Larssen T. Pesticide levels and environmental risk in aquatic environments in China – A review. *Environment International*, 2015, vol. 81, pp. 87–97. https://doi.org/10.1016/j.envint.2015.04.013
13. Guerra R., Pasteris A., Righi S., Ok G. Historical record of polychlorinated biphenyls (PCBs) in the continental shelf of the Korea Strait. *Chemosphere*, 2019, vol. 237, pp. 124438. https://doi.org/10.1016/j.chemosphere.2019.124438
14. Hartwell S. I., Apeti A. D., Pait A. S., Radenbaugh T., Britton R. Benthic habitat contaminant status and sediment toxicity in Bristol Bay, Alaska. *Regional Studies in Marine Science*, 2018, vol. 24, pp. 343–354. https://doi.org/10.1016/j.rsma.2018.09.009
15. Kopko O., Dabrowska H. Variability of biological indices, biomarkers, and organochlorine contaminants in flounder (*Platichthys flesus*) in the Gulf of Gdańsk, southern Baltic Sea. *Chemosphere*, 2018, vol. 194, pp. 701–713. https://doi.org/10.1016/j.chemosphere.2017.12.039
16. Lukyanova O. N., Tsygankov V. Yu., Boyarova M. D. Khristoforova N. K. Bioaccumulation of HCHs and DDTs in organs of Pacific salmon (genus *Oncorhynchus*) from the Sea of Okhotsk and the Bering Sea. *Chemosphere*, 2016, vol. 157, pp. 174–180. https://doi.org/10.1016/j.chemosphere.2016.05.039
17. Lukyanova O. N., Tsygankov V. Yu., Boyarova M. D. Organochlorine pesticides and polychlorinated biphenyls in the Bering flounder (*Hippoglossoides robustus*) from the Sea of Okhotsk. *Marine Pollution Bulletin*, 2018, vol. 137, pp. 152–156. https://doi.org/10.1016/j.marpolbul.2018.10.017
18. Lukyanova O. N., Tsygankov V. Yu., Boyarova M. D., Khristoforova N. K. Pacific salmon as a vector in the transfer of persistent organic pollutants in the Ocean. *Journal of Ichthyology*, 2015, vol. 55, no. 3, pp. 425–429. https://doi.org/10.1134/s0032945215030078
19. Reddy A. V. B., Moniruzzaman M., Aminabhavi T. M. Polychlorinated biphenyls (PCBs) in the environment: Recent updates on sampling, pretreatment, cleanup technologies and their analysis. *Chemical Engineering Journal*, 2019, vol. 358, pp. 1186–1207. https://doi.org/10.1016/j.cej.2018.09.205
20. Trinh M. M., Kuo C. H., Chang M. B. Characterization of PCDD/Fs and dl-PCBs emission from combustion of PCB-containing oil in a fluidized-bed incinerator. *Chemosphere*, 2019, vol. 225, pp. 35–42. https://doi.org/10.1016/j.chemosphere.2019.03.013
21. Tripathi V., Edrisi S. A., Chaurasia R., Pandey K. K., Dinesh D., Srivastava R., Srivastava P., Abhilash P. C. Restoring HCHs polluted land as one of the priority activities during the UN-International Decade on Ecosystem Restoration

(2021–2030): A call for global action. *Science Total Environment*, 2019, vol. 689, pp. 1304–1315. https://doi.org/10.1016/j.scitotenv.2019.06.444

22. Tsygankov V. Yu. Organochlorine pesticides in marine ecosystems of the Far Eastern seas of Russia (2000–2017). *Water Research*, 2019, vol. 161, pp. 43–53. https://doi.org/10.1016/j.watres.2019.05.103
23. Tsygankov V. Yu., Boyarova M. D. Sample preparation method for the determination of organochlorine pesticides in aquatic organisms by gas chromatography. *Achievements in the Life Sciences*, 2015, vol. 9, no. 1, pp. 65–68. https://doi.org/10.1016/j.als.2015.05.010
24. Tsygankov V. Yu., Boyarova M. D., Lukyanova O. N. Bioaccumulation of organochlorine pesticides (OCPs) in the northern fulmar (*Fulmarus glacialis*) from the Sea of Okhotsk. *Marine Pollution Bulletin*, 2016, vol. 110, iss. 1, pp. 82–85. https://doi.org/10.1016/j.marpolbul.2016.06.084
25. Tsygankov V. Yu., Boyarova M. D., Lukyanova O. N. Bioaccumulation of persistent organochlorine pesticides (OCPs) by gray whale and Pacific walrus from the western part of the Bering Sea. *Marine Pollution Bulletin*, 2015, vol. 99, iss. 1–2, pp. 235–239. https://doi.org/10.1016/j.marpolbul.2015.07.020
26. Tsygankov V. Yu., Boyarova M. D., Lukyanova O. N., Khristoforova N. K. Bioindicators of organochlorine pesticides in the Sea of Okhotsk and the western Bering Sea. *Archives of Environmental Contamination and Toxicology*, 2017, vol. 73, no. 2, pp. 176–184. https://doi.org/10.1007/s00244-017-0380-2
27. Tsygankov V. Yu., Lukyanova O. N., Boyarova M. D. Organochlorine pesticide accumulation in seabirds and marine mammals from the Northwest Pacific. *Marine Pollution Bulletin*, 2018, vol. 128, pp. 208–213. https://doi.org/10.1016/j.marpolbul.2018.01.027
28. Tsygankov V. Yu., Lukyanova O. N., Khristoforova N. K. The Sea of Okhotsk and the Bering Sea as the region of natural aquaculture: Organochlorine pesticides in Pacific salmon. *Marine Pollution Bulletin*, 2016, vol. 113, iss. 1–2, pp. 69–74. https://doi.org/10.1016/j.marpolbul.2016.08.060
29. Waszak I., Dabrowska H., Komar-Szymczak K. Comparison of common persistent organic pollutants (POPs) in flounder (*Platichthys flesus*) from the Vistula (Poland) and Douro (Portugal) River estuaries. *Marine Pollution Bulletin*, 2014, vol. 81, iss. 1, pp. 225–233. https://doi.org/10.1016/j.marpolbul.2014.01.044

ХЛОРОРГАНИЧЕСКИЕ СОЕДИНЕНИЯ В КАМБАЛАХ РОДА *HIPPOGLOSSOIDES* GOTTSCHE, 1835 ИЗ ДАЛЬНЕВОСТОЧНЫХ МОРЕЙ РОССИИ

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Хлорорганические пестициды (ХОП) и полихлорированные бифенилы (ПХБ) относятся к группе стойких органических загрязняющих веществ (СОЗ) и являются глобальными суперэкотоксикантами. Рыба и морепродукты — важный источник полноценного белка и полиненасыщенных жирных кислот, особенно для жителей приморских районов. До 90 % всех поллютантов поступают в организм человека с пищей. Конечным депо СОЗ в окружающей среде являются морские экосистемы, а следовательно, эти вещества могут накапливаться в различных объектах морского промысла. В работе представлены сведения о концентрациях ХОП [изомеры ГХЦГ (α-, β-, γ-), ДДТ и его метаболиты (ДДД и ДДЕ)] и ПХБ в мышцах камбал рода *Hippoglossoides* Gottsche, 1835, обитающих в дальневосточных морях России (Охотское море, Татарский пролив, Японское море). Липиды экстрагировали из образцов тканей рыб смесью гексана и ацетона с последующим разрушением жировых компонентов концентрированной серной кислотой. ХОП и ПХБ разделяли при помощи колоночной хроматографии полярным и неполярным растворителями. Ксенобиотики количественно определяли методом газовой хромато-масс-спектрометрии. Для оценки качества выбранной методики применяли метод стандартных добавок. Средняя воспроизводимость концентраций аналитов варьировала от 94,6 до 103,7 %, что говорит о надёжности полученных данных и об эффективности использованных методов. Средние концентрации $\sum$ДДТ, $\sum$ГХЦГ, $\sum$ХОП ($\sum$ДДТ + $\sum$ГХЦГ) и $\sum$ПХБ конгенеров составили: в образцах, отобранных в восточной части Охотского моря, — (62 ± 89), (50 ± 52), (100 ± 125) и (92 ± 45) нг·г$^{-1}$ липидов; в южной части Охотского моря — (20 ± 17), (36 ± 37), (54 ± 41) и (99 ± 43) нг·г$^{-1}$ липидов; в Японском море — (40 ± 29), (62 ± 36), (102 ± 50) и (1616 ± 1177) нг·г$^{-1}$ липидов соответственно. В образцах из Татарского пролива средние уровни $\sum$ГХЦГ, $\sum$ХОП и $\sum$ПХБ составили (221 ± 182), (224 ± 180) и (455 ± 317) нг·г$^{-1}$ липидов соответственно. ДДТ обнаружен в трёх исследованных образцах. В восточной части Охотского моря в камбалах зарегистрированы наибольшие концентрации ДДТ и умеренные — ГХЦГ, что может быть связано с расположением на полуострове Камчатка «могильника» ядохимикатов и пестицидов, в котором захоронены ХОП. Поступление ПХБ в воды южной части Охотского моря может объясняться как активным судоходством, так и наличием стоков с мусорных полигонов, несущих остаточные количества ПХБ в экосистему. Южная часть Охотского моря — самый чистый из исследованных районов, характеризующийся наименьшим содержанием ДДТ, ГХЦГ и ПХБ в организмах. В камбалах из залива Невельского (Татарский пролив) ДДТ практически отсутствовал. В то же время в них выявлен самый высокий уровень содержания ГХЦГ, представленного только β-изомером, что говорит о длительной циркуляции токсиканта в экосистеме. Согласно постановлению Правительства Сахалинской области, на территории о-ва Сахалин есть полигоны размещения пришедших в негодность или запрещённых пестицидов, хранение которых (на момент вступления постановления в силу) осуществлялось с нарушениями, способными привести к серьёзному загрязнению окружающей среды. Скорее всего, источником загрязнения Татарского пролива стали именно они. Другим источником загрязнения ГХЦГ могут быть течения, выносящие воды Японского моря через пролив Невельского в Охотское море. Высокие уровни ПХБ в водах залива могут быть связаны с активным судоходством и, возможно, с влиянием свалок бытовых отходов на о-ве Сахалин. Камбалы из Японского моря характеризуются наибольшим загрязнением СОЗ. Поступление ХОП в акваторию моря может быть связано с поверхностными смывами, речными стоками, утечками из хранилищ запрещённых к применению пестицидов и атмосферным переносом из стран Азии, где до сих пор разрешено применение некоторых ХОП. Найденные уровни содержания ПХБ на порядок величин превышают таковые в камбалах из Охотского моря и Татарского пролива, что может быть объяснено активным судоходством в водах Японского моря, влиянием действующих нефтеналивного и угольного портов в г. Находке, а также местным загрязнением прибрежной полосы (так называемых диких пляжей). Таким образом, исследована аккумуляция хлорорганических пестицидов (ГХЦГ и ДДТ) и полихлорированных бифенилов в мышцах камбал из дальневосточных морей России. При существующем глобальном фоне СОЗ, сформировавшемся на планете, уровни этих соединений в камбалах южной части Охотского моря могут быть приняты как фоновые. Наибольшему антропогенному прессу подвержено Японское море, где концентрации ПХБ значительно превышают таковые как в дальневосточных морях России, так и в сравниваемых регионах мира в целом.

Ключевые слова: ДДТ, ГХЦГ, ПХБ, камбалы, род *Hippoglossoides*, дальневосточные моря России

Морской биологический журнал, 2020, том 5, № 1, с. 43–49
Marine Biological Journal, 2020, vol. 5, no. 1, pp. 43–49
https://mbj.marine-research.org; doi: 10.21072/mbj.2020.05.1.05
ISSN 2499-9768 print / ISSN 2499-9776 online

UDC 581.526.325.3:577.34(262.5)

QUANTITATIVE RELATIONSHIP BETWEEN SOLAR RADIATION INTENSITY AND AVERAGE DAILY VALUE OF PHOTOSYNTHESIS LIGHT SATURATION FOR PHYTOPLANKTON IN THE DEEP-WATER AREA OF THE BLACK SEA

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Received by the Editor 29.08.2019; after revision 26.12.2019;
accepted for publication 27.03.2020; published online 31.03.2020.

According to data obtained during expeditions in the Black Sea (1987–1993), linear relationship between the light flux density incident on the sea surface (E_0) and the starting point of photosynthesis light saturation ($E_{n\ opt}$) is revealed. For calculations, measurements of phytoplankton photosynthesis rate obtained by the radiocarbon method were used. The equation of the relationship between the values reported is presented for the first time for the Black Sea. $E_{n\ opt}$ is the average daily, optimal value of photosynthesis light saturation. The parameters of photosynthesis – light curve, determined in short-period exposures under constant illumination, differ from the parameters obtained in long-term experiments under conditions of variable illumination. This is due to different effects of the intensity and dose on the phytoplankton photosynthesis rate. The values of photosynthetic parameters for a certain time are integrated into a single value which is the optimum for the entire period observed. The approximation of daily data integrated is carried out both separately for seasons and in general for the period of 1987–1993. Using statistical processing of data of average daily values of the intensity of solar radiation incident on the sea surface, slope of the photosynthesis – light curve, and maximum photosynthesis rate, the approximation is determined for the functional dependence of $E_{n\ opt}$ on E_0. The equation is applicable in the range of light intensity 3 to 75 mol quanta·m^{-2}·day^{-1}. It describes with high reliability a change of average daily value of photosynthesis light saturation in the Black Sea during different seasons of the year. The equation includes a parameter easily accessible for measurement. It can be used in analysis of physiological characteristics of phytoplankton and calculation of integrated phytoplankton productivity in euphotic layer with using both satellite and expedition data.

Keywords: phytoplankton, photosynthesis light saturation, photosynthesis rate, photosynthetically active radiation, deep-water area of the Black Sea

It is known that with increasing incident light intensity, the phytoplankton photosynthesis rate increases. Up to some values of photosynthetically active radiation, it grows linearly; then saturation occurs, and the photosynthesis rate becomes constant. A further increase in the light flux density per unit of the surface leads to inhibition of photosynthesis, which can be reversible, while with extremely high light intensity it can become irreversible.

When modeling the photosynthesis – light dependence, the amount of photosynthesis saturation with light (E_n) is an important physiological characteristic showing the light intensity, at which the maximum photosynthesis rate is observed. Studies [9, 14, 18] show that photosynthesis – light parameters in short-period exposures under constant illumination are not equivalent to the parameters if the data are obtained in long-term experiments under conditions of variable solar lighting. These differences often result from different effects of the intensity and dose of the radiation on the phytoplankton photosynthesis rate. The dynamics of photosynthesis during the day or the daylight hours is integrated into a single value, which is the optimum for the entire period observed.

As a rule, a model for calculating phytoplankton primary production includes equations with photosynthetic parameters: the maximum photosynthesis rate, the photosynthesis efficiency, and the amount of light saturation. Depending on the type of the model, different types of parameters are used to calculate the integral primary production [in particular, the values of photosynthesis light saturation both with constant lighting (E_n) and with variable lighting ($E_{n\ opt}$)]. The average daily, or optimal, value of photosynthesis light saturation ($E_{n\ opt}$) deserves special attention. Usually its values are not determined by direct measurements in field studies or remote observations. However, it is convenient to use it when calculating the integral primary production over a long period or evaluating photosynthesis profiles in a water column.

The aim of the research is to determine the relationship between the optimal value of photosynthesis light saturation for phytoplankton and the light incident on the surface in the Black Sea.

MATERIAL AND METHODS

The database formed for the study includes materials of 4 expeditions conducted in 1987–1993 in the Black Sea (Table 1). The basic data set was obtained by D. Sc. Z. Z. Finenko (IBSS RAS) [6, 7] and supplemented by data from the literature [1].

Table 1. Years of the expeditions and number of measurements included in the database

Year	Month	Number of measurements	Year	Month	Number of measurements
1987	12	12	1989	4, 5, 6	52
1988	1	10	1993	4	14
1988	3	48			

The database contains information on the time (year, month, and day) and location (longitude, latitude, and depth) of sampling. The measurements were carried out in the water area of 41° to 46° Northern latitude and 28° to 35° Eastern longitude for the depths of the euphotic zone, which ranged 12 to 100 m in different months at individual stations. The database also includes the parameters measured:

- the solar radiation intensity incident on the sea surface, E_0 (mol quanta$\cdot$m$^{-2}\cdot$day^{-1});
- maximum photosynthesis rate, $P^B{}_{opt}$ (mgC$\cdot$mgChl$^{-1}\cdot$day^{-1});
- slope of the photosynthesis – light curve reflecting the photosynthesis efficiency, α (mgC$\cdot$mgChl$^{-1}\cdot$(mol quanta$\cdot$m^{-2})$^{-1}$).

The optimal photosynthesis light saturation, $E_{n\ opt}$ (mol quanta$\cdot$m$^{-2}\cdot$day^{-1}), is calculated as the ratio: $E_{n\ opt} = P^B{}_{opt} / \alpha$.

Phytoplankton photosynthesis rate was measured by the radiocarbon method during the first or second half of daylight hours [6]. To obtain daily production, the values were doubled, since the phytoplankton photosynthesis rate is a function of light, and the total solar radiation flux on clear days during the first and second half of the day is approximately the same. The flasks were filled with water from the depths where 0.5 to 100 % of surface light penetrated. Then radioactive carbon was added, and the flasks were exposed on the ship deck under natural light which was weakened by neutral filters to the light conditions observed at the depths from which the samples were taken. Lighting was measured with a Yu116 light meter from dawn to sunset with an interval of one hour. Values in luxes were converted to energy units (1 klx = 20 $\mu E \cdot m^{-2} \cdot s^{-1}$; 1 mol quanta$\cdot m^{-2} \cdot s^{-1} = 10^6$ $\mu E \cdot m^{-2} \cdot s^{-1}$) [5].

RESULTS

For all the parameters studied, the sample for each month of 1987–1993 was averaged. The measured values of E_0 have high variability, especially in summer and spring (Fig. 1). Therefore, for calculations, we used the integral average daily values determined for each specific month in the considered period.

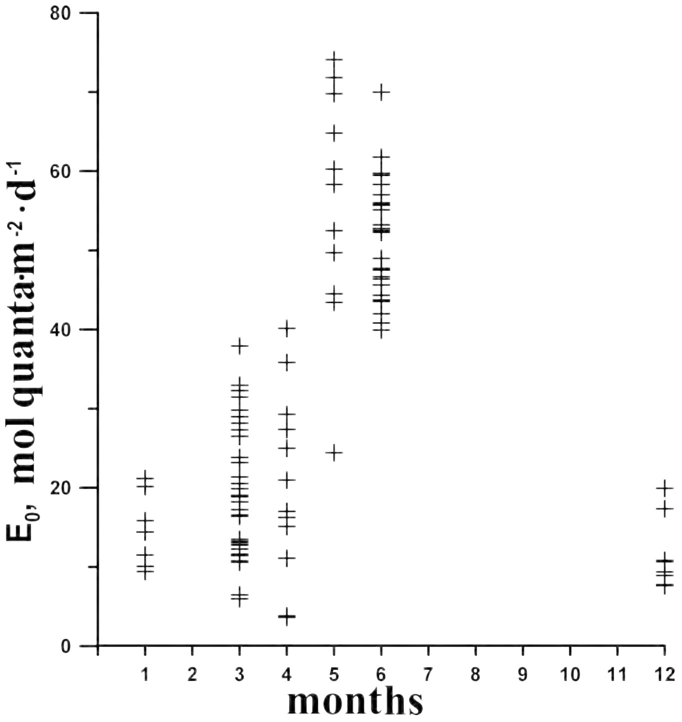

Fig. 1. Variability of solar radiation intensity incident on the sea surface (E_0) in different months of 1987–1993 in the Black Sea

The approximation of daily data was carried out by seasons and in general for the period of 1987–1993. The integration of average daily values of the solar radiation intensity incident on the sea surface, the slope of the photosynthesis – light curve, and the maximum photosynthesis rate for each month in a single year made it possible to determine the best approximation for the functional dependence of $E_{n\,opt}$ on E_0 (Fig. 2).

As a result of the analysis, a linear equation was obtained for the optimal value of photosynthesis light saturation in the absence of inhibition for the deep-water area of the Black Sea. The general equation relating the average daily values of $E_{n\,opt}$ and E_0 is:

$$E_{\mathrm{nopt}} = a \times E_0 + b \,, \tag{1}$$

where $E_{n\,opt}$ is measured in mol quanta$\cdot m^{-2} \cdot day^{-1}$;

a and b are constant coefficients (a = 0.12; b = 1.92).

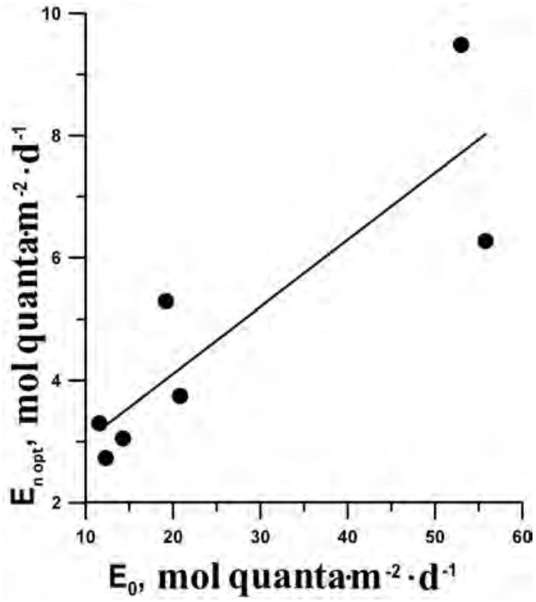

Fig. 2. Variability of optimal value of photosynthesis light saturation ($E_{n\ opt}$) depending on intensity of the solar radiation incident on the sea surface (E_0) in 1987–1993 in the Black Sea

The equation (1) is significant at $p < 0.0001$, $r^2 = 0.76$. The value of $E_{n\ opt}$ is determined for the euphotic zone. The dependence found is applicable in the illumination range 3 to 75 mol quanta·m^{-2}·day^{-1}. The equation (1), obtained from empirical data, has a regional character and is calculated for the first time for the deep-water area of the Black Sea.

DISCUSSION

In our studies, we used empirical data on the values of the optimal maximum photosynthesis rate and the slope of the photosynthesis – light curve integrated over the day for the period of 1987–1993 [1, 6, 7]. Unfortunately, regular measurements of these parameters, which could be used in modeling in the Black Sea for other years, were not carried out. Many studies have estimated the photosynthesis light saturation. This characteristic was used when modeling the photosynthesis rate and the integral primary production of phytoplankton [2, 9, 10, 11, 12, 13, 14, 15, 16, 17]. E_n or $E_{n\ opt}$ parameters were usually determined empirically for various regions of the World Ocean. A detailed analysis of the difference between these two parameters was made by M. Behrenfeld and P. Falkowski [9]. Empirically, they determined relationship between E_0 and the optimal value of light photosynthesis saturation E^*_n in the absence of inhibition; linear relationship was obtained. This function was used in modeling the integral primary production for the analysis of optical depths and vertical profiles of photosynthesis rate [3], since it gave similar results to the Black Sea data [7]. However, for the Black Sea, there was no such mathematical dependence that allowed one to determine $E_{n\ opt}$ using one parameter that is easily accessible for measurements, such as E_0. Previously, we examined the maximum value of photosynthesis light saturation; according to the model calculations, we estimated the greatest influence of the factors on E_n [4]. As a result, a multiple regression was obtained for E_n; it was determined that the maximum E_n values are observed at low chlorophyll concentrations and high P^B_m values, while minimum values are observed at high chlorophyll concentration and low photosynthetic activity. It is indicated that E_n values are more dependent on the maximum photosynthesis intensity than on the chlorophyll concentration. E_n differs from $E_{n\ opt}$, but such an influence of factors qualitatively reflects the change in $E_{n\ opt}$, especially if we take into account the vertical heterogeneity of the phytoplankton distribution in a water column. We found a linear dependence of $E_{n\ opt}$ on E_0. For the dependence (1) obtained empirically, the vertical uniformity of the phytoplankton distribution is assumed. Consequently, the photosynthesis profile in a water column without photoinhibition is presented on the surface by the area of light saturation, and at depth – by the area of light limitation. Such a change in the photosynthesis profile is usually observed in the deep-water area of the Black Sea [7, 8].

Conclusion. According to the results of the analysis of the data obtained during expeditions in the Black Sea (1987–1993), a quantitative relationship between the optimal value of photosynthesis light saturation for phytoplankton and the average daily light incident on the sea surface is obtained. This relationship is considered important in a number of modern studies, since the optimal value of photosynthesis light saturation is one of the fundamental characteristics used in modeling the phytoplankton productivity. For the first time for the Black Sea, an equation is obtained that allows one to determine $E_{n\ opt}$ in the surface layer, having the measurement data of E_0. This is especially convenient when using a large array of satellite observation data.

This work was carried out partially within the framework of government research assignment of IBSS RAS "Functional, metabolic, and toxicological aspects of the existence of hydrobionts and their populations in biotopes with different physical and chemical regimes" (no. AAAA-A18-118021490093-4) and partially – according to the project of the Presidium of the RAS "Influence of physical and chemical processes on the change of species composition and productivity of marine phytoplankton" (no. AAAA-A18-118020790209-9).

Acknowledgement. The author thanks D. Sc. Z. Z. Finenko for the experimental data provided.

REFERENCES

1. Vedernikov V. I. Osobennosti raspredeleniya pervichnoi produktsii i khlorofilla v Chernom more v vesennii i letnii periody. *Izmenchivost' ekosistemy Chernogo morya: estestvennye i antropogennye faktory*. Moscow : Nauka, 1991, pp. 128–147. (in Russ.)
2. Demidov A. B., Sheberstov S. V., Gagarin V. I. Interannual variability of the ice cover and primary production of the Kara Sea. *Okeanologiya*, 2018, vol. 58, no. 4, pp. 578–592. (in Russ.). http://doi.org/10.1134/S0030157418040019
3. Kovalyova I. V. *Modelirovanie sezonnoi i mnogoletnei izmenchivosti pervichnoi produktsii fitoplanktona v Chernom more*. [dissertation]. Sevastopol, 2017, 147 p. (in Russ.)
4. Kovalyova I. V. Relationship of primary production with intensity of sun irradiance. *Ekologiya morya*, 2006, iss. 72, pp. 77–86. (in Russ.)
5. Parsons T. R., Takahashi M., Hargrave B. *Biological Oceanography*. Moscow : Legkaya i pishchevaya promyshlennost', 1982, 432 p. (in Russ.)
6. Finenko Z. Z., Krupatkina D. K. Primary production in the Black Sea in the winter-spring period. *Okeanologiya*, 1993, vol. 33, no. 1, pp. 97–104. (in Russ.)
7. Finenko Z. Z., Churilova T. Ya., Sosik H. M. Vertical distribution of phytoplankton photosynthetic characteristics in the Black Sea. *Okeanologiya*, 2004, vol. 44, no. 2, pp. 222–237. (in Russ.)
8. Finenko Z. Z., Churilova T. Ya., Sosik H. M., Basturk O. Variability of photosynthetic parameters of the surface phytoplankton in the Black Sea. *Okeanologiya*, 2002, vol. 42, no. 1, pp. 60–75. (in Russ.)
9. Behrenfeld M., Falkowski P. A consumer's guide to phytoplankton primary productivity models. *Limnology and Oceanography*, 1997, vol. 42, no. 7, pp. 1479–1491. https://doi.org/10.4319/lo.1997.42.7.1479
10. Blackman F. F. Optimal and limiting factors. *Annals of Botany*, 1905, vol. 19, pp. 281–293.

11. Parker R. A. Empirical functions relating metabolic processes in aquatic systems to environmental variables. *Journal of the Fisheries Research Board of Canada*, 1974, vol. 31, no. 9, pp. 1550–1552. https://doi.org/10.1139/f74-192
12. Platt T., Sathyendranath S. Estimators of primary production for interpretation of remotely sensed data on ocean color. *Journal Geophysical Research*, 1993, vol. 98, iss. C8, pp. 14561–14576. https://doi.org/10.1029/93JC01001
13. Regaudie-de-Gioux A., Huete-Ortega M., Sobrinoc C., Lopez-Sandovald D. C., Gonzaleze N., Fernandez-Carrerac A., Vidalf M., Maranonc E., Cermenog P., Latasah M., Agustid S., Duarted C. M. Multi-model remote sensing assessment of primary production in the subtropical gyres. *Journal of Marine Systems*, 2019, vol. 196, pp. 97–106. http://doi.org/10.1016/j.jmarsys.2019.03.007
14. Rodhe W., Vollenweider R. A., Nauwerk A. The primary production and standing crop of phytoplankton. In: *Perspectives in Marine Biology* / A. A. Buzzati-Travenso (Ed.). Berkeley : University of California Press, 1958, pp. 299–322.
15. Smith E. M. Photosynthesis in relation to light and carbon dioxide. *Proceedings of the National Academy of Sciences*, 1936, vol. 22, no. 8, pp. 504–511. http://doi.org/10.1073/pnas.22.8.504
16. Smith R. C., Baker K. S. The bio-optical state of ocean waters and remote sensing. *Limnology and Oceanography*, 1978, vol. 23, iss. 2, pp. 247–259. https://doi.org/10.4319/lo.1978.23.2.0247
17. Talling J. E. The phytoplankton population as a compound photosynthetic system. *New Phytologist*, 1957, vol. 56, iss. 2, pp. 133–149. https://doi.org/10.1111/j.1469-8137.1957.tb06962.x
18. Vollenweider R. A. Models for calculating integral photosynthesis and some implications regarding structural properties of the community metabolism of aquatic systems. In: *Prediction and Measurement of Photosynthetic Productivity* : proceedings of the IBP/PP technical meeting, Třeboň, 14–21 Sept., 1969. Wageningen : Pudoc, 1970, pp. 455–472.

КОЛИЧЕСТВЕННАЯ СВЯЗЬ ИНТЕНСИВНОСТИ СОЛНЕЧНОЙ РАДИАЦИИ И СРЕДНЕСУТОЧНОЙ ВЕЛИЧИНЫ НАСЫЩЕНИЯ ФОТОСИНТЕЗА ФИТОПЛАНКТОНА ПО СВЕТУ ДЛЯ ГЛУБОКОВОДНОЙ ЧАСТИ ЧЁРНОГО МОРЯ

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По данным экспедиционных исследований, проведённых в Чёрном море в 1987–1993 гг., установлена линейная зависимость между плотностью светового потока, достигающего поверхности моря (E_0), и началом светового насыщения фотосинтеза фитопланктона ($E_{n\,opt}$). Для расчётов использованы измерения скорости фотосинтеза фитопланктона, полученные радиоуглеродным методом. Уравнение связи между указанными величинами представлено впервые для Чёрного моря. $E_{n\,opt}$ — среднесуточная, оптимальная величина насыщения фотосинтеза по свету. Параметры кривой фотосинтез — свет, определяемые в короткопериодных экспозициях при постоянном освещении, отличаются от параметров, полученных в длительных опытах в условиях переменного освещения. Это обусловлено разным действием интенсивности и дозы облучения на скорость фотосинтеза фитопланктона. Величины фотосинтетических параметров за определённое время интегрируются в единственное значение, которое является оптимумом за весь наблюдаемый период. В работе проведена аппроксимация интегрированных суточных данных отдельно за сезоны и в целом за 1987–1993 гг. С помощью статистической обработки данных среднесуточных значений интенсивности солнечной радиации, падающей на поверхность моря, тангенса угла наклона кривой фотосинтез — свет и величины максимальной скорости фотосинтеза определена аппроксимация для функциональной зависимости $E_{n\,opt}$ от E_0. Уравнение с высокой достоверностью описывает изменение среднесуточной величины светового насыщения фотосинтеза в Чёрном море в различные сезоны года, оно применимо в диапазоне освещённостей от 3 до 75 моль квантов$\cdot$м$^{-2}\cdot$сут$^{-1}$. Эта зависимость включает легко доступный для измерения параметр и может использоваться при анализе физиологических характеристик фитопланктона и расчёте интегральной продуктивности фитопланктона в эвфотическом слое как по спутниковым наблюдениям, так и по экспедиционным данным.

Ключевые слова: фитопланктон, световое насыщение фотосинтеза, скорость фотосинтеза, фотосинтетически активная радиация, глубоководная часть Чёрного моря

1871
ИнБЮМ – IBSS

Морской биологический журнал, 2020, том 5, № 1, с. 50–63
Marine Biological Journal, 2020, vol. 5, no. 1, pp. 50–63
https://mbj.marine-research.org; doi: 10.21072/mbj.2020.05.1.06
ISSN 2499-9768 print / ISSN 2499-9776 online

UDC 576.895.121:597.2/.5(262.5)

FISH CESTODES OF THE KARADAG NATURE RESERVE AND ADJACENT WATER AREAS OF THE BLACK SEA

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Received by the Editor 08.07.2019; after revision 04.10.2019;
accepted for publication 27.03.2020; published online 31.03.2020.

The first data on marine fish parasites in Karadag nature reserve water area were published at the beginning of the 20th century. By the beginning of the 21st century, information on the fauna of cestodes in this area of the Black Sea included data on 19 species recorded in 24 fish species. However, taxonomy of this class of helminths has changed significantly over the last decade, and regional fauna needs to be revised. The aim of this work is to revise the species composition of fish cestodes in the water area of the Karadag nature reserve and adjacent areas on the basis of new data obtained and in accordance with current systematics of Cestoda. The material for this study was the collections of cestodes gathered by the staff of IBSS RAS Environmental Parasitology Department in the area of the Karadag nature reserve in different years, as well as our own collections of 1754 specimens of rays and teleosts of 53 species (2005–2018). The area studied is Black Sea coastal area from Meganom Cape to Ordzhonikidze village (southeastern part of Crimea), including various marine biotopes of the Karadag nature reserve. Voucher preparations of all types of cestodes used in this study were deposited in a subcollection of marine parasites of the World Ocean hydrobionts collection of IBSS RAS. Totally 20 cestode species were found in 17 fish species. Nine species, namely *Progrillotia dasyatidis*, *Parachristianella trygonis*, *Dollfusiella aculeata*, *Rhinebothrium walga*, *Caulobothrium* sp., *Rhabdotobothrium* sp., *Acanthobothrium* sp. 5, 7, and Anthocephaliidae gen. sp. 2., were reported for the common stingray *Dasyatis pastinaca* in the area under study for the first time. Cestodes belonging to the new species *Acanthobothrium* sp. 1, 2, 4 were found in the thornback ray *Raja clavata*. Of 19 species previously known in Karadag area, only 8 adult mature cestodes were recorded: “*Bothriocephalus scorpii*”, “*B. gregarius*”, *Echinobothrium typus*, *Grillotia erinaceus*, *Prochristianella papillifer*, *Echeneibothrium variabile*, *Cairaeanthus ruhnkei*, and *C. healyae*. In addition, larvae of the complex species “*Scolex pleuronectis*” were found in teleost fish. Larvae of cestode *Progrillotia dasyatidis* were found for the first time in the water area studied in 8 teleost fish species; this data contribute to the information on the participants in the life cycle of this helminth. Four cestode species, namely *Hepatoxylon trichiuri* larvae, *Nybelinia lingualis* larvae, *Tetrarhynchobothrium tenuicolle*, and *Anthobothrium cornucopia*, which were previously reported from this area, were not found in the present survey. Moreover, recent analysis of the occurrence and synonymy of species of orders Trypanorhyncha and Onchoproteocephalidea revealed that the previous identification of the cestodes in elasmobranchs as *Grillotia* (*Christianella*) *minuta* and *Acanthobothrium coronatum*, as well as identification of the cestodes in teleosts as *Tentacularia* sp. larvae, is incorrect due to the absence of their specific definitive hosts in the Black Sea. On the other hand, among representatives of *Acanthobothrium* spp. registered in *D. pastinaca* and *R. clavata* in Karadag water area, we found 7 morphologically different new taxa identified to the species level. The cestodes found belong to 6 orders: Bothriocephalidea, Diphyllidea, Trypanorhyncha, “Tetraphyllidea” relics, Rhinebothriidea, and Onchoproteocephalidea. The most species diversity of cestodes in both species of rays is registered among representatives of the orders Trypanorhyncha and Onchoproteocephalidea (5 species each), the least – in the orders

Diphyllidea and “Tetraphyllidea” relics (1 species each). Thus, 12 species were added to the fauna of the cestodes parasitizing fish in Karadag area, and 8 of them are obviously representatives of new taxa.

Keywords: cestodes, fish, fauna, systematics, reserve, Crimea, Black Sea

On the territory of Crimea there is a large range of protected natural objects – from natural monuments of local importance to national and international reserves. Fauna of fish parasites in the water area of the Karadag natural reserve began to be studied in the early 20th century, and today it is the most thoroughly studied among fauna of protected water areas of Crimea [5].

The first data on the fauna of fish cestodes of Karadag water area were published in 1931 [4]. In this work, 3 cestode species were recorded, 2 species of which were larvae identified only to the genus level. After almost 30-year break, the study of fish cestodes in this area has been resumed [8, 9, 10, 11, 12, 13, 18]. In most works focused on fish cestodes of Karadag, only the fact of occurrence of a particular type of helminth in a particular host is indicated. Only [13] provides a brief description and schematic drawings of the found adult mature cestode species from the common stingray *Dasyatis pastinaca* (L., 1758) and the thornback ray *Raja clavata* L., 1758, as well as from teleosts: the Black Sea brill *Scophthalmus maeoticus* (Pallas, 1814) and the black scorpionfish *Scorpaena porcus* L., 1758.

At the beginning of the 21st century, the annotated list of fish parasites of Karadag included data on 19 cestode species registered in 24 fish species before 2002 [10]. This list of cestode species is currently inaccurate due to significant changes in their taxonomy [31, 35, 36, 37, 38]; it does not show their current species composition.

The aim of this work is to identify a modern species composition of fish cestodes in the water area of the Karadag nature reserve and adjacent water areas.

MATERIAL AND METHODS

The material for this study was the collections of cestodes obtained by researchers of IBSS RAS Environmental Parasitology Department in the Karadag nature reserve in 1994 and 2006, as well as our own samples collected in 2005–2018 from rays and teleosts (Table 1), caught in biotopes of the Karadag nature reserve (Biostation, Kuzmichev Rocks, and Malaya Putstsolanovaya and Serdolikovaya bays), as well as in water areas adjacent to the reserve (Meganom and Tolsty capes, Lis’ya Bay, and Koktebel and Ordzhonikidze villages).

Totally 1754 fish specimens of 53 species were examined by the method of incomplete parasitological dissection in the waters of Karadag and adjacent areas.

Fishes were identified by [3]. Cestodes were maintained in fresh water for 10–20 minutes to relax muscles and to evaginate tentacles and cirrus before fixing it in 70° ethanol and preparing total slides [2]. The cestodes were stained with acetocarmine and alum carmine by the standard method [19]; after dehydration with alcohol (70–100°) and enlightenment in clove oil, they were put into Canadian balsam. The infestation of fish with cestodes was assessed by the following parameters: prevalence (%) and intensity (worms per host) of infestation, as well as abundance index (worms per individual host) [1]. Calculations of the parameters were performed in Statistica 6 and PAST 3 programmes [30]. All types of cestodes, used in this study, were deposited in a subcollection of marine parasites of the World Ocean hydrobionts collection of IBSS RAS [27].

RESULTS

Of 53 fish species in the area studied, in 17 species 20 cestode species were found (Table 1). The greatest cestode species richness (12 species) was identified in *Dasyatis pastinaca*. Seven species were identified in *Raja clavata*; 1 species of adult mature cestode was identified in the teleost *Scophthalmus maeoticus*, and 1 – in the teleost *Scorpaena porcus*. The larvae of two cestode species (*Progrillotia dasyatidis* and the complex species "*Scolex pleuronectis*") were identified in 14 teleost species.

Table 1. Cestode infestation parameters in fish of the water area of the Karadag nature reserve and adjacent areas (1994–2018)

Cestode species	Species of the host (number of specimens)	II, min – max / mean ± SE	PI[1]	AI, mean ± SE
Parachristianella trygonis Dollfus, 1946	*Dasyatis pastinaca* (L., 1758) (11)	1 – 6 / 4	2 of 11	0.6
Progrillotia dasyatidis Beveridge, Neifar & Euzet, 2004		10 – 73 / 42	2 of 11	8
Dollfusiella aculeata Beveridge, Neifar & Euzet, 2004		1 – 14 / 7	3 of 11	2
Prochristianella papillifer (Poyarkoff, 1909) Dollfus, 1957 (syn. *P. trigonicola* Dollfus, 1946)		2 – 11 / 7	2 of 11	1.2
Acanthobothrium sp. 5		1 – 4 / 3	2 of 11	1
Acanthobothrium sp. 7		2 – 5 / 4	2 of 11	1
Caulobothrium sp.		1 – 1508 / 472	6 of 11	257
Cairaeanthus ruhnkei Kornyushin & Polyakova, 2012		1	1 of 11	0.1
C. healyae Kornyushin & Polyakova, 2012		1 – 3 / 2	3 of 11	0.6
Anthocephaliidae gen. sp. 2		5 – 6 / 5.5	2 of 11	1
Rhinebothrium walga (Shipley & Hornell, 1906)		1 – 12 / 5	3 of 11	1.3
Rhabdotobothrium sp.		1 – 35 / 13	4 of 11	5
Echinobothrium typus Van Beneden, 1849	*Raja clavata* L., 1758 (11)	1	1 of 11	0.1
Progrillotia sp.		3 – 103 / 28	5 of 11	13
Grillotia erinaceus (Van Beneden, 1858)		1 – 13 / 7	5 of 11	3.1
Echeneibothrium variabile Van Beneden, 1850		1 – 11 / 4	4 of 11	1.3
Acanthobothrium sp. 1		2 – 12 / 6.3	4 of 11	2.3
Acanthobothrium sp. 2		3 – 46 / 18	6 of 11	10
Acanthobothrium sp. 4		1 – 4 / 3	4 of 11	1
"*Bothriocephalus gregarius*" Renaud, Gabrion & Romestand, 1984	*Scophthalmus maeoticus* (Pallas, 1814) (3)	42 – 54	2 of 3	–
"*B. scorpii*" (Müller, 1779)	*Scorpaena porcus* L., 1758 (107)	1 – 3 / 2 ± 0.3	6	0.1 ± 0.04

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Cestode species	Species of the host (number of specimens)	II, min – max / mean ± SE	PI[1]	AI, mean ± SE
Progrillotia dasyatidis larvae	*Gobius niger* L., 1758 (6)	1 – 32	2 of 6	–
	Gobius bucchichi Steindachner, 1870 (5)	1 – 8	2 of 5	–
	Mullus barbatus L., 1758 (53)	1 – 86 / 23 ± 12	13	3.4 ± 2
	Trachurus mediterraneus (Steindachner, 1868) (129)	1	0,8	0.01 ± 0.01
	Gaidropsarus mediterraneus (L., 1758) (42)	1 – 4 / 2.5 ± 1.5	5	0.12 ± 0,1
	S. porcus	1 – 9 / 3.4 ± 0.6	16	0,5 ± 0,2
	Atherina boyeri Risso, 1810 (119)	1 – 4 / 2 ± 0.3	12	0.2 ± 0.06
	Salaria pavo (Risso, 1810) (59)	1	2	0.02 ± 0.02
"*Scolex pleuronectis*" Müller, 1788 larvae	*S. porcus*	1 – 8 / 5 ± 1.3	5	0,2 ± 0,1
	M. barbatus	1 – 4 / 2.5 ± 1.5	4	0,1 ± 0.08
	T. mediterraneus	2	0.8	0.02 ± 0.02
	Symphodus ocellatus Forsskål, 1775 (55)	1 – 38 / 20 ± 9	4	0.7 ± 0.7
	G. mediterraneus	1 – 7 / 4 ± 3	5	0.21 ± 0.18
	Atherina hepsetus L., 1758 (52)	1	2	0.02 ± 0.02
	Gobius niger	1	2 of 6	–
	Gobius bucchichi	3 – 38	3 of 5	–
	Neogobius melanostomus (Pallas, 1814) (14)	1	1 of 14	0.07
	Ponticola eurycephalus (Kessler, 1874) (44)	1	2.3	0.02 ± 0.02
	Aidablennius sphynx (Valenciennes, 1836) (301)	1 – 2 / 1.7 ± 0.3	1	0.02 ± 0.01
	Spicara smaris (L., 1758) (36)	14	3	0.4 ± 0.4

Note: II is intensity of infestation, worms per host; PI is prevalence of infestation, %; AI is abundance index, worms per individual host. [1] – if less than 15 fishes were dissected, then the number of fish infested from the total number of fish studied is given.

For the first time in the area under study, 9 cestode species were found in *D. pastinaca*: *Progrillotia dasyatidis*, *Parachristianella trygonis*, *Dollfusiella aculeata*, *Caulobothrium* sp., *Rhinebothrium walga*, *Rhabdotobothrium* sp., *Acanthobothrium* sp. 5, 7, and Anthocephaliidae gen. sp. 2 (Table 1). In this water area, cestodes *Acanthobothrium* sp. 1, 2, 4 were found in *R. clavata*. Two species of cestode larvae [*Hepatoxylon trichiuri* (Holten, 1802) and *Nybelinia lingualis* (Cuivier, 1817)], previously recorded, were not found in teleosts, whereas two species of adult mature cestodes [*Tetrarhynchobothrium tenuicolle* Diesing, 1854 and *Anthobothrium cornucopia* (Rud., 1819)] were not found in rays.

For the first time in Karadag water area, *Progrillotia dasyatidis* larvae were found in 8 teleost species. Previously, we found the larvae of this cestode in the same fish species in Sevastopol water area. The larvae were without a blastocyst; they localized in the gallbladder, sometimes in the intestinal lumina of fish. These teleosts are the second intermediate hosts for *P. dasyatidis*, which ends its development in the definitive host *D. pastinaca*. The larvae of cestode *P. dasyatidis* were found in demersal fish (*Gobius* spp., *Scorpaena porcus*, *Gaidropsarus mediterraneus*, and *Salaria pavo*), as well as in pelagic fish (*Mullus barbatus*, *Trachurus mediterraneus*, and *Atherina boyeri*). The highest abundance of *P. dasyatidis* larvae was identified in *M. barbatus*, *S. porcus*, and *A. boyeri* (Table 1). No data on the species composition of the first intermediate hosts of *P. dasyatidis* are available in literature. Free larvae, having no blastocyst, of this cestode from 7 species of demersal teleosts of three families (Soleidae Bonaparte, 1833, Scophthalmidae Chabanaud, 1933, and Batrachoididae Jordan, 1896), caught off the coast of Portugal, have recently been described [36]. Our data on the finding of *P. dasyatidis* larvae in Black Sea teleosts contribute to the information on the participants of this helminth's life cycle.

The analysis of cestode infestation of 2 ray species in the area under study revealed that the mass species found in most *D. pastinaca* studied was *Caulobothrium* sp., with the maximum abundance of 1508 worms per individual host (Table 1). Cestodes, following in number and occurrence in the common stingray, were *Dollfusiella aculeata*, *Progrillotia dasyatidis*, and *Rhabdotobothrium* sp. The most abundant cestode in *Raja clavata* was *Progrillotia* sp., with the abundance reaching 103 specimens in one thornback ray. The second most abundant and most common species was *Acanthobothrium* sp. 2.

Adult mature forms of cestodes of the genus *Bothriocephalus* Rud., 1808, "*B. gregarius*" and "*B. scorpii*", were found in their definitive hosts: the Black Sea brill and the black scorpionfish. "*B. gregarius*" intensity of infestation (42–54 worms per host) of the Black Sea brill in Karadag water area is comparable to that of other areas along the coast of Crimea. The definitive host of "*B. scorpii*" in the Black Sea is *Scorpaena porcus*. According to [13], "*B. scorpii*" was found in Karadag water area in 28 % of black scorpionfish with an intensity of 1–2 worms per host. According to [9], up to 30 % of black scorpionfish were infested with this cestode with an intensity of 1–3 worms per host and an abundance of 0.5 worms per individual host. In [11, p. 10] there are no quantitative data on the infestation of the black scorpionfish with this cestode, and it is only stated that its occurrence in fish is high. During our study, "*B. scorpii*" was found on average in 6 % of black scorpionfish; compared with the 1960s [13] and 2000s [9], the occurrence decreased by almost 5 times. To date, this species is quite rare not only in the area under study, but along the whole coast of Crimea as well.

DISCUSSION

As a result of the audit of the cestode species composition in fish of Karadag water area and adjacent areas, the representatives of 6 orders were found: Bothriocephalidea, Diphyllidea, Trypanorhyncha, "Tetraphyllidea" relics, Rhinebothriidea, and Onchoproteocephalidea (Table 2).

Previously, in Karadag water area the infestation with only one cestode representative of the order Bothriocephalidea, "*Bothriocephalus scorpii*", was recorded in the black scorpionfish and the Black Sea brill [11, 13]. The only and incomplete description with schematic drawings of this species in the Black Sea is based on cestodes from the black scorpionfish and the Black Sea brill [13]. To date, this species is a complex one; it is registered in more than 50 genera of marine fish from families and orders that are not related phylogenetically.

Table 2. Fauna of fish cestodes of the Karadag nature reserve and adjacent water areas of the Black Sea (according to own and literary data)

Cestode taxa	Fish species
Bothriocephalidea Kuchta, Scholz, Brabec & Bray, 2008[1]	
Bothriocephalidae Blanchard, 1849	
“*Bothriocephalus scorpii*”	*Scorpaena porcus*
“*B. gregarius*”	*Scophthalmus maeoticus*
Diphyllidea Van Beneden in Carus, 1863	
Echinobothriidae Perrier, 1897	
Echinobothrium typus	*Raja clavata*
Trypanorhyncha Diesing, 1863	
Eutetrarhynchidae Guiart, 1927	
Parachristianella trygonis	*Dasyatis pastinaca*
Prochristianella papillifer	
Dollfusiella aculeata	
Dollfusiella aculeata larvae	*Chelidonichthys lucernus*, *Scomber scombrus*, *Mullus barbatus*, *Belone belone*, *Trachinus draco*, *Pegusa nasuta*
Tetrarhynchobothrium tenuicolle[2]	*Raja clavata*
Progllotiidae Palm, 2004	
Progrillotia dasyatidis	*Dasyatis pastinaca*
Progrillotia dasyatidis larvae	*Gobius niger*, *G. bucchichi*, *Mullus barbatus*, *Trachurus mediterraneus*, *Gaidropsarus mediterraneus*, *Scorpaena porcus*, *Atherina boyeri*, *Salaria pavo*
Lacistorhynchidae Guiart, 1927	
Grillotia erinaceus	*Raja clavata*
Tentaculariidae Poche, 1926	
Nybelina lingualis[2] larvae	*Sarda sarda*
Sphyriocephalidae Pintner, 1913	
Hepatoxylon trichiuri[2] larvae	*Trachinus draco*
“Tetraphyllidea” Van Beneden, 1850 relics: Family incertae sedis	
Anthobothrium cornucopia[2]	*Dasyatis pastinaca*
Caulobothrium sp.	
Scolex pleuronectis larvae	*Scorpaena porcus*, *Neogobius syrman*, *Gobius niger*, *G. bucchichi*, *Pomatoschistus minutus*, *Crenilabrus ocellatus*, *C. scina*, *C. tinca*, *Trachurus mediterraneus*, *Merlangius merlangus*, *Mullus barbatus*, *Platichthys flesus*, *Sciaena umbra*, *Uranoscopus scaber*, *Ophidium rochei*, *Pegusa nasuta*, *Spicara flexuosa*, *Syngnatus abaster*, *Chelon auratus*, *C. saliens*, *Mugil cephalus*, *Arnoglossiis kessleri*, *Gymnammodytes cicerellus*, *Atherina hepsetus*
Onchoproteocephalidea Caira, Jensen, Waeschenbach, Olson & Littlewood, 2014	
Onchobothriidae Braun, 1900	
Acanthobothrium sp. 1	*Raja clavata*
Acanthobothrium sp. 2	
Acanthobothrium sp. 4	
Acanthobothrium sp. 5	*Dasyatis pastinaca*
Acanthobothrium sp. 7	

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Cestode taxa	Fish species
Rhinebothriidea Healy, Caira, Jensen, Webster & Littlewood, 2009	
Rhinebothriidae Euzet, 1953	
Rhinebothrium walga	*Dasyatis pastinaca*
Rhabdotobothrium sp.	
Anthocephaliidae Ruhnke, Caira & Cox, 2015	
Cairaeanthus ruhnkei	*Dasyatis pastinaca*
C. healyae	
Anthocephaliidae gen. sp. 2	
Echeneibothriidae de Beauchamp, 1871	
Echeneibothrium variabile	*Raja clavata*

Note: [1] – classification of orders and families according to [25, 34, 37]; [2] – cestode species, not found by us in fish in Karadag water area.

A type species of the genus, *Bothriocephalus scorpii* sensu stricto, parasitizes only in the shorthorn sculpin *Myoxocephalus scorpius* (L., 1758) (Scorpaeniformes: Cottidae) inhabiting the northern parts of the Pacific and Atlantic oceans [34]. On the basis of the results of the electrophoresis of protein composition of cestodes named "*B. scorpii*" from the Black Sea brill in the Black Sea, these cestodes were redefined as "*B. gregarius*", but without describing morphological features [20]. According to [33, 34], "*B. gregarius*" is *nomen nudum*, as its description from a typical host from the native range has not yet been published. As a result of studying the morphological features of cestodes named "*B. scorpii*" (from the black scorpionfish) and "*B. gregarius*" (from the Black Sea brill) in the Black Sea (Crimea, Caucasus), as well as investigations of the ribosomal genes 18S and 28S of these cestodes [16], both morphological and genetic features were revealed which confirm independence of these species and their inconsistency with the type species *Bothriocephalus scorpii* sensu stricto. So far, we use the names of cestodes "*B. scorpii*" and "*B. gregarius*" in this study (Table 2).

Another species of this genus, *B. atherinae* Chernyschenko, 1949, was first observed in the big-scale sand smelt *Atherina boyeri* in Odessa area [21], and then – in Karadag water area [11]. The taxonomic status of this species is still not clear. It was transferred to the genus *Ptychobothrium* Lönnberg, 1889 as *P. atherinae* (Chernyschenko, 1949) [7]. However, some researchers consider the old name to be valid [33], and others consider this cestode as a species with an undefined generic status [34]. It should be noted that the registration of this species off the coast of Karadag is doubtful, since it prefers brackish-water biotopes. Thus, in the Black Sea *B. atherinae* was identified in the big-scale sand smelt only in three areas of the northwestern part of the sea: in Odessa water area (salinity of 3–14 ‰), as well as in Berezansky (4–12 ‰) and Tiligulsky (4–12 ‰) estuaries [21, 22]. Our data also confirm the association of this species to low salinity biotopes. Throughout our studies, only 2 specimens of *B. atherinae* were found and only in 2 of 280 big-scale sand smelts studied in the brackish-water biotope of Karkinitsky Bay. In polyhaline waters along the coast of Crimea [in Sevastopol water area (17–18 ‰, 545 specimens of the big-scale sand smelt were dissected), Karadag (17–18 ‰, 119 specimens), and the Kerch Strait (12–15 ‰, 65 specimens)], this cestode was not found. Therefore, the recording of *B. atherinae* in Karadag polyhaline area (16–18 ‰) is doubtful. Thus, only two of three cestode species of the order Bothriocephalidea in Karadag fish are evidently parasitic: "*B. scorpii*" and "*B. gregarius*" (Table 2).

Two specimens of *Echinobothrium typus*, the only representative of the order Diphyllidea in the Black Sea, were first found in *Raja clavata* in Karadag water area in the early 1960s [13]. This cestode species was registered again in this ray species in Karadag water area in the late 1980s [12]. According to some authors, *E. typus* is common for this host (without indicating the quantitative parameters of infestation). However, *E. typus* was found by us only in Sevastopol water area (124 specimens of rays were examined, a prevalence of 15 %, with an abundance of 19 worms per individual host). In other areas, rays (166 specimens) were not infested with this cestode. For the first time, in 2018 one immature specimen of *E. typus* was found in *R. clavata* (Table 1).

According to [5, 9, 10, 11, 13], 13 cestode species of order Trypanorhyncha are found in fish in this water area. Of them, 8 are represented by adult mature forms (*Christianella minuta* (Van Beneden, 1849), *Tetrarhynchobothrium minutus* Van Beneden, 1850, *T. erinaceus* Van Beneden, 1861, *T. tenuicolle*, *Tetrarhynchus tenuicolle* Diesing, 1854, *Progrillotia louiseuzeti* Dollfus, 1969, *Grillotia erinaceus*, and *Prochristianella trigonicola*) parasitizing in two ray species and in the shark Picked dogfish *Squalus acanthias* L., 1758; 5 are larvae (*Hepatoxylon trichiuri*, *Nybelinia lingualis*, *Tetrarhynchobothrium* sp., *Tentacularia* sp., and *Eutetrarhynchus* sp.) recorded in teleosts.

Totally 5 cestode species of this order were identified by us in *Raja clavata* and *Dasyatis pastinaca*: *Dollfusiella aculeata*, *Grillotia erinaceus*, *Prochristianella papillifer*, *Parachistianella trygonis*, and *P. dasyatidis* (*P. louiseuzeti* sensu [6, 11]) (Tables 1, 2). The larvae of the cestode *P. dasyatidis* were identified in teleosts for the first time, while the larvae of *N. lingualis* and *H. trichiuri*, previously found in these fish, were not identified. According to [36], of the fish inhabiting the Black Sea, the second intermediate hosts of the latter two species are the Atlantic bonito *Sarda sarda* (Bloch, 1793) and the greater weever *Trachinus draco* L., 1758; the definitive hosts are *Raja clavata* and *Squalus acanthias*. We did not study the Atlantic bonito, the greater weever, and the shark Picked dogfish in Karadag water area, and the amount of *R. clavata* studied was small (11 specimens). This may explain the absence of these cestode species in our samples.

Previous analysis [15] of the occurrence and synonymy of Trypanorhyncha species showed that the identification of *Grillotia (Christianella) minuta* in elasmobranchs and *Tentacularia* sp. larvae in teleosts in the Black Sea is incorrect due to the absence of their specific definitive hosts in this water body. The names *Tetrarhynchobothrium erinaceus*, *T. minutus*, and *Tetrarhynchus tenuicolle* are not valid [36]. The representatives of the genus *Eutetrarhynchus* Pintner, 1913 were recorded in the Black Sea only in teleosts at the larval stage [5, 9, 10]. The adult mature specimens of these cestodes were first recorded in *D. pastinaca* in Sevastopol water area and identified as *Eutetrarhynchus spinifer* Dollfus, 1969 [6], but without description. The analysis of the morphology of Black Sea cestodes, previously identified as *E. spinifer*, showed inconsistency with the redescription of *Dollfusiella spinifer* (syn. *E. spinifer*), and these cestodes were redefined as *D. aculeata* [15].

Another trypanorinch species, *Tetrarhynchobothrium tenuicolle*, in *R. clavata* in the Black Sea (Romanian coast) was identified as *Rhynchobothrium tenuicolle* [23]. Once it was found by T. P. Pogorel'tseva in Karadag water area [13]. This species was not registered in the sea again. Thus, of 13 species of the order Trypanorhyncha, previously observed in fish in Karadag water area [10], in fact only 5 adult mature species parasitize in both ray species, and larvae of 2 cestode species were found in teleosts (Table 2).

Before our studies, 7 cestode species from 4 genera of the order "Tetraphyllidea" relics (*Echeneibothrium* Van Beneden, 1850, *Anthobothrium* Van Beneden, 1850, *Phyllobothrium* Van Beneden, 1849, and *Acanthobothrium* Van Beneden, 1849) were found in fish in Karadag water area [9, 11, 13, 18]. Three new orders were isolated from "Tetraphyllidea": Rhinebothriidea, Phyllobothriidea, and Onchoproteocephalidea [25]. Therefore, of the order "Tetraphyllidea" in Karadag water area only one representative of *Anthobothrium*, *A. cornucopia*, parasitizes in *D. pastinaca* [13]. Previously, *A. auriculatum* (Rud., 1819) and *A. cornucopia* were identified in Black Sea rays. However, in our samples of tetraphyllideans from both ray species off the coast of Crimea these cestodes were not found.

Other representatives of the order "Tetraphyllidea" relics, cestodes of the genus *Caulobothrium* Baer, 1948, were first recorded by us in the Black Sea in *D. pastinaca* in the waters of Sevastopol (Kazach'ya Bay); they were found again in Karadag and Kerch Strait areas [14]. The specimens studied differ from 7 valid species of this genus [37], and in this work they are defined as *Caulobothrium* sp. To date, it is not clear to which family cestodes of this genus belong; so far, they have been identified as a separate group designated as Clade 4 [37, p. 378]. The authors claim that *Caulobothrium* spp. are found only in rays of the family Myliobatidae Bonaparte, 1838 [*Myliobatis* L., 1758], and the identification of cestodes of this genus in rays of the families Dasyatidae Jordan, 1888 [*Himantura* (Bleeker, 1852)] and Urolophidae Müller & Henle, 1841 [*Urolophus* Müller & Henle, 1837] in the Caribbean Sea, as well as in the Pacific and Atlantic oceans, requires additional confirmations. Thus, in the area studied the infestation of rays with only one species of the genus *Caulobothrium* was confirmed (Table 2).

According to [9, 10, 11, 13, 23] and analysis of our samples, cestodes of 3 families of the order Rhinebothriidea are found in Karadag rays: Echeneibothriidae (*Echeneibothrium*), Anthocephaliidae (*Cairaeanthus* Kornyushin & Polyakova, 2012 and Anthocephaliidae gen. sp. 2), and Rhinebothriidae (*Rhinebothrium* Linton, 1890 and *Rhabdotobothrium* Euzet, 1953) [14, 15].

The only representative of the family Echeneibothriidae recorded in *Raja clavata* in the Black Sea is *Echeneibothrium variabile*, and it is described only on the basis of immature specimens [13, 23]. According to [39], "*E. variabile*" is a complex species, and *E. variabile* sensu stricto is highly specific to *R. clavata*; the identification of this cestode species in different ray species and in other areas requires confirmation. In the cestode samples from *R. clavata* from Karadag and Sevastopol water areas, mature specimens, morphologically identical to this species from a typical host from the native range, were found [39]. It should be noted that *E. variabile* was found in Karadag water area in both ray species [9, 10, 11]. We have never registered parasitization of *E. variabile* in *D. pastinaca*. Given the specificity of *E. variabile* to *R. clavata*, we consider its identification in *D. pastinaca* in the Black Sea to be incorrect.

The cestode species composition of *D. pastinaca* in Karadag water area was replenished with new representatives: Anthocephaliidae gen. sp. 2, *Rhinebothrium walga*, and *Rhabdotobothrium* sp. [14, 15]. In cestode samples of the common stingray, caught along the coast of Crimea, cestodes of the genus *Cairaeanthus* (*Phyllobothrium* sensu [13, 23]) with two species, *C. ruhnkei* (syn. *P. lactuca* sensu [13, 23]) and *C. healyae* (syn. *P. gracilis* sensu [13, 23]), were identified by us [32]. In Karadag water area, *C. healyae* was first found in the late 1980s in 45 % of rays *D. pastinaca* and *R. clavata* with an intensity of 1–17 worms per host [12]. The annotated list indicates the identification of another species of this genus, *C. ruhnkei* [10]. According to [10, p. 476], 35 % of *Raja clavata* and *Scorpaena porcus* were infested with *C. ruhnkei* with an intensity of 1–5 worms per host; it was noted that for the first time both *Cairaeanthus* species were found in Karadag water area by T. P. Pogorel'tseva. However, according to [13, pp. 148–150], both cestode species were found only in *D. pastinaca* and in other water areas: *C. ruhnkei* was recorded in the Kerch Strait, and *C. healyae* – in Kerch and Novorossiysk water areas. Cestodes of the genus *Cairaeanthus* spp. are highly specific parasites of *D. pastinaca* [32]. Therefore, identifications of adult mature *Cairaeanthus* spp. not only in *R. clavata* [11], but also in the black scorpionfish [10], are obviously incorrect, since teleosts are the second, or paratenic, hosts for elasmobranch cestodes.

Both species of *Cairaeanthus* are found in the Black Sea, and only *C. ruhnkei* is registered in the Sea of Azov. According to [24, p. 17], this group of cestodes (*Cairaeanthus* spp.) parasitizes in cooler waters, since, while studying cestodes of rays of the genus *Dasyatis* Rafinesque, 1810 from tropical and subtropical areas, cestodes of the genus *Cairaeanthus* were not identified. Thus, 6 cestode species of the order Rhinebothriidea parasitize in rays of Karadag water area: *Echeneibothrium variabile*, *Cairaeanthus healyae*, *C. ruhnkei*, *Rhinebothrium walga*, *Rhabdotobothrium* sp., and Anthocephaliidae gen. sp. 2 (Table 2).

According to [10, 11, 13], only two cestode species of the genus *Acanthobothrium* parasitize in two ray species in Karadag water area: *A. coronatum* (Rud., 1819) and *A. dujardinii* Van Beneden, 1849. Prior to our studies, three species of this genus were recorded in the Black Sea: besides the species mentioned above, the only Black Sea endemic, *A. ponticum* Borcea, 1934, was identified [10, 11, 13, 23]. It is necessary to emphasize, that in our samples of cestodes of this genus from both ray species off the coast of Crimea and Caucasus, we did not find cestodes that would correspond, according to their morphological characteristics, to *A. coronatum*, *A. dujardinii*, and *A. ponticum* previously found. On the other hand, among representatives of *Acanthobothrium* spp. found in *D. pastinaca* and *R. clavata* in Karadag water area, we identified 7 morphologically different species-level taxa. *Acanthobothrium* sp. 1, 2, 4 were registered in *R. clavata*, and *Acanthobothrium* sp. 5, 7 were found in *D. pastinaca* (Tables 1, 2) [14, 17].

The analysis of the morphology, specificity, and synonymy of *A. coronatum*, *A. dujardinii*, and *A. ponticum* in Black Sea rays [15] revealed that the identification of the highly specific species *A. coronatum* [40] in these fish in this water area is incorrect due to the absence of the definitive hosts of this cestode species, sharks of the genus *Scyliorhinus* (Blainville, 1816). Descriptions of cestodes identified as *A. dujardinii* [13, 23] from rays of the Black Sea do not correspond to typical descriptions of this species from the English Channel [28, 29, 40]. The systematic position of *A. ponticum* is still not clear. Some researchers indicated it as a possible junior synonym for *A. crassicolle* Wedl, 1855 [29], others – as a species with an unclear systematic position [40], and others – as a valid species [26], without restudying the typical material, which location is unknown.

In our samples of *Acanthobothrium* spp. from *D. pastinaca* off the coast of Crimea (Karkinitsky Bay, Sevastopol, Karadag, and the Kerch Strait) and Caucasus, specimens found were initially identified as *A. crassicolle* [5]. However, having studied the additional material on the morphology of these cestodes and the sequence of their 18S and 28S ribosomal genes, we have revealed morphological and genetic features [17] which allowed us to identify these cestodes as a new species. So far, in this work, we use the name *Acanthobothrium* sp. 7. Thus, in the composition of the order Onchoproteocephalidea, 5 cestode species infest Karadag rays (Table 2).

Conclusion. As a result of the revision of the fauna of fish cestodes of the Karadag nature reserve and adjacent areas, it is recorded that the current species composition of these helminths includes 20 species from 19 genera and 6 orders. For the first time, 12 species were recorded in Karadag rays: *Progrillotia dasyatidis*, *Parachristianella trygonis*, *Dollfusiella aculeata*, *Rhinebothrium walga*, *Acanthobothrium* sp. 1, 2, 4, 5, 7, *Rhabdotobothrium* sp., *Caulobothrium* sp., and Anthocephaliidae gen. sp. 2. Larvae of *Progrillotia dasyatidis* were found in teleosts of Karadag for the first time. This new data supplement the knowledge about the features of the life cycles of cestodes of this genus. In fish of this water area, of the previously recorded 19 cestode species, finding of only 8 was confirmed: “*Bothriocephalus scorpii*”, “*B. gregarius*”, *Echinobothrium typus*, *Grillotia erinaceus*, *Prochristianella papillifer*, *Echeneibothrium variabile*, *Cairaeanthus ruhnkei*, and *C. healyae*. The larvae of cestodes *Hepatoxylon trichiuri* and *Nybelinia lingualis* were not found in teleosts; *Tetrarhynchobothrium tenuicolle* and *Anthobothrium cornucopia* were not found in rays. As a result of the analysis of current taxonomy, synonymy, and specificity to the definitive hosts of cestodes of the orders Trypanoryncha and Onchoproteocephalidea, it was found out, that the initial identification and description of 3 species, *Tentacularia* sp. larvae, *Grillotia (Christianella) minuta*, and *Acanthobothrium coronatum*, from Black Sea fish were incorrect. The greatest cestode species richness was identified among the representatives of the orders Trypanorhyncha and Onchoproteocephalidea (5 species each), and the lowest cestode species richness was identified among the representatives of the orders Diphyllidea and "Tetraphyllidea" relics (1 species each). An increase in the species composition of elasmobranch cestodes of Karadag water area can occur due to the study of these helminths infesting *Squalus acanthias*, which was practically not studied in this area.

This work was carried out within the framework of government research assignment of IBSS RAS no. AAAA-A18-118020890074-2.

REFERENCES

1. Anikanova V. S., Bugmyrin S. V., Ieshko E. P. *Methods for Collection and Study of Helminths in Small Mammals.* Petrozavodsk : KarNTs RAN, 2007, 145 p. (in Russ.)
2. Bykhovskaya-Pavlovskaya I. E. *Parazitologicheskoe issledovanie ryb.* Leningrad : Nauka, 1985, 120 p. (in Russ.)
3. Vasil'eva E. D. *Fish of the Black Sea. Key to Marine, Brackish-water, Euryhaline, and Anadromous Species With Color Illustrations Collected by S. V. Bogorodsky.* Moscow : VNIRO Publ., 2007, 238 p. (in Russ.)
4. Vlasenko P. V. K faune paraziticheskikh chervei ryb Chernogo morya. *Trudy Karadagskoi biologicheskoi stantsii*, 1931, iss. 4, pp. 88–136. (in Russ.)
5. Dmitrieva E. V., Belofastova I. P., Korniichuk Yu. M., Machkevskii V. K., Pron'kina N. V., Polyakova T. A. Gel'mintofauna ryb Karadagskogo prirodnogo zapovednika. In: *Karadag-2009* : sbornik nauchnykh trudov, posvyashch. 95-letiyu Karadagskoi biologicheskoi stantsii i 30-letiyu Karadagskogo prirodnogo zapovednika NAN Ukrainy. Sevastopol : EKOSI-Gidrofizika, 2009, pp. 150–157. (in Russ.)
6. Kornyushin V. V. Nekotorye itogi izucheniya fauny tsestod pozvonochnykh Ukrainy. In: *IX konferentsiya ukrainskogo respublikanskogo obshchestva parazitologov* : tez. dokl., Lvov, Sept. 1980. Kiev : Naukova dumka, 1980, pp. 156–158. (in Russ.)
7. Kornyushin V. V., Kulakovskaya O. P. O geterogennosti roda *Bothriocephalus* (Cestoda, Pseudophyllidea). *Vestnik zoologii*, 1984, no. 3, pp. 11–15. (in Russ.)
8. Kornyushin V. V., Solonchenko A. I. Redescription of cestodes *Grillotia erinaceus* (Beneden, 1858) and *Christianella minuta* (Beneden, 1849) from the Black Sea cartilaginous fishes. *Biologiya morya*, 1978, iss. 45, pp. 26–34. (in Russ.)
9. Miroshnichenko A. I. Parazity morskikh ryb Karadagskogo prirodnogo zapovednika. In: *Karadag. Gidrobiologicheskie issledovaniya* : sb. nauch. tr., posvyashch. 90-letiyu Karadagskoi nauchnoi stantsii im. T. I. Vyazemskogo i 25-letiyu Karadagskogo prirodnogo zapovednika NAN Ukrainy. Simferopol : SONAT, 2004, book 2, pp. 86–101. (in Russ.)
10. Miroshnichenko A. I. Parazity morskikh ryb i bespozvonochnykh. In: *Karadag. Gidrobiologicheskie issledovaniya* : sb. nauch. tr., posvyashch. 90-letiyu Karadagskoi nauchnoi stantsii im. T. I. Vyazemskogo i 25-letiyu Karadagskogo prirodnogo zapovednika NAN Ukrainy. Simferopol : SONAT, 2004, book 2, pp. 468–495. (in Russ.)
11. Naidenova N. N., Solonchenko A. I. Parazitofauna ryb. In: *Flora i fauna zapovednikov SSSR. Fauna Karadagskogo zapovednika* : operativno-informatsionnyi material. Lyubertsy : VINITI, 1989, pp. 6–19. (in Russ.)

12. *Opredelitel' parazitov pozvonochnykh zhivotnykh Chernogo i Azovskogo morei: paraziticheskie bespozvonochnye ryb, ryboyadnykh ptits i morskikh mlekopitayushchikh.* Kiev : Naukova dumka, 1975, 552 p. (in Russ.)
13. Pogorel'tseva T. P. Materialy k izucheniyu lentochnykh chervei – parazitov ryb Chernogo morya. *Trudy Karadagskoi biololgicheskoi stantsii*, 1960, iss. 16, pp. 143–159. (in Russ.)
14. Polyakova T. A. *Tsestody skatov (Elasmobranchii: Batoidea) krymskogo poberezh'ya Chernogo morya (sistematika, fauna, ekologiya)* : avtoref. dis. … kand. biol. nauk : 03.02.11. Sevastopol, 2014, 24 p. (in Russ.)
15. Polyakova T. A., Gaevskaya A. V., Kornyushin V. V., Biserova N. M. Cestodes of elasmobranchs (Chondrichthyes: Elasmobranchii) in the Black Sea: The state and perspectives of study. *Parazitologiya*, 2017, vol. 51, no. 3, pp. 189–205. (in Russ.)
16. Polyakova T. A., Slynko Yu. V., Slynko E. E. Molecular-genetic identification and taxonomic position of *Bothriocephalus* Rud., 1808 (Cestoda: Bothriocephalidea) of the fish in the Black Sea. In: *Contemporary Parasitology – Major Trends and Challenge* : Proceeding of the VI Congress of the Society of Parasitologists, Russia : International Conference, Saint Petersburg, 15–19 Oct., 2018 / K. V. Galaktionov, S. G. Medvedev, A. Yu. Ryss, A. O. Frolov (Eds). Saint Petersburg : Izd-vo "Lema", 2018, p. 189. (in Russ.)
17. Polyakova T. A., Slynko Yu. V., Slynko E. E., Sarkisov D. G. Taxonomic status and molecular characteristic of *Acanthobothrium* Balanchard, 1848 (Cestoda: Onchoproteocephalidea) from *Dasyatis pastinaca* (L., 1758) in the Black Sea, Crimea. In: *Biodiversity of Parasites*. Moscow : Tov-vo nauch. izd. KMK, 2018, pp. 199–200. (Proceedings / Center for Parasitology, A. N. Severtsov Institute of Ecology and Evolution of RAS ; vol. 50). (in Russ.)
18. Reshetnikova A. V. K izucheniyu parazitofauny ryb Chernogo morya. *Trudy Karadagskoi biologicheskoi stantsii*, 1955, no. 13, pp. 105–124. (in Russ.)
19. Roskin G. I., Levinson L. B. *Mikroskopicheskaya tekhnika* : ucheb. posobie dlya vuzov / G. I. Roskin (Ed.). 3rd ed. Moscow : Sovetskaya nauka, 1957, 466 p. (in Russ.)
20. Solonchenko A. I., Rudneva I. I. Sistematicheskoe polozhenie tsestody "*Bothriocephalus scorpii*" ot kambal Chernogo i Azovskogo morei i ee geneticheskie variatsii. *Ekologiya morya*, 1997, iss. 46, pp. 75–77. (in Russ.)
21. Chernyshenko A. S. Novye gel'minty ryb Chernogo morya. *Pratsi Odeskoho derzhavnoho universytetu*, 1949, vol. 4, no. 57, pp. 79–91. (in Russ.)
22. Chernyshenko A. S. Materialy po parazitofaune ryb Odesskogo zaliva. *Trudy Odesskogo gosudarstvennogo universiteta*, 1955, vol. 14, no. 7, pp. 214–222. (in Russ.)
23. Borcéa L. Note preliminaire sur les cestodes des elasmobranches ou sélaciens de la Mer Noire. *Annales Scientifiques de l'Université de Jassy*, 1934, vol. 19, pp. 345–369.
24. Caira J. N., Healy C. J., Marques F. P. L., Jensen K. Three new genera of rhinebothriidean cestodes from stingrays in Southeast Asia. *Folia Parasitologica*, 2017, vol. 64, article 008 (18 p.). https://doi.org/10.14411/fp.2017.008
25. Caira J. N., Jensen K., Waeschenbach A., Olson P. D., Littlewood D. T. J. Orders out of chaos – Molecular phylogenetics reveals the complexity of shark and stingray tapeworm relationships. *International Journal for Parasitology*, 2014, vol. 44, iss. 1, pp. 55–73. https://doi.org/10.1016/j.ijpara.2013.10.004
26. Campbell R. A., Beveridge I. The genus *Acanthobothrium* (Cestoda: Tetraphyllidea: Onchobothriidae) parasitic in Australian elasmobranch fishes. *Invertebrate Systematics*, 2002, vol. 16, no. 2, pp. 237–344. https://doi.org/10.1071/IT01004
27. Dmitrieva E. V., Lyakh A. M., Kornyychuk Yu. M., Polyakova T. A., Popyuk M. P. *IBSS Collection of Marine Parasites: The Collection of Marine Parasites Maintained by the Institute of Biology of the Southern Seas.* 2020 : [site]. URL: http://marineparasites.org/ (accessed 03.10.2019).
28. Euzet L. *Recherches sur les cestodes tétraphyllides des sélaciens des côtes de France* / Université de Montpellier. [PhD dissertation]. Montpellier : Causse, Graille & Castelneau, 1959, 263 p.

29. Goldstein R. J. The genus *Acanthobothrium* van Beneden, 1849 (Cestoda: Tetraphyllidea). *Journal of Parasitology*, 1967, vol. 53, no. 3, pp. 455–483. https://doi.org/10.2307/3276705
30. Hammer Ø., Harper D. A. T., Ryan P. D. PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica*, 2001, vol. 4, iss. 1, article 4 (9 p.).
31. Healy C. J., Caira J. N., Jensen K., Webster B. L., Littlewood D. T. J. Proposal for a new tapeworm order, Rhinebothriidea. *Journal of Parasitology*, 2009, vol. 39, no. 4, pp. 497–511. https://doi.org/10.1016/j.ijpara.2008.09.002
32. Kornyushin V. V., Polyakova T. A. *Cairaeanthus* gen. n. (Cestoda, Rhinebothriidea), with the description of two new species from *Dasyatis pastinaca* in the Black Sea and the Sea of Azov. *Vestnik zoologii*, 2012, vol. 46, no. 4, pp. 291–308.
33. Kuchta R., Scholz T. Diversity and distribution of fish tapeworms of the “Bothriocephalidea” (Eucestoda). *Parassitologia*, 2007, vol. 49, no. 3, pp. 129–146.
34. Kuchta R., Scholz T., Bray R. A. Revision of the order Bothriocephalidea Kuchta, Scholz, Brabec & Bray, 2008 (Eucestoda) with amended generic diagnoses and key to families and genera. *Systematic Parasitology*, 2008, vol. 71, pp. 81–136. https://doi.org/10.1007/s11230-008-9153-7
35. Olson P. D., Caira J. N., Jensen K., Overstreet R. M., Palm H. W., Beveridge I. Evolution of the trypanorhyncha tapeworms: Parasite phylogeny supports independent lineages of sharks and rays. *International Journal for Parasitology*, 2010, vol. 40, iss. 2, pp. 223–242. https://doi.org/10.1016/j.ijpara.2009.07.012
36. Palm H. W. *The Trypanorhyncha Diesing, 1863.* Bogor : PKSPL-IPB Press, 2004, 710 p.
37. *Planetary Biodiversity Inventory (2008–2017): Tapeworms from Vertebrate Bowels of the Earth* / Caira J. N., Jensen K. (Eds). Lawrence, KS, USA : University of Kansas, Natural History Museum, 2017, 463 p. (Special Publication ; no. 25).
38. Ruhnke T. R., Caira J. N., Cox A. The cestode order Rhinebothriidea no longer family-less: A molecular phylogenetic investigation with erection of two new families and description of eight new species of *Anthocephalum*. *Zootaxa*, 2015, vol. 3904, no. 1, pp. 51–81. https://doi.org/10.11646/zootaxa.3904.1.3
39. Williams H. H. The ecology, functional morphology and taxonomy of *Echeneibothrium* Beneden, 1849 (Cestoda: Tetraphyllidea), a revision of the genus and comments on *Discobothrium* Beneden, 1870, *Pseudanthobothrium* Baer, 1956, and *Phormobothrium* Alexander, 1963. *Parasitology*, 1966, vol. 56, iss. 2, pp. 227–285. https://doi.org/10.1017/S0031182000070864
40. Williams H. H. The genus *Acanthobothrium* Beneden, 1849 (Cestoda: Tetraphyllidea). *Nytt Magasin for Zoologi*, 1969, vol. 17, no. 1, pp. 1–56.

ЦЕСТОДЫ РЫБ АКВАТОРИИ КАРАДАГСКОГО ПРИРОДНОГО ЗАПОВЕДНИКА И ПРИЛЕГАЮЩИХ РАЙОНОВ ЧЁРНОГО МОРЯ

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Данные о паразитах морских рыб акватории Карадага появились в первой половине XX века. К началу XXI столетия сведения о фауне цестод этого района Чёрного моря включали информацию о 19 видах, зарегистрированных у 24 видов рыб. Между тем за последнее десятилетие таксономия этого класса гельминтов претерпела существенные изменения, в свете чего региональные фауны нуждаются в ревизии. Цель работы — провести ревизию видового состава цестод рыб акватории Карадагского природного заповедника и прилегающих районов на основе новых сборов и в соответствии с современной систематикой класса Cestoda. Материалом для исследования послужили как коллекции цестод, собранные сотрудниками отдела экологической паразитологии ФИЦ ИнБЮМ в районе Карадагского заповедника в разные годы, так и собственные сборы от 1754 экз. скатов и костистых рыб 53 видов (2005–2018). Район исследования — прибрежная акватория Чёрного моря от м. Меганом до пос. Орджоникидзе (Юго-Восточный Крым), в том числе различные морские биотопы Карадагского заповедника. Ваучерные препараты всех видов цестод, использованных в данном исследовании, депонированы в подколлекции морских паразитов коллекции гидробионтов Мирового океана ФИЦ ИнБЮМ. У 17 видов рыб обнаружено 20 видов цестод. Впервые в районе исследования у ската *Dasyatis pastinaca* зарегистрированы цестоды 9 видов: *Progrillotia dasyatidis*, *Parachristianella trygonis*, *Dollfusiella aculeata*, *Rhinebothrium walga*, *Caulobothrium* sp., *Rhabdotobothrium* sp., *Acanthobothrium* sp. 5, 7 и Anthocephaliidae gen. sp. 2. У другого вида ската, *Raja clavata*, найдены цестоды, относящиеся, очевидно, к новым видам *Acanthobothrium* sp. 1, 2, 4. Из 19 ранее известных в районе Карадага видов подтверждены находки только 8 половозрелых цестод: «*Bothriocephalus scorpii*», «*B. gregarius*», *Echinobothrium typus*, *Grillotia erinaceus*, *Prochristianella papillifer*, *Echeneibothrium variabile*, *Cairaeanthus ruhnkei* и *C. healyae*. Кроме того, у костистых рыб обнаружены личинки сборного вида «*Scolex pleuronectis*». Личинки цестоды *Progrillotia dasyatidis* найдены впервые в акватории заповедника у 8 видов костистых рыб, что расширяет сведения об участниках жизненного цикла этого гельминта. У рыб Карадага не обнаружены ранее регистрировавшиеся в районе 4 вида цестод: *Hepatoxylon trichiurid* larvae, *Nybelinia lingualis* larvae, *Tetrarhynchobothrium tenuicolle* и *Anthobothrium cornucopia*. Кроме того, недавно проведённый анализ встречаемости и синонимики видов отрядов Trypanorhyncha и Onchoproteocephalidea выявил, что ранее выполненное определение *Grillotia (Christianella) minuta* и *Acanthobothrium coronatum* у эласмобранхий и *Tentacularia* sp. larvae — у костистых рыб Чёрного моря ошибочно ввиду отсутствия в этом водоёме их специфичных окончательных хозяев. С другой стороны, среди найденных *Acanthobothrium* spp. у скатов *D. pastinaca* и *R. clavata* в районе Карадага мы идентифицировали 7 морфологически различных новых таксонов видового уровня. Обнаруженные цестоды относились к 6 отрядам: Bothriocephalidea, Diphyllidea, Trypanorhyncha, «Tetraphyllidea» relics, Rhinebothriidea и Onchoproteocephalidea. Наибольшее видовое богатство цестод у обоих видов скатов отмечено среди представителей отрядов Trypanorhyncha и Onchoproteocephalidea (по 5 видов), наименьшее — среди Diphyllidea и «Tetraphyllidea» relics (по 1 виду в каждом). Таким образом, фауна цестод, паразитирующих у рыб в районе Карадага, дополнена 12 видами, 8 из которых являются, очевидно, представителями новых таксонов.

Ключевые слова: цестоды, рыбы, фауна, систематика, заповедник, Крым, Чёрное море

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Морской биологический журнал, 2020, том 5, № 1, с. 64–77
Marine Biological Journal, 2020, vol. 5, no. 1, pp. 64–77
https://mbj.marine-research.org; doi: 10.21072/mbj.2020.05.1.07
ISSN 2499-9768 print / ISSN 2499-9776 online

UDC 582.26/.27(477.75)

MICROALGAE OF MUD VOLCANO OF THE BULGANAK SOPOCHNOE FIELD ON THE CRIMEAN PENINSULA

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Received by the Editor 03.09.2019; after revision 03.09.2019;
accepted for publication 27.03.2020; published online 31.03.2020.

Mud volcanoes are one of unique natural phenomena widely spread around the world. They can be found in Crimea, including the Bulganak sopochnoe field – the largest cluster of active mud volcanoes on the peninsula (45°25′29.04″N, 36°27′51.64″E). Study of mud volcano microalgae in Crimea, as well as in other regions of Russia, has not been conducted so far. Therefore, scientific interest is caused by need and urgency of the study of these volcanoes. First data on microalgae species composition of active mud volcanoes are presented in this article. Samples collected by O. Yu. Eremin (03.08.2012 and 13.04.2013) in the upper 2–3-cm layer of suspension and in surface water were investigated. The ranges of salinity and water temperature were 27–32 g per L and +28…+31 °C, respectively. Microalgae species composition was determined in water preparations using Axioskop 40 (Carl Zeiss) light microscope at magnification of 10×40 with software AxioVision Rel. 4.6. Totally 16 taxa were found: Cyanobacteria (1), Dinophyta (2), Bacillariophyta (6), and Euglenophyta (7). Of these, cyanobacteria *Chamaecalyx swirenkoi* (Schirshov) Komárek et Anagnostidis, 1986 was found by us in the mud volcano in August 2012. Pennate species of diatoms were also identified – single living (of genera *Cylindrotheca* (Ehrenberg) Reimann & J. C. Lewin, *Lyrella* Karajeva, and *Nitzschia* Hassall) and colonial species (of genera *Berkeleya* Greville and *Pseudo-nitzschia* H. Peragallo). The brackish-water, benthic, boreal-tropical species *Nitzschia thermaloides* Hustedt was recorded for the algal flora of Crimea, the Black Sea, and the Sea of Azov for the first time. Euglenophytes were also found in the samples – 5 species of the genus *Trachelomonas* Ehrenberg and 2 species of the genus *Strombomonas* Deflandre. Of all the species found in the mud volcano ecotope, 7 species are common for the Black Sea, and 9 species, including 3 euglenophytes, are common for the Sea of Azov. It is shown that by characteristics of halobility, species found in the mud volcano belong to freshwater complex (53 %), with a significant share of marine (27 %) and brackish-water (20 %) species. Of the phytogeographic flora elements, boreal species make up 33 %, boreal-tropical – 47 %, and cosmopolites – 20 %. Three species of potentially toxic algae are recorded: diatom *Pseudo-nitzschia prolongatoides* (Hasle) Hasle, 1993, as well as dinophytes *Prorocentrum lima* (Ehrenberg) Dodge, 1975 and *Alexandrium tamiyavanichii* Balech, 1994. The last species is marine, boreal-tropical, and new to the algology of Crimea, the Black Sea, and the Sea of Azov. In the article, own and literary data on morphology, ecology, and phytogeography of species, as well as on their general distribution in different waterbodies of the world, are also presented. Some microalgae species are indicators of saprobity; they are able to participate in purification of water from organic substances. Photos of mud volcanoes and micrographs of some species are presented.

Keywords: microalgae, euglenophytes, diatoms, dinophytes, mud volcano, Crimean Peninsula

Mud volcanoes are one of unique natural phenomena widely spread around the world. They can be found on the Crimean Peninsula being part of the Bulganak sopochnoe field, which is the largest cluster of active mud volcanoes in Crimea [25]. The term "mud volcano" (in German, *Mudevulkan*) was proposed by G. Helmersen, who was involved in the studies of mud volcanoes, in particular, of Altai and oil fields of the Taman and Kerch peninsulas for 60 years. According to academician I. M. Gubkin, one of the founders and creators of oil geology in Russia, gas and oil manifestations and mud volcanism are functions of the same reasons, special forms of tectonics – of diapir structures (folds and domes arising due to extrusion from the lower horizons of highly plastic rocks, salt and clay). He was the first to establish their single genetic whole; it was used later in a program for the study of mud volcanoes of the Crimean-Caucasian geological province of Dzherelo [25].

Crimea is one of the areas of mud volcanism; there are 33 volcanoes on the territory of the peninsula [8]. Dirt pours out through craters and spreads along slopes in the form of streams. Volcano fields of Bulganak type belong to mud volcano formations with violent eruptions being not characteristic. They are natural monuments of regional significance, as well as tourist attractions.

So far, study of microalgae of mud volcanoes in Crimea has not been carried out. Moreover, there is no information available about similar research in other regions of Russia. Preliminary studies have shown presence of microalgae in the surface layer of mud volcano ejections. Relevance of the work is due to complete lack of data on the study of microalgae communities of Crimean mud volcanoes, which is of significant scientific interest.

Aim of the work is to describe the species composition of microalgae biotopes of the mud volcano located in the eastern part of the Crimean Peninsula.

MATERIAL AND METHODS

Material for the study was high-quality samples (sulfur-clay-silty substrate and water), taken by an employee of A. O. Kovalevsky Institute of Biology of the Southern Seas O. Yu. Eremin on the Crimean Peninsula from the area of active volcanoes of the Bulganak sopochnoe field. Volcanoes are scattered over a vast territory there, and their cones are almost flush with the ground or have relatively large sizes (Fig. 1).

Sampling was carried out on August 3, 2012 and April 13, 2013 in the upper 2–3-cm layer of silt suspension with surface water flowing from the mud volcano. Salinity (27–32 g per L) and water temperature (+28…+31 °C) were measured using a refractometer and digital thermometer, respectively [22].

The species composition of microalgae was determined in water preparations using Axioskop 40 (Carl Zeiss) light microscope at magnification of 10×40 with software AxioVision Rel. 4.6. For species identification, modern guides and atlases were used [4, 12, 14, 15, 16, 20, 24, 36, 39, 40, 41].

RESULTS AND DISCUSSION

A preliminary study of two samples of silty suspension of the mud volcanoes showed the presence of microscopic algae in these habitats belonging to different high rank taxonomic groups. Totally 16 species of different genera were identified: cyanobacteria (*Chamaecalyx swirenkoi* (Shirshov) Komárek et Anagnostidis), 2 dinophyte species (*Alexandrium* Halim and *Prorocentrum* Ehrenberg), 6 pennate diatom species (1 species of *Lyrella* Karajeva, *Nitzschia* Hassall, *Cylindrotheca* (Ehrenberg) Reimann et J. C. Lewin, and *Pseudo-nitzschia* H. Peragallo; 2 colonial species of *Berkeleya* Greville). Euglenophytes lodges were often found in samples; we identified 5 species of *Trachelomonas* Ehrenberg and 2 species of *Strombomonas* Deflandre.

Classification of the species identified, their size, ecology, phytogeography, and general distribution are given below.

Fig. 1. General view of the mud volcano of the Crimean Peninsula and its crater vents (photos from O. Yu. Eremin personal archive)

Phylum Cyanobacteria (Cyanoprokaryota), class Cyanophyceae, order Pleurocapsales, family Hyellaceae, genus *Chamaecalyx* J. Komárek et K. Anagnostidis, 1986. ***Chamaecalyx swirenkoi*** **(Schirshov**[*]**) Komárek et Anagnostidis, 1986** (basionym: *Dermocarpa swirenkoi* Shirshov, 1929; synonyms: *Dermocarpa clavata* Geitler, 1932; *D. clavata* var. *aquaedulcis* Geitler, 1932; *Dermocarpella clavata* (Geitler) J. Feldmann et Feldmann, 1953; *Cyanocystis swirenkoi* (Sirsov[*]) G. Hällfors et R. Munsterhjelm, 1982) [32]. Found in the mud volcano on August 03, 2012 (Fig. 2). Met often, singly. Sizes: 41.8 μm long, 13.4 μm wide. Cell sizes [according to: 5, 48]: 20–30 μm (less often up to 40 μm) long, 6.0–10.5 μm wide. This species was first described by P. P. Shirshov from the Kodyma River, a tributary of the Bug River (Ukraine) [47]. Ecology, phytogeography, and general distribution. Freshwater and brackish-water species, found in stagnant freshwater bodies, as well as in seas; boreal-tropical species. It is recorded in supralittoral [23] and microphytobenthos of the Kazantip Nature Reserve of the Sea of Azov [21], in cystoseira

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[*] The author's spelling of the surname Schirshov (Shirshov) [5, 47] is specified in contrast to Širšov used in Komárek et Anagnostidis, 1986 [32, 38].

epiphyton, and on other substrates of the Black and Aegean seas [17], as well as on algae and higher aquatic plants in Dniester River mouth and the Dniester Estuary in Odessa region [7], in epiphyton of green algae and higher aquatic plants near water edge in water bodies of Leningrad Region, in the Chikhachev Bay of the Sea of Japan [1], in a lagoon of the Gulf of Finland of the Baltic Sea [34], in Austria, Japan, Mexico, and Western Slovakia, on the Java Island [38].

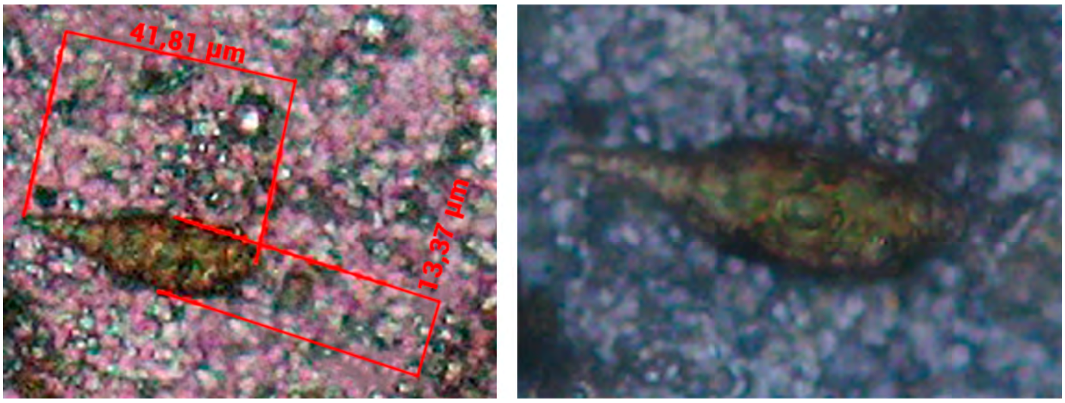

Fig. 2. Cyanobacteria *Chamaecalyx swirenkoi* in the mud volcano in the eastern part of the Crimean Peninsula

Phylum Bacillariophyta, order Naviculales Bessey, family Berkeleyaceae D. G. Mann, 1990, genus *Berkeleya* Greville, 1827. ***Berkeleya micans* (Lyngbye) Grunow, 1868** (basionym: *Bangia micans* Lyngbye, 1819; synonym: *Amphipleura micans* (Lyngbye) P. Cleve, 1894). The benthic species of diatom was found in the mud volcano on August 03, 2012. Sizes: 35 µm long, 3.5 µm wide. Sizes of valves: 39–81 µm long, 4–5 µm wide [17]. Ecology, phytogeography, and general distribution. Marine and brackish-water, boreal and natal species inhabiting mainly the southern European seas, including shallow waters near southern Crimea and the Caucasian coast of the Black Sea, on stones, rocks, and invertebrates' shells [17, 21]. The species was first described from phytoplankton and microphytobenthos of the Sea of Azov [3, 13].

***Berkeleya rutilans* (Trentepohl) Grunow, 1880** (basionym: *Conferva rutilans* Trentepohl ex Roth, 1806; synonym: *Amphipleura rutilans* (Trentepohl) Cleve, 1894). The benthic species of diatom algae was first found in the mud volcano on August 03, 2012. Sizes of valves: 35.1 µm long, 3.5 µm wide. Sizes [according to: 9, 21]: 6–38 long, 2.5–5.0 wide; 26–30 striae and 16–20 rims in 10 µm. Ecology, phytogeography, and general distribution. Marine and brackish-water, littoral and sublittoral, eurythermal species, cosmopolitan. Known in the North, White, Baltic, Barents, Kara, Mediterranean, Black, Caspian, Japanese, and East China seas, as well as in the Sea of Azov, off the coast of Romania, England, North America, Greenland, Iceland, Sweden, China, Kuwait, Japan, New Zealand, and Antarctic Australia [20].

Phylum Bacillariophyta, order Bacillariales Hendey, family Bacillariaceae Ehrenb., genus *Cylindrotheca* L. Rabenhorst, 1859. ***Cylindrotheca closterium* (Ehrenberg) Reimann et J. Lewin, 1964** [45] (basionym: *Ceratoneis closterium* Ehrenb. 1839; synonyms: *Nitzschia closterium* (Ehrenb.) W. Smith, 1853; *N. reversa* W. Smith, 1853; *N. closterium* var. *reversa* (W. Smith) Hauck, 1872; *Nitzschiella closterium* Rabenhorst, 1864; *Nitzschia rostratum* Grunow, 1880; *N. longissima* var. *closterium* (Ehrenb.) Van Heurck, 1885; *N. curvirostris* var. *closterium* (Ehrenb.) De Toni, 1892; *Nitzschiella longissima* var. *closterium* (Ehrenb.) Peragallo et Peragallo, 1897; *Homoeocladia closterium* (Ehrenb.) Kuntze, 1898; *Nitzschiella tenuirostris* Mereschk., 1901; *Nitzschia longissima* Gran, 1930; *N. closterium* var. *recta* Gran, 1931). Found in the mud volcano on August 03, 2012 (Fig. 3). Sizes: 25–260 µm long, 1.5–8.0 µm wide, 12–16 striae in 10 µm [46]. Ecology, phytogeography, and general distribution. The species is eurythermic, euryhaline, marine and brackish-water, benthic-planktonic. It can be found in plankton of neritic waters, littoral, and sublittoral of seas; cosmopolite; identified in all geographical zones of the World Ocean [20, 35].

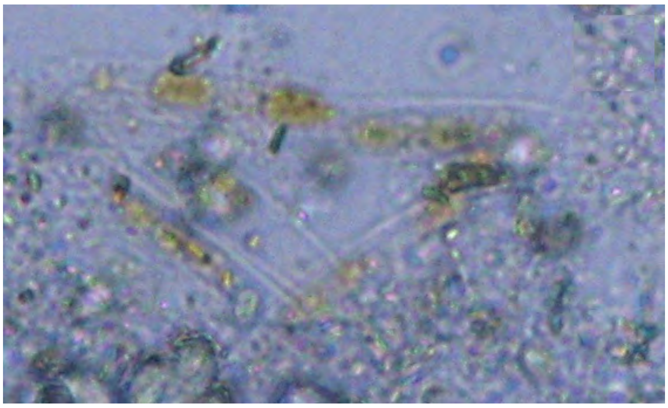

Fig. 3. *Cylindrotheca closterium* cells with chloroplasts in the mud volcano

Phylum Bacillariophyta, order Lyrellales D. G. Mann, 1990, family Lyrellaceae D. G. Mann, 1990, genus *Lyrella* N. I. Karajeva, 1978. ***Lyrella atlantica* (Gregory) D. G. Mann, 1990** (basionym: *Navicula atlantica* A. W. F. Schmidt, 1874; synonyms: *Navicula lyra* var. *atlantica* A. Schmidt, 1874; *Lyrella lyra* var. *atlantica* (Schmidt) Karajeva, 1988). Found in the mud volcano on August 03, 2012 (Fig. 4). Sizes: frustules of 60–100 µm long, 26–32 µm wide, 9–11 striae in 10 µm [4]; 59–65 µm long, 29–35 µm wide, 10 striae in 10 µm [16]. Ecology, phytogeography, and general distribution. Marine, sublittoral species, boreal and natal. Found in the coastal waters of Britain [36] and Australia, as well as in the Black and North seas [16].

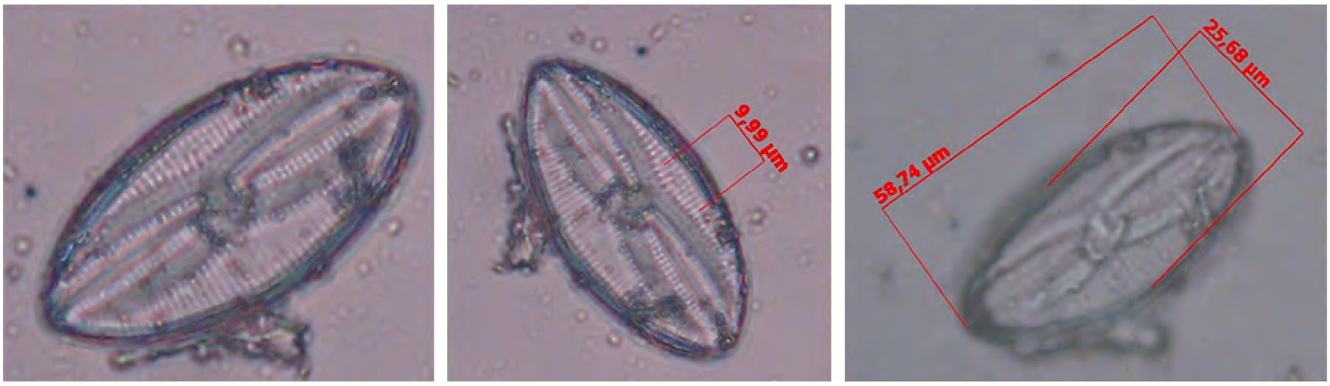

Fig. 4. Benthic diatom *Lyrella atlantica* from the mud volcano

Phylum Bacillariophyta, order Bacillariales Hendey, family Bacillariaceae Ehrenb., genus *Nitzschia* Hassall, 1845. ***Nitzschia thermaloides* Hustedt, 1955** (= *Nitzschia translucida* Hustedt, 1959) (Fig. 44: 1–7 [39]). Found in the mud volcano on August 03, 2012 (Fig. 5). Sizes: 34.7–49.7 µm long, 3–7 µm wide, 1 fibula, 14–16 striae in 10 µm. Sizes: 20–73 µm long, 4–6 µm wide, 16–20 fibulae in 10 µm [39]; 43.2–59.5 µm long, 3.8–5.9 µm wide (light microscope); 52 µm long, 6 µm wide, 1 fibula, 21 striae in 10 µm (scanning electronic microscope) [41]. Ecology, phytogeography, and general distribution. Brackish-water, benthic, and boreal-tropical species. Identified for Crimea, the Black Sea, and the Sea of Azov for the first time. Species marked as halophilic one in Kuril Islands thermal waters, recorded at water temperature of +50…+60 °C [11]. It is common in river estuaries and in the northern fjords of the Sweden coast in winter, spring, and autumn in the supralittoral up to 1.5 m in different ecotopes (silty sand, silt, and sand), as well as on the surface of *Phormidium* and *Mytilus* [40].

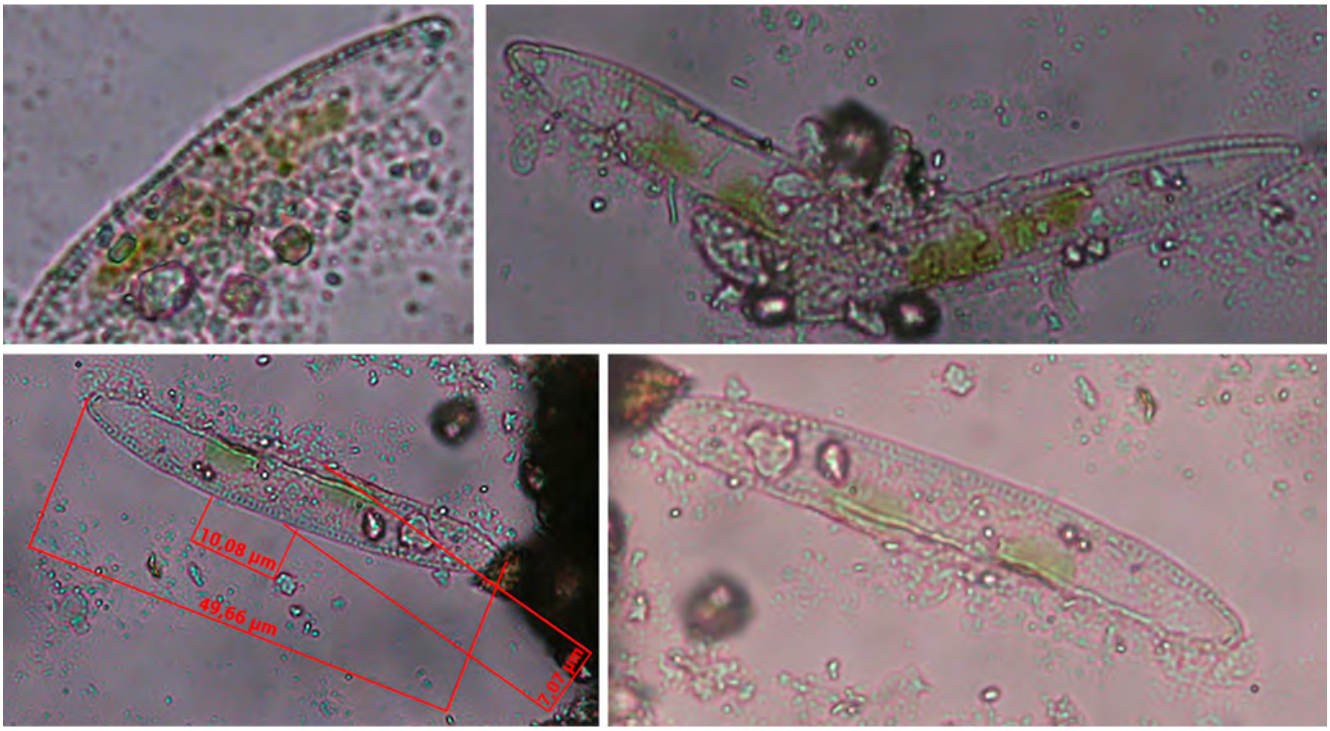

Fig. 5. Benthic diatom *Nitzschia thermaloides* with chloroplasts from the mud volcano

Phylum Bacillariophyta, order Bacillariales Hendey, family Bacillariaceae Ehrenb., genus *Pseudo-nitzschia* H. Peragallo, 1900. ***Pseudo-nitzschia prolongatoides* (Hasle) Hasle, 1993** (basionym: *Nitzschia prolongatoides* Hasle, 1965; synonym: *Nitzschia prolongata* Manguin, 1957). Found in the mud volcano on August 03, 2012. Sizes: 18.5 µm long, 3.3 µm wide. Sizes [according to: 35]: 60 µm long, 16 fibulae and 28 striae at 10 µm. It was recorded relatively recently in the Sea of Azov and in the Black Sea [2, 17]. In Kazantip nature reserve coast (depth up to 1 m) of the Sea of Azov, the species was found on: April 9, 2006 (in the epiphyton of *Enteromorpha* sp., a colony of 2 cells, 123.2 µm long, 2.8 µm wide); October 28, 2011 (in sand ground, a colony of 2 cells was identified being of 117.7 µm long and of 2.2 µm wide, as well as individual cells of 55 µm long, 2.5 µm wide); August 07, 2014 (in the epiphyton of the red alga *Ceramium rubrum*). Ecology, phytogeography, and general distribution. Marine, planktonic, belongs to potentially toxic algae. Due to poor knowledge, the species can still be attributed to boreal and natal. The species is recorded in Antarctic waters [19].

Phylum Dinophyta, class Dinophyceae, order Prorocentrales Lemmermann, family Prorocentraceae F. Stein, genus *Prorocentrum* Ehrenberg, 1834. ***Prorocentrum lima* (Ehrenberg) Dodge, 1975** (basionym: *Cryptomonas lima* Ehrenberg, 1860; synonyms: *Exuviaella marina* Cienkowski, 1881; *Dinopyxis laevis* Stein, 1883; *E. lima* (Ehrenberg) Bütschli, 1885; *E. laevis* (Stein) Schröder, 1900; *E. chathamensis* Lemmermann, 1907; *E. cincta* Schiller, 1918; *E. caspica* I. Kisselev, 1927; *E. marina* var. *lima* (Ehrenberg) Schiller, 1931; *E. ostenfeldii* Schiller, 1933; *Prorocentrum marinum* Dodge et Bibby, 1973; *P. marinum* (Cienkowski) Abé in Bodeanu, 1987–1988; *P. marinum* var. *lima* (Schiller) Krachmalny, 1994).

Found in the mud volcano on April 13, 2013 (Fig. 6). Sizes: 25 μm long, 14.7 μm wide. Sizes [according to: 18]: 30–50 μm long, 18–45 wide; Black Sea specimens: 36–44 μm long, 21–30 μm wide. Ecology, phytogeography, and general distribution. Marine, benthic species, cosmopolitan. Recorded in the Mediterranean, Adriatic, Black, Caspian seas, as well as in the Sea of Azov and in the Indian Ocean. The species is toxic and produces okadaic acid [18, 40, 47].

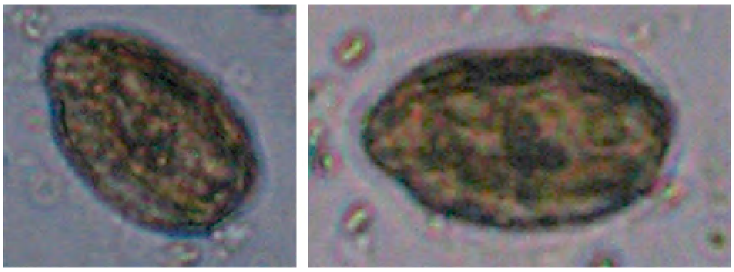

Fig. 6. Different views of dinoflagellate *Prorocentrum lima* (Ehrenberg) Dodge shells from the mud volcano

Phylum Dinophyta, class Dinophyceae, order Gonyaulacales F. J. R. Taylor, family Ostreopsidaceae Lindemann, genus *Alexandrium* Halim, 1960. ***Alexandrium tamiyavanichii* Balech, 1994.** Found in the mud volcano on April 13, 2013 (Fig. 7). Sizes: 32.3 μm long, 29.5 μm wide. Sizes [according to: 29]: 40.8–41.7 μm long, 37.5–43.8 μm wide. Ecology, phytogeography, and general distribution. Marine, planktonic, boreal-tropical, and potentially toxic species [47]. Found in water bodies in Thailand and the Philippines [29]. This is a new species for algal flora of Crimea and the Black Sea.

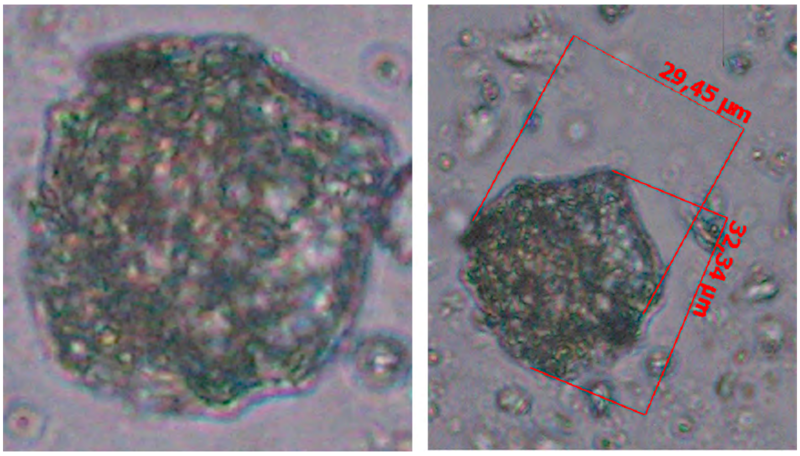

Fig. 7. Dinoflagellate *Alexandrium tamiyavanichii* Balech from the mud volcano

Euglenophytes species, first identified in the mud volcano in the eastern part of the Crimean Peninsula, their ecology, phytogeography, and general distribution [22] are given below.

Phylum Euglenophyta, class Euglenophyceae, order Euglenales, family Euglenaceae, genus *Trachelomonas* Ehrenberg, 1838. ***T. armata* (Ehrenberg) Stein, 1878** (basionym: *Pantotrichum armatum* Ehrenberg; synonym: *Chaetotyphla armata* Ehrenberg, 1833). Found in the mud volcano on April 13, 2013. Sizes: 32.4 μm long, 22.3 μm wide. Sizes [according to: 14]: 30–39 μm long, 25–28.5 μm wide. Ecology, phytogeography, and general distribution. Freshwater, boreal-tropical, natal species. Recorded in Russia [14, 15], Romania [31], China [37], Singapore [43], North and South America [49], Australia and New Zealand [33], Turkey [27], the Netherlands, Slovakia, Sweden, Brazil, Caribbean Islands, Britain, Germany, Spain, Bangladesh, in Africa [32], as well as in the Baltic Sea [34].

***T. hexangulata* Svirenko, 1914** [48]. Found on April 13, 2013 in the surface water of the mud volcano. Sizes: 32.2–34 µm long, 12.6–14 µm wide; neck: 4.2 µm high, 2.8 µm wide. Sizes [according to: 14]: 27–34 µm long, 12–16 µm wide. Ecology, phytogeography, and general distribution. Freshwater, boreal species. Found among cyanobacteria communities in lakes plankton, in the swampy sedge hummock, in forest ditches, puddles along channels of dried forest streams, at the edges of sedge marshes, in sphagnum swamps of the forest-tundra [14]. It is recorded in the marsh waters of European Russia, Western Siberia [14, 15], Far East [10], and Chelyabinsk region [26], in waters of Great Lakes of the USA [42], in water bodies of Romania [30], Turkey [27], the Netherlands, Poland, Romania, Slovakia, Sweden, Britain, Spain, India, Brazil, Argentina, North America, Cuba, Tajikistan, Thailand, Taiwan, Bangladesh, Mexico, and Iraq [32], as well as in the Sea of Azov [6] and the Baltic Sea [34].

***T. planctonica* Svirenko, 1914.** Found in the mud volcano on April 13, 2013. Lodge sizes: 23–27 µm long, 18–21 µm wide. Sizes [according to: 14]: 21–31 µm long, 17–22 µm wide. Ecology, phytogeography, and general distribution. Freshwater, boreal-tropical. Can be found in small water bodies with fresh stagnant water, occasionally in rivers, mainly in plankton of rivers in Tomsk and Chelyabinsk regions, Western Siberia [14, 26], Russian Far East [10], in water bodies of the Netherlands, Poland, Romania, Slovakia, Sweden, Britain, Spain, India, Brazil, Argentina, North America, Cuba, Tajikistan, Thailand, Taiwan, and Bangladesh [32], as well as in the Baltic Sea [34] and the Sea of Azov [6].

***T. scabra* Playfair, 1915** (= *Trachelomonas scabra* var. *latior* Skwortzow, 1925). Found in the mud volcano on August 03, 2012. Sizes: 23–27 µm long, 18–21 µm wide. Sizes [according to: 14]: 18–33 µm long, 15–20 µm wide. Ecology, phytogeography, and general distribution. Freshwater species, boreal-tropical and natal. Found in the coastal lakes of Georgia and Chelyabinsk region [26], Britain, North America [50], Romania [31], Spain [27], Turkey [28], and China [37], as well as in Australia and New Zealand [32]. Recorded in the Sea of Azov [6].

***T. volvocina* (Ehrenberg) Ehrenberg, 1834** (= *Microglena volvocina* Ehrenb.). Found in the mud volcano on April 13, 2013 with cell diameter of 8–9 µm (Fig. 8). Lodges are spherical, with diameter of (4)–8–23–(32) µm [14]. Ecology, phytogeography, and general distribution. It is a freshwater species, mainly inhabiting stagnant water, less commonly found in weakly brackish water at pH of (4.4)–5.5–8.4. It is characterized as β-mesosaprob-oligosaprob, has mixotrophic nutrition. Boreal species. It is recorded in Odessa Region and Crimea [14].

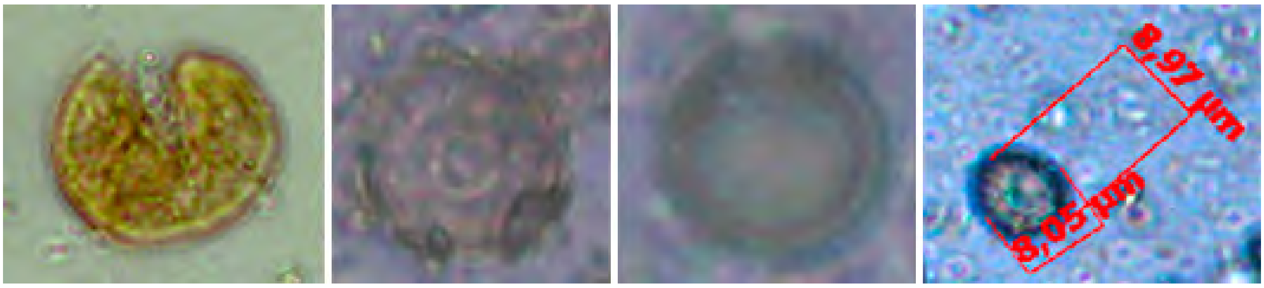

Fig. 8. *Trachelomonas volvocina* from the mud volcano, view from different angles

Phylum Euglenophyta, class Euglenophyceae, order Euglenales, family Euglenaceae, genus *Strombomonas* Deflandre, 1930 (= *Trachelomonas* Ehrenberg). Lodge sizes of species of this genus are larger and more variable in shape compared to those of the genus *Trachelomonas* [14]. *Strombomonas* were found in the intravital state in the mud volcano. Representatives of this genus were often recorded in samples, but it was difficult to identify them to species. Micrographs of some of them are given below.

Strombomonas acuminata **(Schmarda) Deflandre, 1930** (basionym: *Lagenella acuminata* Schmarda; synonym: *Strombomonas acuminata* var. *verricosa* Teodoresco). Found in the mud volcano on April 13, 2013 (Fig. 9). Lodge sizes: 21.5–27.6 µm long, 20.7–32 µm wide; neck: 14×9.8 µm. Sizes [according to: 14]: 38.0–55.5 µm long, 28–33 µm wide. Ecology, phytogeography, and general distribution. The species is freshwater, boreal. It is recorded in the water bodies of Romania [31], Crimea, the Caucasus, Central Asia, and Western Siberia [14].

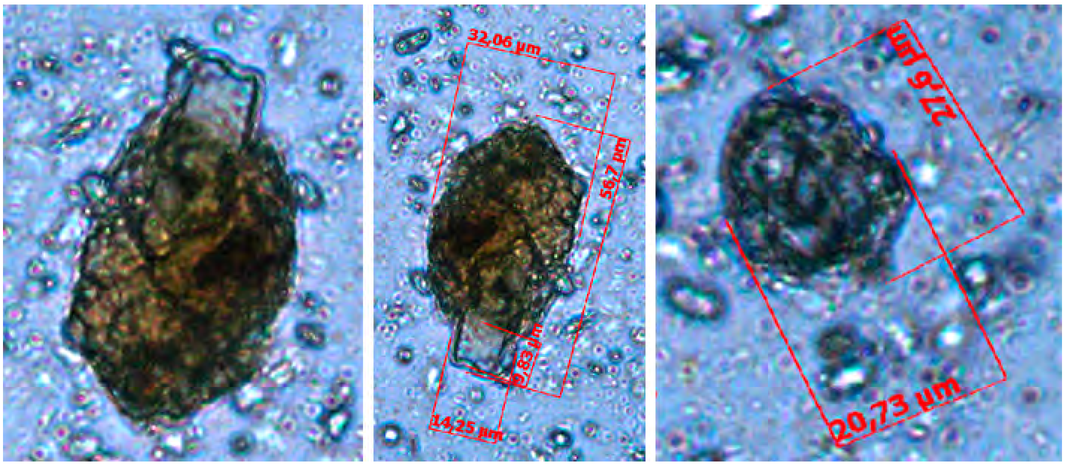

Fig. 9. View of lodges of *Strombomonas acuminata* from the mud volcano

St. tambowica **(Swirenko) Deflandre, 1930** (synonyms: *Trachelomonas zmiewica* Swirenko; *Tr. tambowica* var. *granulata* Skvortzov; *Strombomonas verrucosa* var. *zmiewica* (Swirenko) Deflandre, 1930). Found in the mud volcano on April 13, 2013 (Fig. 10). Lodge sizes: 38–55.5 µm long, 28–34 µm wide. Sizes [according to: 14]: 47.5–56 µm long, 26.6–32 µm wide. Ecology, phytogeography, and general distribution. The species is freshwater, boreal. Recorded in the plankton of freshwater bodies of Ukraine, as well as Tambov, Rostov and Tomsk regions of Russia [14].

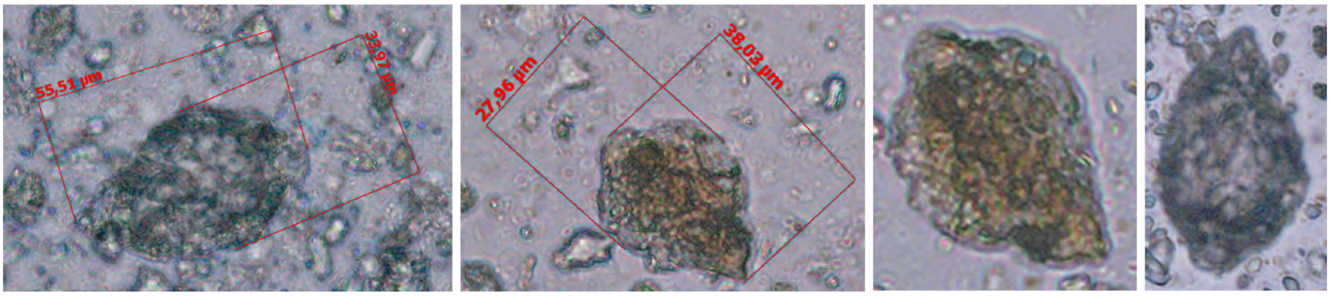

Fig. 10. Lodges of *Strombomonas tambowica* from the mud volcano, view from different angles

Euglenophytes, uniting unicellular, less often colonial microscopic algae, inhabit freshwater bodies throughout the world. A small number of them are confined to brackish and sea waters. These are representatives of the genera *Eutreptia* Perty, *Eutreptiella* da Cunha, *Klebsina* P. C. Silva, *Trachelomonas* Ehrenberg, and *Strombomonas* Deflandre. They can be found in plankton, in thickets of coastal algae and interstitial of sandy beaches, in rivers and lakes, and at sea coasts with low salinity. *Trachelomonas caudata* (Ehrenberg) F Stein, *T. volvocina* (Ehrenberg) Ehrenberg, and *T. volvocina* var. *papillata* Lemmermann species were identified in the Black Sea [17]. Totally 24 species were found in the Sea of Azov, 7 of them (*Trachelomonas borodiniana* Swirenko, *T. globularis* (Averintsev) Lemmermann, *T. hispida* (Perty) F. Stein, *T. planctonica* Svirenko, *T. scabra* Playfair, *T. verrucosa* A. C. Stokes, and *T. volvocina* (Ehrenberg) Ehrenberg) were mainly recorded in summer and autumn [6, 21]. Most species of microalgae from the mud volcano having a mixotrophic or totally saprophytic type of nutrition are actively involved in self-cleaning of habitats.

Conclusion. A preliminary study of microalgae of the mud volcano in the region of the Bulganak sopochnoe field on the Crimean Peninsula showed the diversity of their species composition in watered habitats.

We found cyanobacteria *Chamaecalyx swirenkoi* and 15 species of eukaryotic microalgae: 2 dinoflagellate species (of genera *Prorocentrum* and *Alexandrium*), 6 diatom species (1 of genera *Lyrella*, *Pseudo-nitzschia*, *Nitzschia*, and *Cylindrotheca*; 2 of genus *Berkeleya*), as well as 7 species of euglenophytes (5 of genus *Trachelomonas*; 2 of genus *Strombomonas*). Some of them are widespread in the microphytobenthos of the Sea of Azov and the Black Sea. Of all the types of algae found in the mud volcano, 7 species are common for the Black Sea, while 9 species, including 3 species of euglenophytes, are common for the Sea of Azov.

Three species considered to be potentially toxic were identified: diatom *P. prolongatoides*, as well as dinophytes *Pr. lima* and *A. tamiyavanichii*. The last species is marine, boreal-tropical, and new to Crimean flora. By characteristics of halobility, species found in the mud volcano belong to freshwater complex (53 %), with a significant share of marine (27 %) and brackish-water (20 %) species. Taking into account phytogeographic features, it can be concluded that boreal species make up 33 %, boreal-tropical species – 47 %, and cosmopolite – 20 %.

The work was carried out within the framework of government research assignment of IBSS RAS "Investigation of the mechanisms of controlling production processes in biotechnological complexes with the aim of developing the scientific foundations for the production of biologically active substances and technical products of marine genesis" (no. AAAA-A18-118021350003-6).

Acknowledgment. The article is dedicated to IBSS employee Oleg Yuryevich Eremin who was an enthusiastic participant of many scientific expeditions aimed at studying of hypersaline lakes of Crimea and always helped his colleagues with hydrobiological samplings. He died tragically in 2014 when returning from one of the expeditions. The idea of studying microalgae on the surface of silty substrates in a mud volcano near hypersaline water bodies of Crimea belongs to him. It was he who took samples from the mud volcano and insisted on their analysis.

REFERENCES

1. Beljakova R. N. Synopsis of the benthic cyanophyta flora of the Russian Far-Eastern Seas. 1. Chroococcales (Microcystaceae, Chroococcaceae). *Botanicheskii zhurnal*, 2000, vol. 85, no. 2, pp. 106–118. (in Russ.)
2. Bondarenko A. V., Ryabushko L. I. Diatomovye vodorosli bentosa Krymskogo poberezh'ya Azovskogo morya. In: *Sovremennye problemy al'gologii* : materialy Mezhdunar. nauch. konf. i VII Shkoly po morskoi biologii, Rostov-on-Don, 9–13 June, 2008. Rostov-on-Don : YuNTs RAN, 2008, pp. 61–63. (in Russ.)
3. Bondarenko A. V. Vidovoe i ekologo-geograficheskoe raznoobrazie bentosnykh diatomovykh vodoroslei krymskogo poberezh'ya Azovskogo morya. In: *Bioraznoobrazie i ustoichivoe razvitie* : materialy nauch.-praktich. konf., Simferopol, 20–22 May, 2010. Simferopol : KNTs, 2010, pp. 19–21. (in Russ.)
4. *Diatomovyi analiz. Kniga 3. Opredelitel' iskopaemykh i sovremennykh diatomovykh vodoroslei* / A. N. Krishtofovich, A. I. Proshkina-Lavrenko

(Eds). Leningrad : Gosizdat, 1950, 398 p. (in Russ.)

5. Elenkin A. A. *Sinezelenye vodorosli SSSR.* Moscow ; Leningrad : AN SSSR, 1938, iss. 1, 984 p. (in Russ.)
6. Kovaleva G. V. Sistematicheskii spisok mikrovodoroslei bentosa i planktona pribrezhnoi chasti Azovskogo morya i prilegayushchikh vodoemov. In: *Sovremennye problemy al'gologii* : materialy Mezhdunar. nauch. konf. i VII Shkoly po morskoi biologii, Rostov-on-Don, 9–13 June, 2008. Rostov-on-Don : YuNTs RAN, 2008, pp. 174–192. (in Russ.)
7. Kondrat'yeva N. V., Kovalenko O. V., Prykhod'kova L. P. *Vyznachnyk prisnovodnykh vodorostei Ukrainskoi RSR. Iss. I. Synozeleni vodorosti – Cyanophyta. Pt. 1. Zahalna kharakterystyka synozelenykh vodorostei. Klas khrookokovi vodorosti – Chroococcophyceae. Klas khamesyfonovi vodorosti – Chamaesiphonophyceae* / N. V. Kondratyeva (Ed.). Kyev : Naukova dumka, 1984, 388 p. (in Ukr.)
8. Korzhenevsky V. V., Korzhenevskaya Yu. V. Sinecology of communities of the Hill of Odelnburg. Bulganaksky mud-volcanic field. In: *Actual Problems of Biodiversity and Nature Management* : materials of the II National scientific-practical conference, dedicated to the 20th anniversary of the Marine Ecology Department of FGBOU VO "KGMTU", Kerch, 15–17 May, 2019. Simferopol, 2019, pp. 584–587. (in Russ.)
9. Korotkevich O. S. Diatomovaya flora litorali Barentseva morya. *Trudy Murmanskogo morskogo biologicheskogo instituta*, 1960, iss. 1, pp. 68–338. (in Russ.)
10. Medvedeva L. A., Nikulina T. V. *Catalogue of Freshwater Algae of the Southern Part of the Russian Far East.* Vladivostok : Dal'nauka, 2014, 271 p. (in Russ.)
11. Nikulina T. V. Biodiversity of algae of hot springs from Kuril Islands (Russia). *Al'gologia*, 2010, vol. 20, no. 3, pp. 334–356. (in Russ.). https://doi.org/10.1615/InterJAlgae.v12.i4.10
12. *Opredelitel' presnovodnykh vodoroslei. Iss. 4. Diatomovye vodorosli* / A. I. Proshkina-Lavrenko (Ed.). Moscow : Sovetskaya nauka, 1951, 619 p. (in Russ.)
13. Pitsyk L. K. O kachestvennom sostave fitoplanktona Azovskogo morya. *Trudy Sevastopol'skoi biologicheskoi stantsii*, 1963, vol. 14, pp. 71–89. (in Russ.)
14. Popova T. G. *Opredelitel' presnovodnykh vodoroslei SSSR. Iss. 7. Evglenovye vodorosli.* Moscow : Sovetskaya nauka, 1955, 282 p. (in Russ.)
15. Popova T. G. *Flora planktonnykh kriptogamnykh vodoroslei SSSR. Euglenophyta.* Vol. 8, no. 1. Moscow ; Leningrad : Nauka, 1966, 412 p. (in Russ.)
16. Proshkina-Lavrenko A. I. *Diatomovye vodorosli bentosa Chernogo morya.* Moscow ; Leningrad : Nauka, 1963, 243 p. (in Russ.)
17. Ryabushko L. I. *Microphytobenthos of the Black Sea.* Sevastopol : EKOSI-Gidrofizika, 2013, 416 p. (in Russ.)
18. Ryabushko L. I. *Potentially harmful microalgae of the Azov and Black Sea basin* / V. I. Ryabushko (Ed.). Sevastopol : EKOSI-Gidrofizika, 2003, 288 p. (in Russ.)
19. Ryabushko L. I. The state of knowledge microphytobenthos Argentine Islands of Antarctica. *Trudy Belorusskogo gosudarstvennogo universiteta*, 2016, vol. 11, pt. 1, pp. 337–350. (in Russ.)
20. Ryabushko L. I., Begun A. A. *Diatoms of Microphytobenthos of the Sea of Japan (Synopsis and Atlas)* : in 2 vols. Vol. 2. Sevastopol : PK "KIA", 2016, 324 p. (in Russ.)
21. Ryabushko L. I., Bondarenko A. V. *Microalgae of the Plankton and Benthos of the Sea of Azov (Check-list, Synonymics, and Comment).* Sevastopol : EKOSI-Gidrofizika, 2011, 211 p. (in Russ.)
22. Ryabushko L. I., Shadrin N. V. The first discovery of microalgae of the genus *Trachelomonas*

Ehrenberg, 1834 (Euglenophyta) in the crater of an active mud volcano (Kerch Peninsula, Crimea). In: *Problemy botaniki Yuzhnoi Sibiri i Mongolii* : sb. nauch. statei po materialam XVII mezhdunar. konf. (Barnaul, 24–27 May, 2018). Barnaul : Izd-vo Altaiskogo gos. universiteta, 2018, pp. 124–127. (in Russ.)

23. Sadogurskaya S. A. Flora Cyanophyta supralitorali Kazantipskogo prirodnogo zapovednika (Azovskoe more). *Trudy Nikitskogo botanicheskogo sada*, 2001, vol. 120, pp. 124–131. (in Russ.)
24. Safonova T. A. *Evglenovye vodorosli Zapadnoi Sibiri.* Novosibirsk : Nauka, 1987, 192 p. (in Russ.)
25. Shnyukov E. F., Sobolevskii Yu. V., Gnatenko G. I., Naumenko P. I., Kutnii V. A. *Gryazevye vulkany Kerchensko-Tamanskoi oblasti. Atlas.* Kiev : Naukova dumka, 1986, 152 p. (in Russ.)
26. Yarushina M. I., Tanaeva G. B., Eremkina T. V. *Flora vodoroslei vodoemov Chelyabinskoi oblasti.* Ekaterinburg : UrO RAN, 2004, 308 p. (in Russ.)
27. Alvárez C. M. Catálogo de las algas continentales españolas. II. Craspedophyceae, Cryptophyceae, Chrysophyceae, Dinophyceae, Euglenophyceae, Haptophyceae, Phaeophyceae, Rhodophyceae, Xanthophyceae. *Acta Botanica Malacitana*, 1984, vol. 9, pp. 27–40.
28. Aycel V. Check-list of the freshwater algae of Turkey. *Journal of the Black Sea / Mediterranean Environment*, 2005, vol. 11, pp. 1–124.
29. Balech E. Three new species of the genus *Alexandrium* (Dinoflagellata). *Transactions of the American Microscopical Society*, 1994, vol. 113, no. 2, pp. 216–220. https://doi.org/10.2307/3226651
30. Brosnan S., Brown J. P., Farmer M. A., Triemer R. E. Morphological separation of the euglenoid genera *Trachelomonas* and *Strombomonas* (Euglenophyta) based on lorica development and posterior strip reduction. *Journal of Phycology*, 2005, vol. 41, iss. 3, pp. 590–605. https://doi.org/10.1111/j.1529-8817.2005.00068.x
31. Caraus I. *The algae of Romania. A Distributional Checklist of Actual Algae.* Version 2.3. Bacău: Universitatea Bacău, 2012, 809 p. http://www.vliz.be/imisdocs/publications/270678.pdf
32. Guiry M. D., Guiry G. M. *AlgaeBase*. World-wide electronic publication. Galway : National University of Ireland, 2018. URL: https://www.algaebase.org (accessed 30.08.2019).
33. Day S. A., Wickham R. P., Entwisle T. J., Tyler P. A. *Bibliographic Check-list of Non-marine Algae in Australia*. Canberra : Australian Biological Resources Study, 1995, 276 p. (Flora of Australia Supplementary Series ; 4).
34. Hällfors G. *Checklist of Baltic Sea Phytoplankton Species (Including Some Heterotrophic Protistan Groups)*. Helsinki : Baltic Marine Environment Protection Commission, 2004, 210 p. (Baltic Sea Environment Proceedings ; no. 95).
35. Hasle G. R. *Nitzschia pungiformis* (Bacillariophyceae), a new species of the *Nitzschia seriata* group. *Norwegian Journal of Botany*, 1971, vol. 18, pp. 139–144.
36. Hendey N. I. *An Introductory Account of the Smaller Algae of British Coastal Waters. Part 5. Bacillariophyceae (Diatoms)*. Koenigstein : Otto Koeltz Science Publishers, 1976, 317 p. (Fishery Investigations ; Series 4).
37. Hu H., Wei Y. *The Freshwater Algae of China. Systematics, Taxonomy, and Ecology.* Beijing : Science Press, 2006, 1023 p. (in Chinese).
38. Komárek J., Anagnostidis K. *Cyanoprokaryota.* Tail 1 / Part 1. *Chroococcales. Süsswasserflora von Mitteleuropa.* [Berlin] : SpringerSpektrum, 2008, 548 p. (Süßwasserflora von Mitteleuropa ; Bd. 19/1).
39. Krammer K., Lange-Bertalot H. *Bacillariophyceae.* Tail 2 / Part 2. *Bacillariaceae, Epithemiaceae, Surirellaceae.* [Stuttgart] :

SpringerSpektrum, 1988, 610 p. (Süsswasserflora von Mitteleuropa ; vol. 2/2).

40. Kuylenstierna M. *Benthic Algal Vegetation in the Nordre Älv Estuary (Swedish West Coast).* [Dissertation]. Göteborg, Sweden : Department of Mar. Bot. University of Göteborg, 1989, vol. 1, 244 p.
41. Kuylenstierna M. *Benthic Algal Vegetation in the Nordre Älv Estuary (Swedish West Coast).* [Dissertation]. Göteborg, Sweden : Department of Mar. Bot. University of Göteborg, 1990, vol. 2 [plates], 76 p.
42. Murakami Y., Oshima Y., Yasumoto T. Identification of okadaic acid as a toxic component of a marine dinoflagellate *Prorocentrum lima*. *Bulletin of the Japanese Society for the Science of Fish*, 1982, vol. 48, iss. 1, pp. 69–72. https://doi.org/10.2331/suisan.48.69
43. Pham M. N., Tan H. T. W., Mitrovic S., Yeo H. H. T. *A checklist of the algae of Singapore*. 2nd edition. Singapore : Raffles Museum of Biodiversity Research, National University of Singapore, 2011, 100 p.
44. Prescott G. W. *Algae of the Western Great Lakes Area.* With an illustrated key to the genera of desmids and freshwater diatoms. Revised [second] ed. Dubuque, Iowa : Wm. C. Brown Company Publishers, 1962, 977 p.
45. Reimann B. E. F., Lewin J. C. The diatom genus *Cylindrotheca* Rabenhorst (with a reconsideration of *Nitzschia closterium*). *Journal of the Royal Microscopical Society*, 1964, vol. 83, iss. 3, pp. 283–296. https://doi.org/10.1111/j.1365-2818.1964.tb00542.x
46. Ryabushko L. I., Balycheva D. S., Bondarenko A. V., Zheleznova S. N., Begun A. A., Stonik I. V. Different aspects of studying a diatom *Cylindrotheca closterium* (Ehrenberg) Reimann et Lewin 1964 in natural and laboratory conditions. *Morskoj biologicheskij zhurnal*, 2019, vol. 4, no. 2, pp. 52–63. http://doi.org/10.21072/mbj.2019.04.2.06
47. Schirshov P. P. Über zwei interessante Algen aus dem fluss Kodyma (Nebenfluss des Süd-Bug). *Die Nachrichten der Hydrobiologischen Station zu Dnjepropetrowsk*, 1930, I, pp. 195, fig. 2.
48. Svirenko D. Zur Kenntnis der russischen Algenflora, I. Die Euglenaceen Gattung *Trachelomonas*. *Archiv für Hydrobiologie und Planktonkunde*, 1914, vol. 9, pp. 630–647.
49. Taylor F. J. R., Fukuyo Y., Larsen J. Taxonomy of Harmful Dinoflagellates In: G. M. Hallegraeff, D. M. Anderson, A. D. Cembella (Eds). *Manual on Harmful Microalgae*. 2nd revised ed. Paris : UNESCO Publ., 2003, pp. 389–433. (Monographs on Oceanographic Methodology ; 11).
50. Wolowski K., Walne P. L. *Strombomonas* and *Trachelomonas* species (Euglenophyta) from south-eastern USA. *European Journal of Phycology*, 2007, vol. 42, iss. 4, pp. 409–431. https://doi.org/10.1080/09670260701702508

МИКРОВОДОРОСЛИ ГРЯЗЕВОГО ВУЛКАНА БУЛГАНАКСКОГО СОПОЧНОГО ПОЛЯ КРЫМСКОГО ПОЛУОСТРОВА

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Грязевые вулканы — одно из уникальных явлений природы. Они широко распространены по всему миру. Грязевые вулканы встречаются и на территории Крыма, в том числе на Булганакском сопочном поле — крупнейшем скоплении действующих вулканов на полуострове (45°25′29.04″ с. ш., 36°27′51.64″ в. д.). Изучение одноклеточных водорослей грязевых вулканов в Крыму, как и в других регионах России, до настоящего времени не проводили. Необходимость и актуальность исследований продиктована отсутствием сведений о видовом составе микроводорослей грязевых вулканов. Пробы собраны О. Ю. Ерёминым 03.08.2012 и 13.04.2013 в верхнем 2–3-сантиметровом слое суспензии вместе с приповерхностной водой, вытекающей из него. Диапазон солёности и температуры воды составлял 27–32 г·л$^{-1}$ и +28…+31 °C. Видовой состав микроводорослей определяли в водных препаратах в прижизненном состоянии водорослей с помощью светового микроскопа Axioskop 40 (Carl Zeiss) при увеличении 10×40, используя программное обеспечение AxioVision Rel. 4.6. Обнаружено 16 видов, принадлежащих к высшим таксономическим группам: Cyanobacteria (1 вид), Dinophyta (2), Bacillariophyta (6) и Euglenophyta (7). Из них цианобактерия *Chamaecalyx swirenkoi* (Schirshov) Komárek et Anagnostidis, 1986 найдена нами в августе 2012 г. В пробах отмечены пеннатные диатомовые водоросли — как одиночно живущие (родов *Cylindrotheca* (Ehrenberg) Reimann & J. C. Lewin, *Lyrella* Karajeva и *Nitzschia* Hassall), так и колониальные (родов *Berkeleya* Greville и *Pseudo-nitzschia* H. Peragallo). Солоноватоводный, бентосный, бореально-тропический вид *Nitzschia thermaloides* Hustedt впервые отмечен для альгофлоры Крыма, Чёрного и Азовского морей. Также обнаружены эвгленовые водоросли — 5 видов рода *Trachelomonas* Ehrenberg и 2 вида *Strombomonas* Deflandre. Из всех видов, найденных в экотопе грязевого вулкана, 7 являются общими с Чёрным морем, а 9, включая 3 вида эвгленовых водорослей, — с Азовским. Показано, что по характеру галобности в грязевых вулканах преобладают виды, типичные для пресноводного комплекса (53 %), при существенной доле морских (27 %) и солоноватоводных видов (20 %). Из фитогеографических элементов флоры бореальные виды составляют 33 %, бореально-тропические — 47 %, космополиты — 20 %. Отмечено три вида потенциально токсичных водорослей — диатомея *Pseudo-nitzschia prolongatoides* (Hasle) Hasle, 1993 и динофитовые *Prorocentrum lima* (Ehrenberg) Dodge, 1975 и *Alexandrium tamiyavanichii* Balech, 1994. Последний вид является морским, бореально-тропическим и новым для альгофлоры Крыма и Чёрного моря. В статье также представлены собственные и литературные данные по морфологии, экологии, фитогеографии видов и их общему распространению в разных водоёмах мира. Некоторые виды микроводорослей относятся к индикаторам сапробности; они способны участвовать в очищении вод от избытка растворённых органических веществ. Приведены фотоснимки грязевых вулканов и микрофотоснимки некоторых видов.

Ключевые слова: микроводоросли, эвгленовые, диатомовые, динофитовые, грязевой вулкан, Крымский полуостров

1871
ИнБЮМ – IBSS

Морской биологический журнал, 2020, том 5, № 1, с. 78–89
Marine Biological Journal, 2020, vol. 5, no. 1, pp. 78–89
https://mbj.marine-research.org; doi: 10.21072/mbj.2020.05.1.08
ISSN 2499-9768 print / ISSN 2499-9776 online

UDC 581.526.323(262.5)

TO THE STUDY OF MACROPHYTOBENTHOS OF COASTAL WATERS OF KARADZHINSKY PLOT ON TARKHANKUT PENINSULA (CRIMEA, BLACK SEA)

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Received by the Editor 27.05.2019; after revision 27.12.2019;
accepted for publication 27.03.2020; published online 31.03.2020.

Composition and distribution of seaweeds in coastal waters of the Karadzhinskaya Bay, adjacent to the southern cluster of a specially protected natural area "Tarkhankut" Nature Park (Crimea, Tarkhankut Peninsula, Black Sea), are described and discussed. Peculiarities of the coastal zone geomorphology determine structural features of macrophytobenthos and its intermediate position in relation to macrophytobentos of adjacent abrasive and accumulative coasts. Totally 57 species of seaweeds were identified (Chlorophyta – 12, Ochrophyta (cl. Phaeophyceae) – 12, Rhodophyta – 33); 21 of them occur in pseudolittoral, and 56 – in sublittoral. Biomass ranges from 0.5 kg per m^2 in pseudolittoral to 4.5 kg per m^2 in sublittoral. Nature and quantity of benthic vegetation, flora composition, and ratio of main ecological-floristic groups of macrophytobenthos are generally characteristic for the Tarkhankut-Sevastopol hydrobotanical region of the Black Sea. A rare fraction of flora includes 14 taxa listed in nature conservation lists of various ranks. The biotope subjects to special protection according to the EU Habitats Directive (Directive 92/43/EEC). The territorial-aquatic complex is important both from nature protection and recreation points of view. Its conservation will contribute to structural and functional integrity of Black Sea coastal zone ecosystem.

Keywords: Black Sea, Crimea, Tarkhankut Peninsula, macrophytobenthos, species composition, biomass, distribution

The Tarkhankut Peninsula of Crimea has a dynamic geological past and is characterized with a variety of geomorphologic structures, which, along with weak anthropogenic transformation, determine a high level of both landscape and biological diversity [17]. As part of specially protected natural areas, unique areas of true sod-cereal (poor-forbs) and desert (wormwood-cereal) steppes are preserved here [12]. Biological and landscape diversity is also characteristic of Black Sea waters washing Tarkhankut Peninsula coast. This determines the sozological significance of the region and its potential for optimizing protected areas and ecological networks of various types. On the other hand, it makes the area attractive to adherents of unorganized and extreme tourism. They are usually followed by "traditional" recreants, and it means that infrastructure is inevitably formed. Some areas in a sea coastal zone, transformed (recreational) ones, as well as priority areas for biodiversity conservation (including existing and planned protected objects), have already become closely adjacent and alternating. Taking into consideration dynamics of coastal development, including plans for construction of large-scale recreational complexes, a yacht marina, etc., it can be concluded that the area biological diversity is under threat. Allocation of sites for conservation or limited ecological tourism requires special comprehensive studies. Integral territorial-aquatic complexes of the sea coastal zone with preserved natural or quasi-natural vegetation are of the greatest significance [15]. At the same time, both water areas off Tarkhankut Peninsula coast and macrophytobenthos forming the foundation of coastal marine biotopes have been poorly studied. To fill this gap, we characterized bottom vegetation cover on abrasive and accumulative coast within the boundaries of the vast Karadzhinsky Plot located in the far west of Tarkhankut [13, 28, 29]. At this stage, we set an aim: within the framework of a comprehensive hydrobotanical survey of the Karadzhinsky Plot, to give both a detailed description of marine phytobenthos at the abrasive and accumulative coast and recommendations for their effective use.

Research area. The Tarkhankut Peninsula is of tectonic origin and is a gently sloping rampart formed by tertiary limestones of pontic tier [4]. The Karadzhinskaya Bay is located in the central part characterized by a diverse geomorphological structure of the coast and limited by Karamrun and Tarkhankut capes (Fig. 1).

The bay depression is continued by a lagoon salt Karadzha Lake (Tarkhankut group of lakes), separated by above water sandbar, and a broad arroyo of the same name [5]. Northern and southern coasts of the bay are abrasive, composed of dense upper sarmatian limestones, with their retreat not exceeding 0.1–0.2 m per year and bottom abrasion being the main source of sediment supply [2, 3, 17]. Limestones at Karamrun Cape form high cliffs while at the Cape Tarkhankut they have only a small storm berm. Correspondingly, at the northern shallow-bay coast, bottom is steep covered with block and block-boulder bulk; the southern coast is bordered by a strip of flat limestone bench, forming a number of wide steps under water. In shallow water, a boulder bulk is not observed, while at depths of 4–7 m it can be recorded fragmentary. At a distance of about 1.5–2 km from the coast at depths of 15–20 m, there is a side face of the rock plate, and at its foot at depths of 35–40 m, there is a shelf plain covered with loose sediments [5]. At the adjoining points of the sandbar, the limestones are covered with clays; a dead cliff can be observed here, and it indicates that sandbar body had previously been at a longer distance off the sea coast. In the area surveyed, cliff height does not exceed 1–1.5 m. The sandbar itself, now having a length of about 1 km, a width of up to 400 m, and a height of up to 1.5 m, is composed of oolitic limestone sands with an admixture of broken shells, with the same sediments covering shallow bottom of the bay.

Fig. 1. Map of the Karadzhinsky Plot on the Tarkhankut Peninsula: 1 – sampling area is marked with a circle; hydrobotanical profile is in the circle center

Hydrodynamics off the coast of the Tarkhankut Peninsula is high due to intensive wave activity and alongshore currents with speeds of up to 0.25–0.30 m per s [11]. Moreover, our observations show that at the southern coast of the bay, wave deformation and burrowing of their ridges above a gently sloping bottom begin at a considerable distance off the coast. In summer, winds of the western, northern, and northwestern rhombuses dominate in this sea region. In July, average long-term values are: water temperature of +19.8 °C, mineralization of 17.47 g per L (average annual fluctuations within +4.8…+21.6 °C and 17.21–17.55 g per L, respectively) [1]. Low coastal cliff, adjoining of accumulative beach of sandbar, and availability of roads attract spontaneous recreants to this area. At the same time, the area adjoins southern cluster of protected areas (“Charivna havan” national nature park since 2009; “Tarkhankut” regional nature park since 2015).

MATERIAL AND METHODS

A survey of the coastal zone with a total length of about 1 km along water edge (Fig. 1) was carried out in summer period of 2012 by generally accepted hydrobotanical methods [7, 8]. Location of hydrobotanical profile base (at the intersection of the surf line), along which macrophytobenthos samples were taken, was 45°21′33.2″N, 32°30′35″E. Material was collected during dives using light-diving equipment: in pseudolittoral – along the water edge (station no. 1: distance from the coast $l \approx 0$ m, height above sea level – depth $h \approx \pm (0.05\ldots0.15)$ m), and in sublittoral – along three isobaths (st. no. 2: $l \approx 25\ldots30$ m, $h \approx 1$ m; st. no. 3: $l \approx 100\ldots120$ m, $h \approx 3$ m; st. no. 4: $l \approx 250\ldots300$ m, $h \approx 5$ m). Visual observation of the bottom was carried out up to a depth of 10 m. Thus, the profile covered all areas of the benthal where vegetation was recorded. Totally 10 pseudolittoral samples were taken using a frame with an area of 0.01 m^2; 5 sublittoral samples were taken at each station using a frame with an area of 0.04 m^2. During the work, at a distance of 5 m from the coast in the water surface layer, mineralization was of 17.5 g per L, temperature was of +22.5 °C.

Benthic macrophytes were studied. Nomenclature of macroalgae of phylum Chlorophyta, Ochrophyta (class Phaeophyceae), and Rhodophyta is given according to AlgaeBase [22]; taxa authors names are in the standard abbreviation in accordance with the recommendations of IPNI [23]. If necessary, nomenclature combinations are given in addition according to A. D. Zinova ([6] is used as a basic guide for identifying taxa). Ecological and floristic characteristics of algae are given according to A. A. Kalugina-Gutnik [8]; saprobiological and halobility characteristics – according to data unpublished by A. A. Kalugina-Gutnik and T. I. Eremenko (provided by the authors to Nikitsky Botanical Gardens staff). The projective cover (hereinafter PC) was set visually; average biomass values (wet weight) of macrophytes ($\bar{x}$) and error of the mean ($\pm S_{\bar{x}}$) were determined by statistical processing.

RESULTS AND DISCUSSION

Depending on intensity of wind surge of sea level fluctuations, pseudolittoral vegetation in the Sea of Azov – Black Sea region exhibits various structural features [27]. *Cladophora sericea* + *Ulva linza* community (station no. 1) develops in the pseudolittoral of the area surveyed on a solid substrate, represented by fragmented wave-breaking niche and individual clusters of boulder bulk, in form of narrow, 0.1–0.3 m wide belt undifferentiated into subzones. Totally 21 macrophyte species with biomass of about 490 g per m^2 and PC of up to 75 % were identified (Table 1, Fig. 2). In general, the vegetation cover of the pseudolittoral zone resembles that of the Cape Tarkhankut [29]; however, in the area surveyed, the zone is wider and somewhat less fragmented; therefore, the quantitative values are higher (though they are significantly lower than in Cape Karamrun area [28], while at the top of the bay on loose sediments the pseudolittoral is not observed at all [16]).

Table 1. List and biomass of macrophytobenthos species in the water area surveyed

Taxa	Biomass, g per m^2 (stations no. 1–4)			
	PSL (± 0.15 m)	SBL (−0.5…5 m)		
	no. 1	no. 2	no. 3	no. 4
Chlorophyta				
Bolbocoleon piliferum Pringsh.		L	L	
Chaetomorpha aërea (Dillwyn) Kütz. [*Chaetomorpha chlorotica* (Mont.) Kütz., *Chaetomorpha crassa* (C. Agardh) Kütz.]		L	L	L
Chaetophora pisiformis (Roth) C. Agardh		L	L	
Cladophora albida (Nees) Kütz. [*C. albida* (Huds.) Kütz.]	33.33 ± 25.17	0.42	L	1.22 ± 1.01
Cladophora sericea (Huds.) Kütz.	300.00 ± 55.68	4.50	2.92 ± 1.91	16.51 ± 3.15
Cladophora vagabunda (L.) C. Hoek	5.00	0.67	L	
Ulva intestinalis L. [*Enteromorpha intestinalis* (L.) Link nom. illeg.?]	2.33 ± 1.89	2.50		
Ulva linza L. [*Enteromorpha linza* (L.) J. Agardh, *Enteromorpha ahlneriana* Bliding nom. illeg.]	111.67 ± 41.93	97.08 ± 42.74	12.46	39.87 ± 3.02
Ulva prolifera O. F. Müll. [*Enteromorpha prolifera* (O. F. Müll.) J. Agardh]	L	12.50 ± 9.92		
Ulvella lens P. Crouan et H. Crouan	L			L
Ulvella leptochaete (Huber) R. Nielsen, O’Kelly & B. Wysor [*Ectochaete leptochaete* (Huber) Wille]	L	L	L	
Ulvella viridis (Reinke) R. Nielsen, O’Kelly & B. Wysor [*Entocladia viridis* Reinke] ✪		L	L	
Ochrophyta (кл. Phaeophyceae)				
Cladostephus spongiosum f. *verticillatum* (Lightf.) Prud’homme [*Cladostephus verticillatus* (Lightf.) C. Agardh nom. illeg.?] ✱		32.92 ± 18.93	850.00 ± 143.16	
Corynophlaea umbellata (C. Agardh) Kütz.		L	L	
Cystoseira crinita Duby [*C. crinita* (Desf.) Bory] ★✪▲○		1683.33 ± 95.93	3042.50 ± 415.02	
Dictyota fasciola (Roth) J. V. Lamour. [*Dilophus fasciola* (Roth) M. Howe]	1.35	31.25 ± 28.26		
Ectocarpus siliculosus (Dillwyn) Lyngbye [*E. confervoides* (Roth) Le Jolis]	L	L	L	
Feldmannia irregularis (Kütz.) Hamel [*Ectocarpus arabicus* Fig. et De Not.]				L
Myriactula rivulariae (Suhr ex Aresch.) Feldmann		L	L	
Myrionema seriatum (Reinke) Kylin		L	L	

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Padina pavonica (L.) Thivy [*Padina pavonia* (L.) Gaill. nom. illeg.?] □		3.75		
Spermatochnus paradoxus (Roth) Kütz. ✱		4.58		
Sphacelaria cirrosa [cirrhosa] (Roth) C. Agardh	L	L	L	L
Stilophora tenella (Esper) P. C. Silva [*Stilophora rhizodes* (Ehrh.) J. Agardh nom. illeg.?] ✱✚▲		5.00		
Rhodophyta				
Acrochaetium parvulum (Kylin) Hoyt [*Kylinia parvula* (Kylin) Kylin]			L	L
Acrochaetium secundatum (Lyngb.) Nägeli [*Kylinia virgatula* (Harv.) Papenf., *K. secundata* (Lyngb.) Papenf.]	L	L	L	
Apoglossum ruscifolium (Turner) J. Agardh			1.00	
Callithamnion granulatum (Ducluz.) C. Agardh ✱	28.33 ± 10.41	4.67		
Choreonema thuretii (Bornet) F. Schmitz			L	
Ceramium ciliatum (J. Ellis) Ducluz.	6.43 ± 4.97	0.83	0.92	L
Ceramium diaphanum (Lightf.) Roth. [*Ceramium tenuissimum* (Lyngbye) J. Agardh]		9.82 ± 3.15	L	L
Ceramium virgatum Roth [*Ceramium pedicellatum* (Duby) J. Agardh nom. illeg.?; *Ceramium rubrum* (Huds.) C. Agardh nom. illeg.?]		12.92 ± 3.15	1.42 ± 1.01	
Chondria capillaris (Huds.) M. J. Wynne [*Ch. tenuissima* (Gooden. et Woodw.) C. Agardh]		10.83 ± 4.39	6.08	0.42
Chroodactylon ornatum (C. Agardh) Basson [*Asterocytis ramosa* (Thwaites) Gobi ex F. Schmitz]	L	L	L	
Colaconema savianum (Menegh.) R. Nielsen [*Acrochaetium savianum* (Menegh.) Nägeli]	L	L	L	
Gelidium crinale (Hare ex Turner) Gaillon [*G. crinale* (Turner) J. V. Lamour.]		L	L	
Gelidium spinosum (S. G. Gmel.) P. C. Silva [*G. latifolium* (Grev.) Bornet et Thur.] ✪	L			
Hydrolithon farinosum (J. V. Lamour.) Penrose & Y. M. Chamb. [*Melobesia farinosa* J. V. Lamour.]			L	
Jania rubens (L.) J. V. Lamour.		L	13.75 ± 11.92	
Jania virgata (Zanardini) Mont. [*Corallina granifera* J. Ellis et Soland.]			8.33 ± 0.72	
Laurencia coronopus J. Agardh ✱▲		4.17		
Laurencia obtusa (Huds.) J. V. Lamour. ✪		47.08 ± 18.30	200.83 ± 61.71	
Lomentaria firma (J. Agardh) Falkenb. [*L. firma* (J. Agardh) Kylin nom. illeg.?]		0.17		
Lophosiphonia obscura (C. Agardh) Falkenb.			L	L

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Peyssonnelia rubra (Grev.) J. Agardh			L	
Phyllophora crispa (Huds.) P. S. Dixon [*Ph. nervosa* (DC.) Grev.] ★✪✚▲	4.25			
Pneophyllum confervicola (Kütz.) Y. M. Chamb. [*Melobesia minutula* Foslie]	L	L	L	
Polysiphonia denudata (Dillwyn) Grev. ex Harv. [*P. denudata* (Dillwyn) Kütz. nom. illeg.?]	1.67	11.67 ± 8.78	0.50	1.34
Polysiphonia elongata (Huds.) Spreng. [*P. elongata* (Huds.) Harv. nom. illeg.?]				1.58
Vertebrata fucoides (Huds.) Kuntze [*Polysiphonia fucoides* (Huds.) Grev., *Polysiphonia nigrescens* (Dillwyn) Grev. nom. illeg.?]	0.67	7.50 ± 2.50		
Polysiphonia opaca (C. Agardh) Moris et De Not. [*P. opaca* (C. Agardh) Zanardini nom. illeg.?]				0.20
Polysiphonia subulata (Ducluz.) Kütz. [*Polysiphonia violacea* var. *subulata* (Ducluz.) L. Batten] ✪	L	82.50 ± 47.70	0.83	
Vertebrata subulifera (C. Agardh) Kuntze [*Polysiphonia subulifera* (C. Agardh) Harvey]		500.83 ± 77.51	310.83 ± 146.49	
Rhodochorton purpureum (Lightf.) Rosenv. ✱			0.08	0.02
Sahlingia subintegra (Rosenv.) Kornmann [*Erythrocladia subintegra* Rosenv.]			L	
Stylonema alsidii (Zanardini) K. M. Drew [*Goniotrichum elegans* (Chauv.) Zanardini] ✱✪		L	L	
Titanoderma pustulatum (J. V. Lamour.) Nägeli [*Dermatolithon pustulatum* (J. V. Lamour.) Foslie]			L	

Note: PSL – pseudolittoral; SBL – sublittoral. Empty cellsindicate absence of the species in the samples; L – little (less than 0.01 g in the sample). Standard error of the mean (± $S_{\bar{x}}$) is indicated for cases where $v < 100$ % (v is coefficient of variation). There is an opinion that *Cystoseira crinita* is Mediterranean endemic and does not occur in the Black Sea, and specimens identified as *C. crinita* f. *crinita* and *C. crinita* f. *bosphorica* actually refer to *Cystoseira bosphorica* Sauv. [19]. This issue requires a special study, with Crimean coast including. The conservation status of taxa in the Sea of Azov – Black Sea region: ✚ – Red Book of the Russian Federation [10]; □ – Red Data Book of the Republic of Bulgaria [26]; ✱ – Red Data Book of Ukraine [18]; ★ – Black Sea Red Data Book [20]; ✪ – Black Sea Red Data List [21]; ○ – Convention for the Protection of the Mediterranean Sea Against Pollution (Barcelona Convention, 1976) [25], ▲ – Red Book of the Republic of Crimea [9].

In shallow water of the sublittoral, surf waves transform when interacting with a gentle bottom, and their kinetic energy increases. As a result, macroscopic vegetation is damaged on a hard-flat surface of a rocky bench, and during storms it is actually abraded by pebbles, gravel, etc. Therefore, the bench up to a depth of 0.3–0.4 (0.5) m is almost devoid of macroscopic vegetation. Moreover, here and below (even under canopy of vegetation), it is more or less covered with a thin layer of sand brought from the bay (it was not observed south to the Cape Tarkhankut). At some distance off the coast multi-tiered (PC of 90–100 %), well developed, having no signs of mechanical damage "*Cystoseira* belt" communities were registered on hard, even ground. At shallow depths (station no. 2), *Cystoseira crinita* + *Vertebrata subulifera* community develops, with 42 macroalgae taxa recorded with biomass of a little over 2.5 kg per m^2 (Table 1, Fig. 2). *V. subulifera* develops abundantly over almost the entire spectrum of depths, and it dominates in the epiphyton at the distal ends of the branches of the largest *Cystoseira* specimens. Deeper (station no. 3)

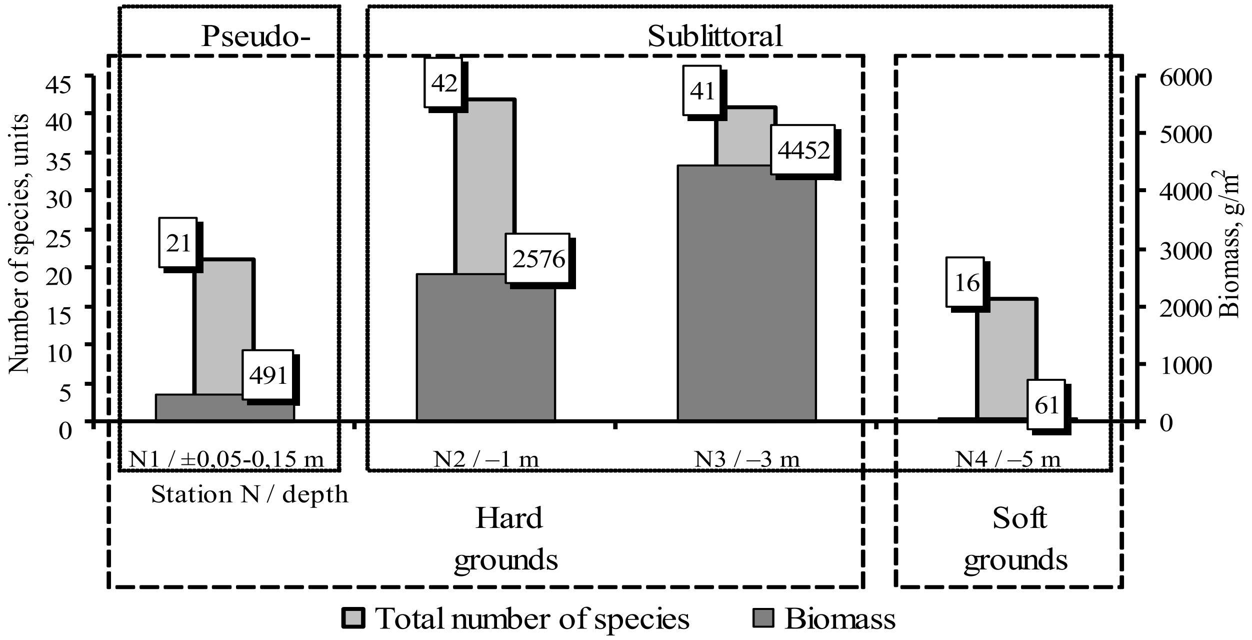

Fig. 2. Changes in total number of species and average biomass of macrophytobenthos with depth change in the water area surveyed

up to the lower boundary of distribution of solid grounds, at a depth of about 4 m at a distance of 190–200 m off the coast, a flat bottom is covered with *Cystoseira crinita* + *Vertebrata subulifera* – *Cladostephus spongiosum* community with biomass of about 4.5 kg per m^2 and PC of 90–95 %, and in this community 41 taxa were identified. Up to this point, picture in general terms was similar to that registered at the Cape Tarkhankut [29]. However, at the Cape Tarkhankut, a deeper flat bench is covered with a block bulk of *Cystoseira* thickets, and in the area surveyed it plunges gradually into loose sediments (there is no clear line between rocks and sand).

In bays in summer period, which is relatively windless, on molluscs shells and on other solid objects located on loose grounds surface, at a depth of not lower than 3 m, an ephemeral vegetation cover develops predominantly from green and, to a lesser extent, red algae [14]. We previously showed that in the central part of the Karadzhinskaya Bay under conditions of reduced hydrodynamics at a certain distance off the surf zone with its increased hydrodynamics (and with lots of recreants, who also have a mechanical effect on the ground and vegetation), *Ulva linza* + *Cladophora sericea* community develops [16]. It was registered in the area surveyed under similar conditions (station no. 4). With biomass of more than 60 g per m^2 and PC of 10–15 %, 16 macrophyte species were recorded there (Table 1, Fig. 2). That is, during the period of research throughout the bay (visually to a depth of at least 8 m), a fairly uniform ephemeral vegetation cover of the same composition and structure developed on loose grounds. Empirically, it can be concluded that its density (and biomass) depends on the amount of shells on the ground surface and on the duration of relatively windless period, since damage is usually observed even after short storms.

Totally 57 macroalgae species were observed within the boundaries of the area surveyed (Table 1): Chlorophyta – 12 (21.05 %), Ochrophyta (Phaeophyceae) – 12 (21.05 %), and Rhodophyta – 33 (57.9 %). At the same time, 56 taxa were registered in the sublittoral, and it is comparable with the values obtained for other points of the Karadzhinsky Plot. In the pseudolittoral, there were 21 taxa, and is not much more than on the Cape Tarkhankut (where the coast structure is similar to that of the area under discussion), but 1.5 times less than in Cape Karamrun area (coast is deep there, and bench is not expressed) [13, 28, 29].

In a range of depths of 1–3 m in the sublittoral *Cystoseira* communities, values of species diversity and phytobenthos biomass are the highest (Table 2). In terms of biomass, share of Phaeophyceae in these communities is within 68–87 %, and share of epiphyton decreases with depth while total values increase. Contribution of Chlorophyta to formation of the biomass of these communities is minimal, while in pseudolittoral and on loose grounds in the sublittoral they form more than 90 % of the biomass. Over the entire spectrum of the depths surveyed, in the ratio of systematic groups by the species number, the share of Rhodophyta is half or more.

In general, oligosaprobic macroalgae dominate the area (Table 2). Their share in terms of the species number (61–64 %) and biomass (94–99 %) is maximal in *Cystoseira* communities, while in pseudolittoral and on loose grounds in the sublittoral, almost the entire biomass is formed by mesosaprobionts. By the species number, their share is also the highest (33–43 %).

By the species number, short vegetation algae dominate in the area surveyed, but with an increase in depth, share of perennial taxa increases (Table 2). They form 69 to 92 % of the biomass in *Cystoseira* communities, while in pseudolittoral and on loose grounds in the sublittoral, up to 100 % is formed by short vegetation taxa.

The analysis of halobility groups ratio shows that marine macroalgae dominate by the species number, with a tendency to increase their share with depth increase (Table 2). Representatives of this group form 94–99 % of the biomass of *Cystoseira* communities, but in pseudolittoral and on loose grounds in the sublittoral, this result is shown by brackish-marine taxa. It should be noted that composition of marine groups varies greatly from station to station, while brackish-marine and brackish groups taxa are actually the same at all stations (Table 1).

In general, representatives of warm-water complex dominate over the area, as well as at some stations, especially by biomass. With depth increase, share of warm-water macroalgae in the total number of species increases, but their contribution to biomass formation decreases (the latter is true for the sublittoral) (Table 2). On the whole, the picture resembles that previously recorded at the Cape Tarkhankut [29].

Macrophytobenthos of the area surveyed has 14 rare taxa (taking into consideration relatively small size of the water body and international cooperation of the Black Sea states in the field of protection of the sea, all published national and international phytososological lists, as well as the regional Red Book, are taken into account) (Table 1). Biotope based on macrophyte communities falls under the EU Directive on the conservation of natural habitats and wild fauna and flora (Directive 92/43/EEC; code 1170 – Reefs) [24].

Comparison of the results of this survey with the data obtained earlier for other points of the Karadzhinsky Plot shows that the vegetation cover of this area has an intermediate position. It reveals features typical for the Cape Tarkhankut: in the pseudolittoral zone it shows relatively weak development and some fragmentation of the vegetation cover; in the sublittoral zone it is absent in the shallowest part, while monotonous *Cystoseira* thickets with lower biomass and relatively simplified vertical structure (two tiers) develop up to the lower boundary of hard ground (for example, at the Cape Karamrun with longer thalli and larger *Cystoseira* biomass, three tiers are formed in communities). As we indicated earlier, this is due to the peculiarities of the geomorphological structure of the coastal zone: at the Cape Karamrun, the narrow coastal bottom strip covered with block-boulder and (deeper) with block bulk has a significant slope angle, while both the area surveyed and the Cape Tarkhankut are characterized by extensive shallow rocky bench, almost devoid of block clusters. Above its surface, an early transformation of surf waves occurs, when oscillatory motion of water mass is converted into translational one. This enhances the mechanical effect on the coastal *Cystoseira* thickets resulting in not only reduced production values, but also in eliminating

Table 2. Distribution of the species number and biomass of macrophytes in ecological-floristic groups in the water area surveyed

GR	Species number, units / % (stations no. 1–4)					Biomass, g per m^2 / % (stations no. 1–4)				
	PSL	SBL			**total**	PSL	SBL			**mean**
	no. 1	no. 2	no. 3	no. 4		no. 1	no. 2	no. 3	no. 4	
Chl	8 38.10	11 26.19	9 21.95	5 31.25	**12** **21.05**	452.33 92.17	117.67 4.57	15.38 0.35	57.60 94.18	**160.74** **8.48**
Oh	3 14.29	11 26.19	7 17.07	2 12.25	**12** **21.05**	1.35 0.28	1760.83 68.36	3892.50 87.42	L 0	**1413.67** **74.60**
Rh	10 47.62	20 47.62	25 60.98	9 56.25	**33** **57.89**	37.10 7.56	697.24 27.10	544.57 12.23	3.56 5.82	**320.62** **16.92**
Os	10 47.62	27 64.29	25 60.98	8 50.00	**37** **64.91**	36.78 7.49	2423.66 94.10	4435.15 99.61	2.02 3.30	1724.40 91.00
Ms	7 33.33	8 19.05	11 26.83	7 43.75	**13** **22.81**	446.67 91.01	113.67 4.41	15.88 0.36	59.14 96.70	158.84 8.38
Ps	4 19.05	7 16.67	5 12.20	1 6.25	**7** **12.28**	7.33 1.50	38.41 1.49	1.42 0.03	L 0	11.79 0.62
Pr	2 9.52	8 19.05	11 26.83	4 25.00	**16** **28.07**	L 0	1770.74 68.75	4116.49 92.45	1.80 2.94	**1472.25** **77.70**
Sh	19 90.48	33 78.57	28 68.29	12 75.00	**39** **68.42**	490.78 100	805.00 31.25	335.96 7.55	59.36 97.06	422.78 22.31
?	0 0	1 2.38	2 4.88	0 0	**2** **3.51**	0 0	L 0	L 0	0 0	**L** **0**
Cl	8 38.10	15 35.71	15 36.59	6 37.50	**19** **33.33**	339.00 69.07	138.09 5.36	854.83 19.20	19.33 31.61	**337.81** **17.83**
Wr	10 47.62	21 50.00	23 56.10	10 62.50	**32** **56.14**	149.45 39.45	2405.56 93.39	3596.20 80.77	41.83 68.39	**1548.26** **81.70**
Cs	3 14.29	5 11.90	3 7.32	0 0	**5** **8.77**	2.33 0.47	27.92 1.08	1.42 0.03	0 0	**7.92** **0.42**
En	0 0	1 2.38	0 0	0 0	**1** **1.75**	0 0	4.17 0.16	0 0	0 0	**1.04** **0.05**
Mr	11 52.38	28 66.67	29 70.73	10 62.50	**43** **75.44**	36.78 7.49	2423.66 94.10	4435.15 99.61	2.22 3.63	**1724.45** **91.00**
Bm	6 28.57	10 23.81	10 24.39	6 37.50	**10** **17.54**	446.67 91.01	136.41 5.30	17.30 0.40	58.94 96.37	**164.83** **8.70**
Br	4 19.05	4 9.52	2 4.88	0 0	**4** **7.02**	7.33 1.49	15.67 0.61	L 0	0 0	**5.75** **0.30**
Σ	**1** **100**	**2** **100**	**1** **100**	**6** **100**	**7** **100**	**90.78** **100**	**575.74** **100**	**452.45** **100**	**1.16** **100**	**1895.03** **100**

Note: GR – groups. Systematical: Ch – Chlorophyta; Oh – Ochrophyta (cl. Phaeophyceae); Rh – Rhodophyta. Saprobiological: Os – oligosaprobes; Ms – mesosaprobes; Ps – polysaprobes. By the duration of the vegetation period: Pr – perennial; Sh – short-vegetating; ? – no data. Phytogeographical: Cl – cold-water; Wr – warm-water; Cs – cosmopolitans; En – endemics. In relation to halobility: Mr – marine; Bm – brackish-marine; Br – brackish.

quantitative differences between stations [29]. At the same time, an ephemeral vegetation cover develops deeper on a loose substrate, which is similar in composition and structure to that of the central part of the Karadzhinskaya Bay [13, 16].

Conclusion. As a result of hydrobotanical survey carried out in the coastal water area near the Cape Tarkhankut, it has been revealed that macrophytobenthos develops on a solid substrate (class of formations of hard grounds community is Thalassophycion sclerochthonophytia) and on a loose substrate (class of formations of soft grounds community is Thalassophycion malacochthonophytia), and it determines the common nature of vegetation cover. At the same time, A. A. Kalugina-Gutnik when classifying Black Sea benthic vegetation referred to the last class of formations only communities of char algae developing on silty sediments. Our observations show that development of ephemeral communities of green algae in summer (often with a significant share of red algae) is characteristic of soft (sandy, admixture of shells) grounds localized in vast bays at the Black Sea and the Sea of Azov coasts. Nature and quantity of benthic vegetation, flora composition, and ratio of ecological-floristic groups are generally characteristic for the Tarkhankut-Sevastopol hydrobotanical region of the Black Sea. Specific features of geomorphological structure of the coastal zone of the area surveyed determine certain structural characteristics of macrophytobenthos and its intermediate position concerning vegetation cover of adjacent abrasive and accumulative coastal areas. At the same time, vegetation cover demonstrates quite a high level of preservation; taxa and biotopes, that are of particular value in the framework of regional and international legislation, are registered within its borders. Previously, we recommended absolutely reserved status for the waters adjacent at the Tarkhankut and Karamrun capes. However, taking into account the recreational use of the area surveyed, in the future it can be included in the zones of regulated recreation of the nature park covering regime marine water areas, at least along the contour of distribution of solid grounds occupied by *Cystoseira* thickets. We would like to emphasize that we are discussing not only some protected water areas, but also the whole territorial-aquatic complex of the Cape Tarkhankut. Such an approach will make it possible to control and regulate anthropogenic load from full preservation to limited recreation and ecotourism in certain areas, which is to slow down (and even to prevent) structural and functional transformation of one of few preserved territorial-aquatic complexes in the Steppe Crimea.

REFERENCES

1. *Gidrometeorologiya i gidrokhimiya morei SSSR. Vol. IV. Chernoe more. Iss. 1. Gidrometeorologicheskie usloviya* / A. I. Simonov, E. N. Altman (Eds). Saint Petersburg : Gidrometeoizdat, 1991, 426 p. (in Russ.)
2. Goryachkin Yu. N., Ivanov V. A. Modern conditions of the Black Sea coast of the Crimea. *Dopovidi NAN Ukrainy*, 2010, no. 10, pp. 87–92. (in Russ.)
3. Dzens-Litovskii A. I. Morskaya abraziya, ee tipy i formy. *Trudy Laboratorii gidrogeologicheskikh problem*, 1955, vol. 12, pp. 64–87. (in Russ.)
4. Zenkovich V. P. *Berega Chernogo i Azovskogo morei.* Moscow : Geografgiz, 1958, 373 p. (in Russ.)
5. Zenkovich V. P. *Morfologiya i dinamika sovetskikh beregov Chernogo morya. Vol. 2: Severo-zapadnaya chast'.* Moscow : AN SSSR, 1960, 216 p. (in Russ.)
6. Zinova A. D. *Opredelitel' zelenykh, burykh i krasnykh vodoroslei yuzhnykh morei SSSR.* Moscow ; Leningrad : Nauka, 1967, 400 p. (in Russ.)
7. Kalugina-Gutnik A. A. Issledovanie donnoi rastitel'nosti Chernogo morya s primeneniem legkovodolaznoi tekhniki. In: *Morskie podvodnye issledovaniya*. Moscow : Nauka, 1969, pp. 105–113. (in Russ.)
8. Kalugina-Gutnik A. A. *Phytobenthos of the Black Sea*. Kiev : Naukova dumka, 1975, 248 p. (in Russ.)
9. *Red Book of the Republic of Crimea. Plants, Algae, and Fungi* / A. V. Yena, A. V. Fateryga (Eds). Simferopol : "PP "ARIAL" LCC, 2015, 480 p. (in Russ.)

10. *Red Book of the Russian Federation (Plants and Fungi)* / Yu. P. Trutnev et al. (Eds) ; R. V. Kamelin et al. (Comps). Moscow : Tov-vo nauch. izd. KMK, 2008, 885 p. (in Russ.)
11. Markova N. V. Rekonstruktsiya i analiz polya techenii severo-zapadnogo shel'fa Chernogo morya. In: *Litodinamika donnoi kontaktnoi zony okeanov* : materialy Mezhdunar. konf., posvyashch. 100-letiyu so dnya rozhd. prof. V. V. Longinova. Moscow : GEOS, 2009, pp. 29–31. (in Russ.)
12. Milchakova N. A., Vakhrusheva L. P., Yepikhin D. V. NPP "Charivna havan". In: *Phytodiversity of Nature Reserves and National Nature Parks of Ukraine. Pt. 2. National Nature Parks* / V. A. Onyshchenko, T. L. Andrienko (Eds). Kyiv : Fitosotsiotsentr, 2012, pp. 519–529. (in Ukr.)
13. Sadogurskiy S. Ye. Preliminary data about macrophytobenthos of Lake Karadzha and adjoining water area of Karadzhinskaya Bay (Black Sea). *Algologia*, 2014, vol. 24, no. 3, pp. 334–339. (in Russ.)
14. Sadogurskiy S. Ye., Belich T. V. To description of macrophytobenthos of south coast of Azov Sea (Crimea). *Trudy Nikitskogo botanicheskogo sada*, 2004, vol. 123, pp. 76–84. (in Russ.)
15. Sadogurskiy S. Ye., Belich T. V., Sadogurskaya S. A. Nekotorye aspekty formirovaniya regionalnoi i lokalnykh ekologicheskikh setei v Krymu. In: *Priroda Vostochnogo Kryma. Otsenka bioraznoobraziya i razrabotka proekta lokalnoi ekologicheskoi seti* / S. P. Ivanov (Ed.). Kiev : [s. n.], 2013, pp. 79–85. (in Russ.)
16. Sadogurskiy S. Ye., Sadogurskaya S. A., Belich T. V. Biomass and distribution of macrophytobenthos of Karadzinskaya Bay and laguna lake Karadza (Crimea, Black Sea). *Trudy Nikitskogo botanicheskogo sada*, 2016, vol. 143, pp. 197–208. (in Russ.)
17. *The current state of the coastal zone of Crimea* / Yu. N. Goryachkin (Ed.). Sevastopol : ECOSI-Gidrofisika, 2015, 252 p. (in Russ.)
18. *Red Data Book of Ukraine. Flora* / Ya. P. Didukh (Ed.). Kyiv : Globalkosalting, 2009, 912 p. (in Ukr.)
19. Berov D., Ballesteros E., Sales M., Verlaque M. Reinstatement of species rank for *Cystoseira bosphorica* Sauvageau (Sargassaceae, Phaeophyceae). *Cryptogamie, Algologie*, 2015, vol. 36, iss. 1, pp. 65–80.
20. *Black Sea Red Data Book* / H. J. Dumont (Ed.). New York : United Nations Office for Project Services, 1999, 413 p.
21. *Black Sea Red Data List.* 1997. URL: http://www.grid.unep.ch/bsein/redbook/about/datalist.htm (accessed 20.05.2019).
22. Guiry M. D., Guiry G. M. *AlgaeBase.* Worldwide electronic publication. Galway : Nat. Univ. Ireland, 2019. URL: http://www.algaebase.org (accessed 20.05.2019).
23. *International Plant Names Index* (IPNI). 2019. URL: http://www.ipni.org (accessed 20.05.2019).
24. *Interpretation Manual of European Union Habitats. EUR 27.* European Commission, DG Environment, Brussels, 2007, 144 p.
25. *Proposal for a Council Decision Establishing the Position to be Adopted on Behalf of the European Community With Regard to Proposals for Amending Annexes II and III to the Protocol Concerning Specially Protected Areas and Biological Diversity in the Mediterranean (SPA/BD Protocol) of the Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean (Barcelona Convention) at the Sixteenth Meeting of the Contracting Parties.* COM (2009) 585 final. Brussels, 26.10.2009. 13 p. URL: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52009PC0585&from=EN (accessed 20.05.2019).
26. *Red Data Book of the Republic of Bulgaria. Vol. 1. Plants and Fungi* / D. Peev et al. (Eds). Sofia : BAS & MOEW, 2015, 881 p.
27. Sadogurskiy S. Ye. Macrophytobenthos of the Black Sea coast of the Kerch peninsular (Crimea, Ukraine). *International Journal on Algae*, 2007, vol. 9, no. 4, pp. 365–383.
28. Sadogurskiy S. Ye. Macrophytobenthos of the Coastal Water Area at the Cape Karamrun (Crimean Peninsula, the Black Sea). *International Journal on Algae*, 2017, vol. 19, no. 2, pp. 51–58. https://doi.org/10.1615/InterJAlgae.v19.i2.30
29. Sadogurskiy S. Ye. Macrophytobenthos in the coastal waters near Cape Tarkhankut (Crimean Peninsula, the Black Sea). *International Journal on Algae*, 2018, vol. 20, no. 1, pp. 69–84. https://doi.org/10.1615/InterJAlgae.v20.i1.70

К ИЗУЧЕНИЮ МАКРОФИТОБЕНТОСА ПРИБРЕЖНОЙ АКВАТОРИИ КАРАДЖИНСКОГО УЧАСТКА ПОЛУОСТРОВА ТАРХАНКУТ (КРЫМ, ЧЁРНОЕ МОРЕ)

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Приведены сведения о составе и распределении макроводорослей в прибрежной акватории Караджинской бухты, примыкающей к южному кластеру особо охраняемой природной территории Национальный природный парк «Тарханкутский» (Крым, п-ов Тарханкут, Чёрное море). Специфика геоморфологического строения береговой зоны обусловливает структурные особенности макрофитобентоса и его промежуточное положение по отношению к макрофитобентосу прилегающих абразионных и аккумулятивных участков берега. Всего зарегистрировано 57 видов макроводорослей (Chlorophyta — 12, Ochrophyta (кл. Phaeophyceae) — 12, Rhodophyta — 33), из них 21 — в псевдолиторали и 56 — в сублиторали. Биомасса колеблется от 0,5 кг$\cdot$м$^{-2}$ в псевдолиторали до 4,5 кг$\cdot$м$^{-2}$ в сублиторали. Характер и количественные показатели бентосной растительности, состав флоры и соотношение эколого-флористических группировок макрофитобентоса в целом характерны для Тарханкутско-Севастопольского гидроботанического района Чёрного моря. Раритетная фракция включает 14 таксонов, занесённых в природоохранные списки различного ранга. Биотоп подлежит особой охране согласно Директиве ЕС о местообитаниях (Directive 92/43/EEC). Территориально-аквальный комплекс имеет созологическую и рекреационную ценность, его сохранение необходимо для обеспечения структурно-функциональной целостности экосистемы береговой зоны моря.

Ключевые слова: Чёрное море, Крым, полуостров Тарханкут, макрофитобентос, видовой состав, биомасса, распределение

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Морской биологический журнал, 2020, том 5, № 1, с. 90–98
Marine Biological Journal, 2020, vol. 5, no. 1, pp. 90–98
https://mbj.marine-research.org; doi: 10.21072/mbj.2020.05.1.09
ISSN 2499-9768 print / ISSN 2499-9776 online

UDC 582.232(066)

THE STORAGE OF ANHYDROBIOTIC CULTURES OF MICROALGAE AND CYANOBACTERIA OF A. O. KOVALEVSKY INSTITUTE OF BIOLOGY OF THE SOUTHERN SEAS OF RAS

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Received by the Editor 25.07.2019; after revision 17.09.2019;
accepted for publication 27.03.2020; published online 31.03.2020.

Reliable preservation of microalgae cultures and creation of genetic banks of strains is one of the important tasks of modern biology. To date, 792 collections of various cultivated organisms from 76 countries are registered in the catalog of the World Federation for Culture Collections in the WDCM CCINFO database. This is the most extensive consolidated database of culture collections, which includes both well-known large collections and small repositories of research and educational institutions from all over the world. The database contains 47 algological collections and 80 collections of various microorganisms, which also include microalgae and cyanobacteria cultures. Only 30 biological collections are registered in Russia, from which only 13 contain algae strains. The most common technique of microalgae cultures storage is the method of their periodic re-sowing onto liquid media or agar. It is used in 127 collections (99 % of the total number in the catalog). Other methods used are: cryopreservation – in 33 collections (27 %), lyophilization – in 13 (11 %), L-drying – in 5 (4 %), freezing – in 19 (16 %), and immobilization in alginate beads – in 1 (0.8 %). However, when using these methods, there is a change in morphological and functional features of cells of the cultures stored, as well as their shredding. In addition, cultures maintaining in a viable state is time-consuming and requires expensive equipment. Preservation of microalgae, transferred to the state of anhydrobiosis by dehydration, is simple and cost-effective. Anhydrobiosis is a deep and long-term inhibition of metabolism, reversible under favorable conditions; it is a quite common phenomenon in nature. The only collection in the WDCM CCINFO database that applies the method of transferring cells to a resting state (for soil algae) is the collection of algae cultures of the National University of Kyiv (ACKU WDCM 994). Many years of experiments on the transfer of microalgae to the state of anhydrobiosis allowed us to develop a method of long-term preservation of microalgae without the use of nutrient media. This technique includes cells transfer to the state of anhydrobiosis, their preservation in a dehydrated state, and subsequent removal to an active culture. In order to preserve algological biodiversity, IBSS RAS created a repository of microalgae transferred to the state of anhydrobiosis, which can be converted to active cultures if necessary. The objects of the repository were marine unicellular algae, as well as freshwater and halobic species of lower phototrophs which are perspective for biotechnology and aquaculture. The cultures were obtained as an inoculum from IBSS RAS collection of live cultures of planktonic microalgae. The algae were grown in an accumulative mode under constant lighting. The biomass was collected during cultivation of algologically pure microalgae cultures at the growth retardation or at the stationary stage. Cells were separated from the culture medium by centrifugation or by filtering them on a plankton sieve. Then the algae were dehydrated and maintained in hermetic zipper bags placed in plastic containers of 100 to 500 ml, at a temperature of +18…+21 °C in the dark in a specially equipped room. The main part of the collection is represented by strains from the phyla Chlorophyta, Cyanophyta, Bacillariophyta, and Rodophyta. The list of species, the number of isolates stored, and the information on preservation forms are provided in this article. The technological regulations for maintenance and replenishment of the storage of anhydrobiotic cultures are described. The repository is at the stage of formation. Its future lies in the fund expansion to include marine, freshwater, and halobic species. Optimization of the dehydration method will allow the transfer of microalgae belonging to different systematic phyla to the state of anhydrobiosis.

Keywords: microalgae, anhydrobiosis, viability, dehydration, storage of microalgae and cyanobacteria

One of the important tasks of modern biology is the reliable preservation of microalgae cultures and the creation of genetic banks of strains. According to the catalog of the World Federation of Cultures, to date 792 collections of various cultivated organisms from 76 countries are registered in the WDCM CCINFO database [27]. The most common technique of microalgae cultures storage is the method of their periodic re-sowing onto liquid media [1, 2, 4, 15, 17, 23] or agar [3, 4, 15]. The catalog contains 47 algological collections and 80 collections of various microorganisms, which also include microalgae and cyanobacteria cultures. The method of periodic re-sowing of microalgae cultures onto liquid media or agar is used in 127 collections (99 % of the total number in the catalog). Other methods used are: cryopreservation – in 33 collections (27 %) [5, 10, 13, 14, 16, 18, 19, 22, 24, 25, 28], lyophilization – in 13 (11 %) [26], L-drying – in 5 (4 %) [21], freezing – in 19 (16 %), and immobilization in alginate beads – in 1 (0.8 %) [11, 12]. However, when using these methods, there is a change in morphological and functional features of cells of the cultures stored, as well as their shredding. In addition, cultures maintaining in a viable state is time-consuming and requires expensive equipment.

Preservation of microalgae, transferred to the state of anhydrobiosis by dehydration, is simple and cost-effective. Anhydrobiosis is a deep and long-term inhibition of metabolism, reversible under favorable conditions. This phenomenon, quite common in nature, formed the basis for the method of transferring cells to a resting state. The only collection in the WDCM CCINFO database applying the method of transferring cells to a resting state for soil algae is the collection of algae cultures of the National University of Kyiv (ACKU WDCM 994) [3].

Many years of experiments on the transfer of microalgae to the state of anhydrobiosis allowed us to develop a method of long-term preservation of microalgae without the use of nutrient media. This technique includes cells transfer to the state of anhydrobiosis, their preservation in a dehydrated state, and subsequent removal to an active culture [6].

The method was tested on prokaryotic and eukaryotic microalgae: on marine, halobic, and freshwater species. The technique is successfully used in IBSS RAS. A collection of anhydrobiotic cultures of lower phototrophs, reversible to a viable state and retaining the ability to divide, was created in the Biotechnology and Phytoresources Department in 2005. The initiator of the collection is PhD R. P. Trenkenshu.

The purpose of creating the collection is the reliable preservation of cultures of lower phototrophs that are also suitable for creating a genetic bank of strains. The practical importance of the repository is connected with the ability to constantly have viable cultures at our disposal to provide experimental research work. In future, it is planned to use the collection as a bank of microalgae and cyanobacteria for conservation of rare and endemic species, algae that are rich in biologically active substances and perspective for application in biotesting, biomonitoring, bioremediation, and scientific and educational process.

MATERIAL AND METHODS

The cultures were obtained as an inoculum from IBSS RAS collection of live cultures of planktonic microalgae [9]. The algae were grown in an accumulative mode under constant lighting. The biomass was collected during cultivation of algologically pure microalgae cultures at the growth retardation stage or at the stationary stage. Cells were separated from the culture medium by centrifugation at 3000 rpm on a centrifuge OPN-3-UKhL 42 or by filtering them on a plankton sieve 100 PE [20]. The choice of filtration method depended on the size of the cells and the trichomes of the lower phototrophs.

The preparation for the preservation was carried out in three ways:

1) to remove salts, the cells were washed from the culture medium first with a solution of ammonium carbonate and then with distilled water, and after this, the cells were dehydrated;
2) the algae to be stored for a long period were dehydrated along with the culture medium;
3) the cells of lower phototrophs were suspended with protectors.

For each microalga, the preservation was carried out in different ways to be able to conduct a comparative analysis of the cells viability of the lower phototrophs stored for a long period and to identify the optimal method.

At the initial stage, the microalgae were dehydrated at a temperature of +20…+70 °C in increments of 10 °C. After a series of experiments, the temperature range of +30…+40 °C was selected [8]. The control of the residual moisture level was carried out during drying; its values were in the range of 10–17 % for most dehydrated cells [7]. The dehydrated algae were maintained in hermetic zipper bags placed in plastic containers of 100 to 500 ml, at a temperature of +18…+21 °C in the dark in a specially equipped room.

The repository consists of a box and a room that both serves as an incubation-stabilizing space in front of the entrance to the storage and is intended for handling samples. An air conditioner is installed in the box to dry the air and to maintain the set temperature.

All samples are labeled with information on name of the culture, dehydration conditions (temperature and duration), and date of transfer to the state of anhydrobiosis.

RESULTS AND DISCUSSION

The first collection samples were spirulina tablets given by manufacturers ("Agro-Viktoriya" LTD) and purchased in pharmacies of the city. The cyanoprokaryotic tablets were reactivated, adapted to cultivation conditions, and transferred to intensive culture. Then, the cultures were subject to re-putting into the suspended animation. The method was tested on microalgae of different phyla.

To date, 366 samples of dehydrated microalgae cultures from 4 phyla have been put for long-term maintenance: cyanobacteria Chlorophyta (Cyanobacteria), green microalgae Cyanophyta, red algae Rodophyta, and diatom alg Bacillariophyta (Fig. 1).

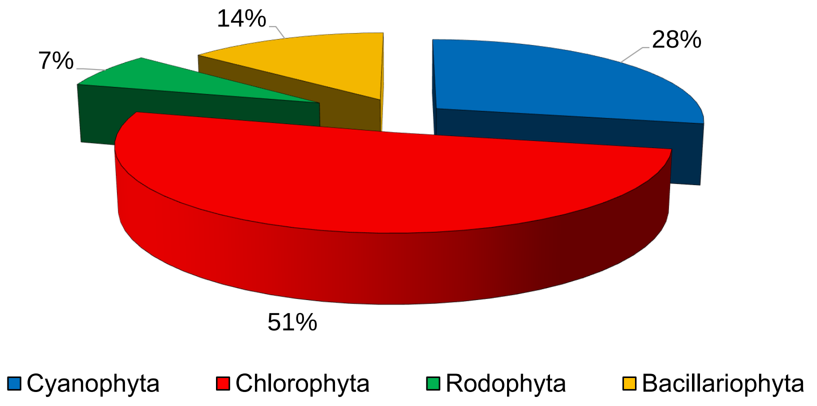

Fig. 1. Ratio of the number of algae strains from different phyla in IBSS RAS collection of anhydrobiotic cultures

The main part of the collection is represented by strains from the phylum Chlorophyta and contains species *Dunaliella salina* (Dunal) Teodorescu, 1905, *Tetraselmis viridis* Rouchijajnen, 1966, *Chlorella vulgaris* f. *suboblonga* V. M. Andreeva, 1975, *Chlorella* sp., and *Scenedesmus* sp. Phylum Cyanophyta is represented by four species (*Arthrospira (Spirulina) platensis* (Nordstedt) Gomont, 1892, *Synechococcus elongates* (Nägeli) Nägeli, 1849, *Oscillatoria amoena* (Kutzing) Gomont, 1892, and *Nostoc commune* var. *flagelliforme* Bornet & Flahault, 1886); phylum Bacillariophyta – by two species (*Phaeodactylum tricornutum* Bohlin, 1897 and *Cylindrotheca closterium* (Ehrenb.) Reimann et Lewin, 1964); phylum Rodophyta – by one species (*Porphyridium purpurerum* (Bory de Saint-Vincent) Drew and Ross, 1965) (Table 1).

Table 1. Taxonomic diversity of cyanobacteria and algae in IBSS RAS collection of anhydrobiotic cultures

Phylum	Order	Genus	Species	When and where from collected/identified	Number of samples stored
CHLOROPHYTA	Chlamydomonadales	*Dunaliella*	*Dunaliella salina*	Salt lakes of Syvash (Crimea)	126
	Sphaeropleales	*Scenedesmus*	*Scenedesmus* sp.	Accompanying the cultivation of chlorella	3
	Chlorodendrales	*Tetraselmis*	*Tetraselmis viridis*	The Black Sea	37
	Chlorellales	*Chlorella*	*Chlorella vulgaris*	The Institute of Botany (Kyiv, Ukraine)	12
			Chlorella sp.	“Ikhlyas-agroenergiya” LTD	20
CYANOBACTERIA	Oscillatoriales	*Oscillatoria*	*Oscillatoria amoena*	Identified while cultivating *Spirulina platensis*	2
			Spirulina (Arthrospira) platensis	MSU (Sochi)	94
	Synechococcales	*Synechococcus*	*Synechococcus elongates*	Accompanying the cultivation of spirulina	3
	Nostocales	*Nostoc*	*Nostoc commune*	The Institute of Botany (Kyiv, Ukraine)	3
BACILLARIOPHYTA	Bacillariales	*Phaeodactylum*	*Phaeodactylum tricornutum*	The Black Sea	23
		Cylindrotheca	*Cylindrotheca closterium*	Algobank (Caen, France), the Mediterranean Sea	3
RHODOPHYTA	Porphyridiales	*Porphyridium*	*Porphyridium purpurerum*	BRI (Saint Petersburg)	74

Microalgae and cyanobacteria stored were transferred to state of anhydrobiosis at different dehydration modes, including temperature and duration of dehydration. Samples of the same algae and cyanobacteria were dehydrated in different years; they were dehydrated with and without various protectors. This is due to the fact that the storage time limits have not yet been determined. Forms of lower phototrophs preservation are presented in Figs 2 and 3. In order to determine the physico-chemical changes of microalgae depending on the storage period, the aliquots of cultures were periodically removed from the collection, and their bioche

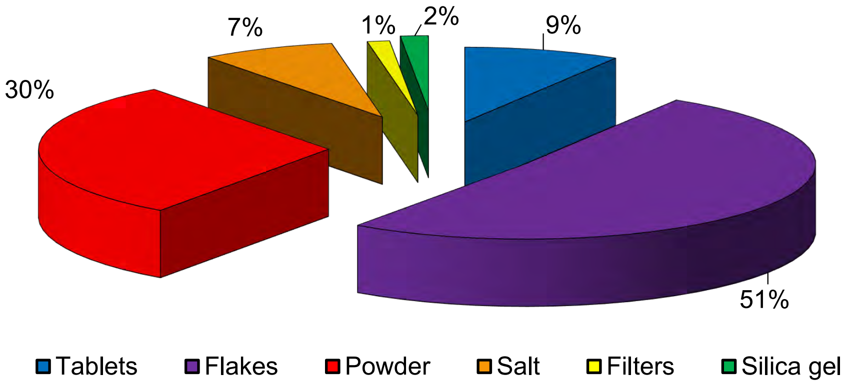

Fig. 2. Forms of microalgae and cyanobacteria preservation for long-term storage

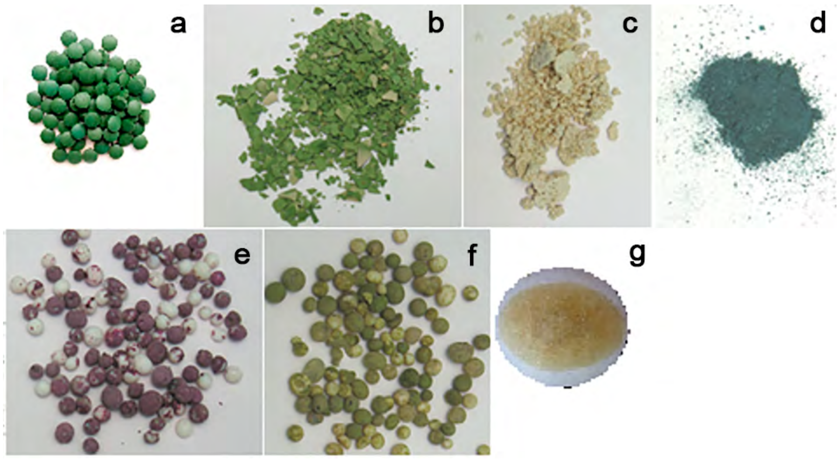

Fig. 3. Appearance of dehydrated samples of microalgae and cyanobacteria stored: a – tablets; b – flakes; c – salt; d – powder; e, f – silica gel; g – filters

In order to preserve the cultures of the lower phototrophs, the following technological regulations for servicing and replenishing the collection of anhydrobiotic cultures was applied (Table 2).

Optimization of the method makes it possible to transfer microalgae belonging to various systematic phyla to the state of anhydrobiosis. The technique can be recommended for use in scientific and educational institutions, as well as in biotechnology, where long-term preservation of strains of museum cultures is required. At this stage, IBSS RAS repository is unique and has no analogues. The collection of anhydrobiotic cultures is constantly replenished with new species of lower phototrophs.

Conclusion. IBSS RAS collection of anhydrobiotic cultures is at the stage of formation. Its future lies in the fund expansion to include marine, freshwater, and halobic species. The development of individual protocols for dehydration and reactivation will make it possible to transfer microalgae belonging to different systematic phyla to the state of anhydrobiosis.

Table 2. Technological regulations for servicing and replenishing the collection of anhydrobiotic cultures of microalgae and cyanobacteria

Stage number	Stage name	Manipulations carried out
I	Obtaining the anhydrobiotic culture	• Obtaining an algologically pure culture from a natural population; • culture certification; • culture adaptation to artificial growing conditions; • intensive cultivation; • transfer of culture to the state of anhydrobiosis; • preparation for long-term storage.
II	Biochemical control of microalgae and cyanobacteria species preserved	Complex biochemical analysis of the lower phototrophs to be stored for a long period (determination of the content of chlorophylls, total carotenoids, total proteins, carbohydrates, lipids, and nucleic acids).
III	Storage and control of viability of cultures preserved	• Identification of living and dead cells of lower phototrophs; • biochemical control of samples preserved; • reactivation and assessment of ability to grow on liquid media.
IV	Maintaining a catalog of anhydrobiotic cultures	Development of the electronic catalog. It includes information on strain name, number, preservation form, date of transferring to the state of anhydrobiosis and dehydration conditions, data on cultivation (including growth characteristics), results of biochemical analysis before the preservation and during the storage, information on previous reactivation, and morphological and biochemical characteristics of algae after reactivation.

This work was carried out within the framework of government research assignment of IBSS RAS "Investigation of the mechanisms of controlling production processes in biotechnological complexes with the aim of developing the scientific foundations for the production of biologically active substances and technical products of marine genesis" (no. AAAA-A18-118021350003-6).

REFERENCES

1. Aizdaicher N. A. Collection of marine microalgae at the A. V. Zhirmunsky Institute of Marine Biology. *Biologiya morya*, 2008, vol. 34, no. 2, pp. 152–155. (in Russ.)
2. Vladimirova M. G., Ignat'evskaya M. I. Izuchenie vliyaniya uslovii khraneniya kul'tur *Chlorella* v kollektsii na posleduyushchuyu ikh produktivnost'. *Mikrobiologiya*, 1966, vol. 35, no. 3, pp. 539–548. (in Russ.)
3. Kostikov I. Yu., Demchenko E. N., Berezovskaya M. A. Microalgae culture collection at the Taras Shevchenko National University, Kyiv. Catalogue of strains (2008). *Chornomorskyi botanichnyi zhurnal*, 2009, vol. 5, no. 1, pp. 37–79. (in Russ.)
4. Novakovskaya I. V., Patova E. N. Collection of living microalgae strains of the Institute of Biology, Komi Science Centre, Ural Branch, RAS, and its perspective using. *Izvestiya Komi nauchnogo tsentra UrO RAN*, 2012, iss. 2, no. 10, pp. 36–41. (in Russ.)
5. Odintsova N. A., Boroda A. V. Cryopreservation of the cells and larvae of marine organisms. *Biologiya morya*, 2012, vol. 38, no. 2, pp. 93–103. (in Russ.)
6. Pat. 2541452 Rossiiskaya Federatsiya. MPK6 S 12 N 1/04. *Sposob dlitel'nogo khraneniya mikrovodoroslei* / Kharchuk I. A. ; zayavitel' i patentoobladatel' Federal'noe gosudarstvennoe byudzhetnoe uchrezhdenie nauki "Institut morskikh biologicheskikh issledovanii imeni A. O. Kovalevskogo RAN". No. 2014149881/93; zayavl. 26.09.2014; prior. 17.03.2008; opubl. 10.02.2015, Byul. no. 4. (in Russ.)

7. Kharchuk I. A. Khranenie mikrovodoroslei v sostoyanii angidrobioza. In: *Mikrovodorosli Chernogo morya: problemy sokhraneniya bioraznoobraziya i biotekhnologicheskogo ispol'zovaniya* / Yu. N. Tokarev, Z. Z. Finenko, N. V. Shadrin (Eds). Sevastopol : EKOSI-Gidrofizika, 2008, chap. 9, pp. 237–267. (in Russ.)
8. Kharchuk I. A. Dynamics of viability and components of biochemical composition *Arthrospira* (*Spirulina*) *platensis* (Nords) Gomont depending on the dehydration temperature transferring at anhydrobiosis state. *Voprosy sovremennoi al'gologii*, 2018, no. 1 (16). URL: http://algology.ru/1258 (accessed 23.07.2019). (in Russ.)
9. *Kollektsiya mikrovodoroslei otdela ekologicheskoi fiziologii vodoroslei FITs InBYuM* [Electronic resource] : site. URL: http://ibss-ras.ru/?page_id=2828 (accessed 24.07.2019). (in Russ.)
10. Cañavate J. P., Lubian L. M. Relationship between cooling rates, cryoprotectant concentrations and salinities in the cryopreservation of marine microalgae. *Marine Biology*, 1995, vol. 124, iss. 2, pp. 325–334. https://doi.org/10.1007/BF00347136
11. Chen Y. C. Immobilized microalgae *Scenedesmus quadricanda* (Chlorophyta, Chlorococcales) for long-term storage and for application for water quality control in fish culture. *Aquaculture*, 2001, vol. 195, no. 1–2, pp. 71–80. https://doi.org/10.1016/S0044-8486(00)00540-8
12. Chen Y. C. Immobilized *Isochrysis galbana* (Haptophyta) for long-term storage and applications for feed and water quality control in clam (*Meretrix lusoria*) cultures. *Journal of Applied Phycology*, 2003, vol. 15, no. 5, pp. 439–444. https://doi.org/10.1023/A:1026071714199
13. Crutchfield A., Diller K., Brand J. Cryopreservation of *Chlamydomonas reinhardtii* (Chlorophyta). *European Journal of Phycology*, 1999, vol. 34, iss. 1, pp. 43–52. https://doi.org/10.1080/09670269910001736072
14. Day J. G., Watanable M. M., Morris G. H., Fleck R. A., McLellan M. R. Long-term viability of preserved eukaryotic algae. *Journal of Applied Phycology*, 1997, vol. 9, no. 2, pp. 121–127. https://doi.org/10.1023/A:1007991507314
15. Hur S. B., Bae J. H., Youn J., Jo M. J. KMMCC – Korea Marine Microalgae Culture Center: List of strains, 2nd edition. *Algae*, 2015, vol. 30, suppl., pp. S1–S188. http://dx.doi.org/10.4490/algae.2015.30.S.S1
16. Kumari N., Gupta M., Singh R. Open encapsulation-vitrification for cryopreservation of algae. *Cryobiology*, 2016, vol. 73, iss. 2, pp. 232–239. https://doi.org/10.1016/j.cryobiol.2016.07.005
17. Marsalek B., Rojickova-Padrtova R. Long-term maintenance of alga strains for use in biomassays and biotechnology. *Archiv fuř Hydrobiologie. Supplementband: Algological Studies*, 1988, vol. 124, pp. 121–136.
18. Meyer M. A. *Cryopreservation of a Marine Diatom*. PhD Thesis. Texas A & M University, College Station, 1985, 112 p.
19. Poncet J.-M., Véron B. Cryopreservation of the unicellular marine alga, *Nannochloropsis oculata*. *Biotechnology Letters*, 2003, vol. 25, no. 23, pp. 2017–2022. https://doi.org/10.1023/B:BILE.0000004395.04116.45
20. Richmond A., Lichtenberg E., Stahl B., Vonshak A. Quantitative assessment of the major limitations on productivity of *Spirulina platensis* in open raceways. *Journal of Applied Phycology*, 1990, vol. 2, no. 3, pp. 195–206. https://doi.org/10.1007/BF02179776
21. Sakane T. Preservation of microorganisms by L-drying. *International Journal of Reefing*, 1982, vol. 57, no. 6, pp. 767–775.
22. Scarbrough C., Wirschell M. Comparative analysis of cryopreservation methods in *Chlamydomonas reinhardtii*. *Cryobiology*, 2016, vol. 73, iss. 2, pp. 291–295. https://doi.org/10.1016/j.cryobiol.2016.07.011
23. von Schwartzenberg K., Bornfleth S., Lindner A., Hanelt D. The Microalgae and Zygnematophyceae Collection Hamburg (MZCH) – living cultures for research on rare streptophytic algae. *Algological Studies*, 2013, vol. 142, pp. 77–108. http://doi.org/10.1127/1864-1318/2013/0131
24. Surek B. Meeting report: International Symposium on the Cryopreservation of Algae (Austin, Texas, USA, 16–17 April, 1988). *Protist*, 1998, vol. 149,

iss. 3. pp. 201–205. https://doi.org/10.1016/S1434-4610(98)70026-4

25. Tessarolli L. P., Day J. G., Vieira A. H. Establishment of a cryopreserved biobank for the Culture Collection of Freshwater Microalgae (CCMA-UFSCar), São Paulo, Brazil. *Biota Neotropica*, 2017, vol. 17, no. 2, e20160299. http://dx.doi.org/10.1590/1676-0611-bn-2016-0299
26. Tsuru S. Preservation of marine and fresh water algae by means of freezing and freeze-drying. *Cryobiology*, 1973, vol. 10, iss. 5, pp. 445–452. https://doi.org/10.1016/0011-2240(73)90074-6
27. *WDCM CCINFO. World Data Centre for Microorganisms, Culture Collections Information Worldwide. 2014.* [Electronic resource.] URL: http://www.wfcc.info/ccinfo/home (accessed 23.07.2019).
28. Zheng L., Lu Z., Zhang Q., Li T., Song L. A fluorescence ratio-based method to determine microalgal viability and its application to rapid optimization of cryopreservation. *Cryobiology*, 2018, vol. 81, pp. 27–33. https://doi.org/10.1016/j.cryobiol.2018.02.014

ХРАНИЛИЩЕ АНГИДРОБИОЗНЫХ КУЛЬТУР МИКРОВОДОРОСЛЕЙ И ЦИАНОБАКТЕРИЙ ИНСТИТУТА БИОЛОГИИ ЮЖНЫХ МОРЕЙ ИМЕНИ А. О. КОВАЛЕВСКОГО РАН

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Надёжное сохранение культур микроводорослей и создание генетических банков штаммов — одна из важных задач современной биологии. В каталоге Всемирной федерации культур в базе WDCM CCINFO на сегодняшний день зарегистрировано 792 коллекции различных культивируемых организмов из 76 стран. Это самая обширная сводная база данных, включающая как известные крупные коллекции, так и небольшие хранилища исследовательских и образовательных учреждений со всего мира. В базе представлено 47 альгологических коллекций и 80 коллекций микроорганизмов, которые также включают культуры микроводорослей и цианобактерий. В России зарегистрировано всего 30 биологических коллекций; фонды только 13 из них включают штаммы водорослей. Самый распространённый способ хранения культур микроводорослей — метод их периодических пересевов на жидкие среды или агар. Его используют в 127 коллекциях (99 % от общего количества в каталоге). Также применяют криоконсервацию — в 33 коллекциях (27 %), лиофилизацию — в 13 (11 %), L-высушивание — в 5 (4 %), замораживание — в 19 (16 %), иммобилизацию в альгинатных бусинках — в 1 (0,8 %). Между тем при использовании этих методов изменяются морфологические и функциональные свойства клеток сохраняемых культур и происходит их измельчание. Кроме того, поддержание культур в жизнеспособном состоянии трудоёмко и требует дорогостоящего оборудования. При этом хранение микроводорослей, переведённых в состояние ангидробиоза путём их обезвоживания, просто и экономически выгодно. Ангидробиоз — глубокое и длительное торможение метаболизма, обратимое при благоприятных условиях; это достаточно распространённое явление в природе. Единственная коллекция из базы WDCM CCINFO, для которой применяют способ перевода клеток в покоящееся состояние путём ангидробиоза (для почвенных водорослей) — коллекция культур водорослей Киевского национального университета (ACKU WDCM 994). Многолетние опыты по переводу микроводорослей в состояние ангидробиоза позволили разработать метод их длительного хранения без использования питательных сред, включающий перевод клеток в состояние ангидробиоза, их сохранение в дегидратированном состоянии и последующее выведение в активную культуру. С целью поддержания альгологического биоразнообразия на базе ФИЦ ИнБЮМ создано хранилище микроводорослей, переведённых в состояние ангидробиоза; их при необходимости можно вывести в активные культуры. Объектами стали морские одноклеточные водоросли, а также пресноводные и галобные виды низших фототрофов, перспективные для аквакультуры и биотехнологии. Культуры получены в виде инокулята из коллекции живых культур планктонных микроводорослей ФИЦ ИнБЮМ.

Водоросли выращивали в накопительном режиме при постоянном освещении. Биомассу собирали во время культивирования альгологически чистых культур микроводорослей на стадии замедления роста или на стационарной стадии. Клетки отделяли от культуральной среды центрифугированием или путём их фильтрации на планктонном сите. Затем водоросли обезвоживали и хранили в герметичных зиплок-пакетах, помещённых в пластиковые ёмкости объёмом от 100 до 500 мл, при температуре +18…+21 °C в темноте в специально оборудованном помещении. Основная часть коллекции представлена штаммами из отделов Chlorophyta, Cyanophyta, Bacillariophyta, Rodophyta. В статье приведены список видов и количество сохраняемых изолятов, представлена информация о формах хранения, описан технологический регламент обслуживания и пополнения хранилища ангидробиозных культур. Хранилище находится на стадии формирования. Его будущее связано с расширением фонда за счёт морских, пресноводных и галобных видов. Оптимизация способа обезвоживания позволит перевести в состояние ангидробиоза микроводоросли, относящиеся к разным систематическим отделам.

Ключевые слова: микроводоросли, ангидробиоз, жизнеспособность, дегидратация, хранение микроводорослей и цианобактерий

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Морской биологический журнал, 2020, том 5, № 1, с. 99–110
Marine Biological Journal, 2020, vol. 5, no. 1, pp. 99–110
https://mbj.marine-research.org; doi: 10.21072/mbj.2020.05.1.10
ISSN 2499-9768 print / ISSN 2499-9776 online

UDC 576.893.1:597.2/.5(262.5+262.54+262.81)

HISTORY OF FORMATION AND PECULIARITIES OF PONTO-CASPIAN FISH MYXOSPOREAN FAUNA

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Received by the Editor 18.07.2019; after revision 20.09.2019;
accepted for publication 27.03.2020; published online 31.03.2020.

History of formation of Ponto-Caspian basin fish myxosporean fauna is examined. This work is based on our own material on myxosporean parasites of fish from the Black Sea (collected in 1987–2018) and the Sea of Azov (1997–2016). Totally, we have investigated 15 368 specimens of 87 species of fish (14 297 specimens of 80 species of fish in the Black Sea and 1071 specimens of 19 species of fish in the Sea of Azov). The material was collected by the method of incomplete parasitological dissections and treated by generally accepted methods. Also, all available literary sources on the myxosporean parasites of fish from the Black Sea, the Sea of Azov, and the Caspian Sea are analyzed (references list contains the most significant publications). A comparative analysis of fish myxosporean fauna in the Black Sea, the Sea of Azov, and the Caspian Sea is performed. It is stated that 108 parasite species are known in the Black Sea, 42 – in the Sea of Azov, and 68 – in the Caspian Sea. Number of myxosporean parasite species common for the Sea of Azov and the Black Sea is 32, for the Caspian and Black seas – 32, and for the Sea of Azov and the Caspian Sea – 20. Totally 16 species of myxosporean parasites are registered in all the mentioned seas. To date, of 108 myxosporean species of Black Sea region, 29 are registered only in freshwater fish in the estuaries with considerable brackish water. For 79 myxosporean species, marine fish species serve as hosts; they are registered mainly in full-salt sea part, and 17 of them are of freshwater origin: 7 species are registered in freshwater fish and in euryhaline mullets; 1 parasitizes on freshwater and marine salmon fishes; the only hosts for 9 species are mullets or other marine fish species. Marine forms are represented by Pontic (22 species), Ponto-Azov (3), and Ponto-Caspian (2) endemics, as well as by Mediterranean invaders (35). Among freshwater myxosporeans, only 1 species is Black Sea endemic; 1 species is Ponto-Azov endemic, and most other species are widely represented in freshwater reservoirs. Among Mediterranean invaders, 23 species found in the Mediterranean Sea should be noted; 12 species still have not been registered in the Mediterranean Sea, mainly due to very few studies on this group of parasites. The fauna of Sea of Azov fish parasites includes 42 myxosporean species; 32 of them are found in the Black Sea, 20 – in the Caspian Sea. Totally 19 species belong to freshwater ones, and they are parasitic only in freshwater fish species. Ten species are of freshwater origin but can be registered in marine fish species (mostly in mullets, and one – in gobies). Of the freshwater species, one is Ponto-Azov endemic. Totally 13 myxosporean species are marine ones: 2 species are Sea of Azov endemics; 3 species are Ponto-Azov endemics; 8 species are Mediterranean invaders. Caspian Sea fauna includes 68 species of myxosporeans: 8 are marine ones (1 is ancient marine species; 1 is Ponto-Caspian endemic; 6 are brackish-water myxosporeans), and 60 are freshwater species. Five endemics of the Caspian Sea are known (2 of marine origin and 3 of freshwater origin). When moving from west to east (from the Black Sea to the Caspian Sea), a gradual impoverishment of marine myxosporean fauna and its replacement by freshwater myxosporean species are observed. Impoverishment of Myxosporea species composition of the Sea of Azov and the Caspian Sea in comparison with the Black Sea one is also found.

Keywords: fauna, Myxosporea, fish, Black Sea, Sea of Azov, Caspian Sea

History of formation of the Ponto-Caspian basin fish myxosporean fauna is connected with a complex geological past of the southern seas. At the Paleogene at the site of the Mediterranean, Black, and Caspian seas, as well as the Sea of Azov, there laid a full-salt sea Tethys, connected openly with the ocean [2, 32, 33, 42, 43]. At Miocene, it gradually isolated, becoming Sarmat basin; it was accompanied by strong desalination. Sharp stratification of waters appeared, leading to development of hydrogen sulfide zone at depth. Under new conditions, rapid extinction of almost the entire fauna in the Sarmat basin and wide development of peculiar mactric fauna took place. Between Sarmat and Pliocene periods, the Meotic basin also connected with the ocean was formed. In its turn, this led to changes in the fauna: Sarmat species began to vanish, and typical Mediterranean species appeared. In the eastern part of the Meotic basin, hydrological conditions were very similar with those of modern Black Sea, and there was a deep-water part contaminated with hydrogen sulfide [1]. The Meotic basin eastern part has separated during Pliocene period. Vast inner brackish-water Pontic Lake was a sea with brackish-water fauna. By the end of Tertiary and the beginning of Quaternary period, this sea split, forming now existing the Black Sea, the Sea of Azov, the Caspian Sea, and the Aral Sea. The last two of them preserved brackish-water character with appropriate fauna, while the Black Sea underwent one more transformation. Being connected with the Mediterranean Sea, it underwent great salinization. As a result, brackish-water fauna partially died out, and its remnants remained in the estuaries and in the Sea of Azov. All the other parts of the sea were inhabited by Mediterranean fauna. Due to low water salinity and uninhabited Black Sea depth resulting from hydrogen sulfide presence, many Mediterranean species and their parasites could not settle there; nevertheless, of fish, only Gobiidae, Atherinidae, Clupeidae, Syngnathidae, and Acipenseridae originate from the brackish water basin mentioned above and are common for the Caspian Sea [17]. All other fish resettled in the Black Sea from the Mediterranean Sea [37].

Absence of many Mediterranean parasite species in Black Sea fish parasite fauna was noted by V. A. Dogel [5]; he based on the results of his own research and data of S. U. Osmanov [22], A. V. Reshetnikova [30], and Z. S. Donets [7, 8] who wrote about impoverishment of Black Sea fish myxosporeans compared to Atlantic and Mediterranean basins fish myxosporeans. A. Reshetnikova relying on myxosporeans origin proposed to divide Black Sea species into following zoogeographic groups: global, arctic-boreal, boreal-Atlantic, Mediterranean, freshwater, and Black Sea endemics. N. N. Naidenova [21] studying parasites of gobies from the Black Sea and the Sea of Azov isolated some other zoogeographic groupings. So, freshwater species are divided into paleo-arctic, Ponto-Caspian-Aral, Black Sea endemics, characteristic for the area and common with Amur species, and Mediterranean with not clear areal. Marine parasites are divided into arctic-boreal, boreal (boreal-Atlantic, amphi-boreal), Mediterranean, Ponto-Azov endemics, tropic-boreal, global, and with not clear areal. In general, according to N. Naidenova, the fauna of gobies parasites unites the following zoogeographic groupings of species: 1 – Mediterranean; 2 – widely spread geographically (arctic and boreal); 3 – freshwater; 4 – characteristic only for the Black Sea and the Sea of Azov. N. Naidenova attributed *Sphaeromyxa sevastopoli*, *Fabespora nana*, and neoendemic *Myxidium melanostomi* to the last paleo-endemics.

Faunistic complexes and groups of the freshwater myxosporeans have another history and, therefore, adhere to different laws. For the southern water basins of the former USSR, they are given in detail by Z. Donets [6], and that is why we will limit ourselves to a brief presentation. In Black Sea area of Ponto-Caspian-Aral province, this problem was studied by Z. Donets mostly for myxosporean fauna of big rivers coming into the Black Sea and the Sea of Azov, as well as Crimean water reservoirs. Z. Donets emphasizes that Ponto-Caspian-Aral province is the richest region with freshwater myxosporeans within the Euro-Asian part of Golarctics, but in this province, when moving from west to east, gradual impoverishment of parasites species composition is observed, especially pronounced in the Aral Sea.

Formation of myxosporean fauna in southern seas of the former USSR is also analyzed by Z. Donets [7, 8]. It is proposed to consider the Black Sea fauna to be impoverished marine with not considerable admixtures of freshwater elements. In the Black Sea, 32 species were divided by Z. Donets into the following groups: ancient-marine (1 species), Ponto-Caspian endemics of marine origin (6), Mediterranean invaders (19), parasites of Gasterosteidae (1), freshwater species (4), and Ponto-Caspian endemics of freshwater origin (1). Analysis of the Caspian and Aral seas myxosporeans has been given [7].

All the authors named above marked that zoogeographical characteristic of parasites is in good accordance with zoogeography of Black Sea free-living organisms. In 1987, a bit different idea on the richness of Black Sea fish myxosporean fauna appeared. M. G. Kolesnikova and Z. S. Donets [18] presented their belief that the idea of fauna's extreme impoverishment is a bit out reality. They supposed that a great number of new species found in the Black Sea can be also found in the Mediterranean Sea. Based on their data (description of 4 new myxosporean species), we continued and made description of 15 new species increasing considerably the list of fish myxosporeans in the Ponto-Azov basin. *Myxidium pulchrum* described in 1991 for the Black Sea was later found in the Adriatic Sea [19]. *Alataspora solomoni* – Black Sea species new for science described by V. Yurakhno in 1988 – was later found in the Ionian Sea [4]. *Zschokkella admiranda* considered previously as Black Sea species was found in the Mediterranean Sea near coast of Spain [39]. The data on species richness of Black Sea fish myxosporean fauna became fuller due to Pacific and Atlantic oceans species, as well as Mediterranean, Red, and Adriatic seas species found in the Pontic basin. Myxosporean species known for the Atlantic Ocean (*Chloromyxum schulmani*, *Sinuolinea rebae*), for the Atlantic and Pacific oceans (*Ch. ovatum*, *Ortholinea orientalis*) [25, 26, 34], and for the Mediterranean and Red seas and for the Pacific Ocean (*Enteromyxum leei*) [28] were later found in the Black Sea. *Ceratomyxa beloneae* – a species described earlier in the Adriatic Sea – was later found by us in the Black Sea [29, 34]. Myxosporean parasites *Sphaerospora dicentrarchi*, *Sphaerospora mugilis*, *Myxobolus spinacurvatura*, *M. ichkeulensis* [23, 39], and *Myxobolus episquamalis* [3, 31] found earlier in Mediterranean Sea mullet were later registered in the Black Sea and in the Sea of Azov. Many myxosporean species were found in new hosts and geographical areas, and their lists of hosts and areas were widened.

In this regard, it is of interest to give modern comparative characteristics of the Ponto-Caspian basin fish parasite fauna, since myxosporeans constitute one of the most interesting groups in it.

MATERIAL AND METHODS

This work is based on our own material on the myxosporeans of fish from the Black Sea collected in 1987–2018 and from the Sea of Azov collected in 1997–2016. Totally, we have investigated 15 368 specimens of 87 species of fish (14 297 specimens of 80 species of fish in the Black Sea and 1071 specimens of 19 species of fish in the Sea of Azov).

The material was collected by the method of incomplete parasitological dissections and treated by generally accepted methods. Also, all available literary sources on the myxosporean parasites of fish from the Black Sea, the Sea of Azov, and the Caspian Sea were analyzed (references list contains only the most significant publications).

RESULTS AND DISCUSSION

Comparison of species composition of Black Sea, Sea of Azov, and Caspian Sea fish myxosporean parasites has shown that 108 species are known in the Black Sea, 42 – in the Sea of Azov, and 68 – in the Caspian Sea (Table 1) [41, modern data]. Number of myxosporean parasite species common for the Sea of Azov and the Black Sea is 32, for the Caspian and the Black seas – 32, and for the Sea of Azov and the Caspian Sea – 20. Totally 16 myxosporean species are registered in all three seas.

Table 1. Myxosporean species of Black Sea, Sea of Azov, and Caspian Sea fishes (according to literary and own data)

Myxosporean species	The Black Sea	The Sea of Azov	The Caspian Sea
Alataspora solomoni	+		
Ceratomyxa agilis	+		
C. arcuata	+		
C. beloneae	+		
C. caspia			+
C. elegans	+		
C. globulifera	+		
C. hepseti	+	+	
C. inaequalis	+		
C. informis	+		
C. markewichi	+		
C. merlangi	+		
C. parva	+		
C. peculiaria	+		
C. reticularis	+		
Chloromyxum cristatum	+		+
Ch. esocinum	+		
Ch. fluviatile	+		+
Ch. legeri	+		+
Ch. osmanovi	+		
Ch. ovatum	+		
Ch. partistriatus	+		
Ch. psetti	+		
Ch. schulmani	+		
Ch. trachuri	+		
Ch. truttae			+
Ch. varicorhini			+
Enteromyxum leei	+		
Fabespora nana	+		
Henneguya chaibulaevi			+
H. creplini	+		
H. gigantea	+		
H. lobosa	+		+
H. oviperda	+		
H. psorospermica	+		+
H. schizura		+	
H. sinova	+		
Hoferellus conifer		+	
H. jurachni	+		
Gadimyxa ovale	+		
Kudoa anatolica	+		

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Myxosporean species	The Black Sea	The Sea of Azov	The Caspian Sea
K. niluferi	+		
K. nova	+	+	
K. quadratum	+		
K. stellula	+		
Myxidium cochleatum	+		
M. benthophili		+	
M. gadi	+	+	
M. incurvatum	+		
M. lieberkühni	+	+	+
M. macrocapsulare	+		+
M. melanostomi	+	+	
M. parvum	+		
M. pfeifferi	+	+	+
M. pulchrum	+		
M. rhodei	+		+
M. salmonis	+		
M. schulmani			+
Myxobilatus convexum	+		
M. gasterostei	+		+
M. medius	+	+	+
M. platessae	+		
M. varicorhini			+
Myxobolus adeli	+	+	
M. albovae	+		+
M. alburni			+
M. alievi			+
M. anurus	+		
M. asymmetricus	+		
M. bliccae		+	+
M. bramae	+	+	+
M. branchialis	+		+
M. carassii			+
M. chondrostomi			+
M. circulus	+		+
M. cycloides			+
M. cyprini	+	+	+
M. cyprinicola			+
M. dispar	+	+	+
M. diversicapsularis			+
M. dogieli		+	+
M. donecae			+
M. dujardini			+
M. elegans	+		
M. ellipsoides	+	+	+

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Myxosporean species	The Black Sea	The Sea of Azov	The Caspian Sea
M. episquamalis	+	+	
M. exiguus	+	+	+
M. gigas			+
M. ichkeulensis	+	+	
M. infundibulatus			+
M. karelicus	+		+
M. kubanicus		+	
M. kuleminae			+
M. lobatus			+
M. macrocapsularis	+	+	+
M. magnus		+	
M. minutus			+
M. muelleri	+	+	+
M. musajevi			+
M. musculi	+	+	+
M. najdenovae	+	+	
M. nemachili			+
M. obesus	+		+
M. oviformis	+	+	+
M. parvus	+	+	
M. percarinae	+		
M. pfeifferi			+
M. pseudodispar	+	+	+
M. rotundus	+		+
M. rutili	+	+	+
M. saidovi			+
M. samgoricus			+
M. sandrae	+	+	+
M. schulmani			+
M. squamae			+
M. sphaericus	+		
M. spinacurvatura	+	+	
M. truttae			+
Myxodavisia cornuta		+	
M. ophidioni	+		
Ortholinea antipae	+		
O. divergens	+		
O. gobiusi	+	+	
O. mullusi	+		
O. orientalis	+		
Pseudalataspora pontica	+		
Sigmomyxa sphaerica	+		
Sinuolinea rebae	+		
S. sakinachanumae	+		+

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Myxosporean species	The Black Sea	The Sea of Azov	The Caspian Sea
Sphaeromyxa atherinae	+		
S. balbiani	+		
S. incurvata	+		
S. sabrazesi	+		
S. sevastopoli	+	+	
Sphaerospora bergi	+		
S. carassii			+
S. caspialosae	+		+
S. caudata	+	+	
S. dicentrarchi	+	+	
S. donecae			+
S. elegans	+		+
S. mugilis	+	+	
Thelohanellus misgurni			+
T. pyriformis		+	+
Zschokkella admiranda	+		
Z. dogieli	+		
Z. iskovi	+		
Z. nova	+	+	+
Z. striata		+	
Z. sturionis		+	+

Black Sea fish myxosporean fauna was depicted earlier in key manuals [9, 16]. Origin and composition of Black Sea fish myxosporean fauna were considered later by V. M. Yurakhno in Russian, Georgian, Abkhazian, and Ukrainian waters [35, 37]. New data on Black Sea fauna were obtained near Turkish shores [12, 24, 27, 28, etc.], as well as in Ukrainian and Moldavian waters of Lower Danube and Dniester basins (for Clupeidae fishes) [20]. New data on Sea of Azov fauna were obtained for the Taganrog Gulf [36, 38]. New information was received for myxosporeans of Mugilidae in the Black Sea and the Sea of Azov [39, 40]. Comparison of the myxosporean fauna in the Black Sea and adjacent seas using Czekanowski – Sørensen index [35] has shown that fish myxosporean fauna in the Black Sea, the Sea of Azov, and the Caspian Sea forms one cluster. It is not quite homogenous by hydrological conditions and hosts species composition. Water salinity in the main part of the Black Sea is of 17–18.5 ‰. The Sea of Azov and the Caspian Sea have highly brackish waters with salinities of 10–14 ‰ and 12.7–12.8 ‰ (less often of 13.2 ‰), respectively. In the Sea of Azov near estuary areas, salinity is of 2–4 ‰ and even lower, and in the Caspian Sea – of 1–2 ‰ (in the north-west).

To date, of 108 myxosporean species of the Black Sea region, 29 are registered only in freshwater fish in the estuaries with considerable brackish water (*Myxidium lieberkühni*, *M. macrocapsulare*, *M. pfeifferi*, *M. rhodei*, *Chloromyxum cristatum*, *Ch. esocinum*, *Ch. fluviatile*, *Ch. legeri*, *Myxobolus albovae*, *M. anurus*, *M. cyprini*, *M. dispar*, *M. elegans*, *M. ellipsoids*, *M. karelicus*, *M. macrocapsularis*, *M. musculi*, *M. obesus*, *M. oviformis*, *M. percarinae*, *M. pseudodispar*, *M. rutili*, *M. sandrae*, *M. sphaericus*, *Henneguya creplini*, *H. gigantea*, *H. lobosa*, *H. oviperda*, and *H. psorospermica*). For 79 myxosporean species, marine fish species serve as hosts; they are registered mainly in full-salt sea part. Totally 17 of them are of freshwater origin: 7 species (*Zschokkella nova*, *Myxobolus branchialis*, *M. bramae*, *M. circulus*, *M. exiguus*, *M. muelleri*, and *M. rotundus*) are registered in freshwater fish and in euryhaline mullets; 1 (*Myxidium salmonis*) parasitizes on freshwater and marine salmon fishes; the only hosts for 9 species are mullets (for *Myxobolus adeli*, *M. episquamalis*, *M. ichkeulensis*, *M. parvus*, and *M. spinacurvatura*) or other marine fish species (for *Myxobolus asymmetricus*, *M. najdenovae*, *Hoferellus jurachni*, and *Henneguya sinova*).

Marine forms are represented by Pontic (22 species), Ponto-Azov (3), and Ponto-Caspian (2) endemics, as well as by Mediterranean invaders (35). Among freshwater myxosporeans, only 1 species (*M. percarinae*) is Black Sea endemic; 1 species (*Myxobolus najdenovae*) is Ponto-Azov endemic, and most other species are widely represented in freshwater reservoirs. Of the marine forms, Pontic endemics are: *Sphaeromyxa atherinae*, *Myxidium cochleatum*, *M. parvum*, *Fabespora nana*, *Gadimyxa ovale*, *Myxodavisia ophidioni*, *Ceratomyxa merlangi*, *C. markewichi*, *C. peculiaria*, *Myxobilatus convexum*, *Chloromyxum osmanovi*, *Ch. partistriatus*, *Ch. psetti*, *Ch. trachuri*, *Ortholinea antipae*, *Ortholinea mullusi*, *Sphaerospora bergi*, *Zschokkella dogieli*, *Pseudalataspora pontica*, *Kudoa anatolica*, *K. niluferi*, and *K. stellula*. Ponto-Azov endemics are: *Sphaeromyxa sevastopoli*, *Myxidium melanostomi*, and *Ortholinea gobiusi*. Ponto-Caspian endemics are *Sphaerospora caspialosae* and *Sinuolinea sakinachanumae*. Among Mediterranean invaders, 23 species were found in the Mediterranean Sea (*Sphaeromyxa balbiani*, *S. incurvata*, *S. sabrazesi, Myxidium gadi*, *M. incurvatum*, *M. pulchrum*, *Sigmomyxa sphaerica*, *Enteromyxum leei*, *Zschokkella admiranda*, *Ortholinea divergens*, *Ceratomyxa arcuata*, *C. beloneae*, *C. globulifera*, *C. inaequalis*, *C. parva*, *C. reticularis*, *C. agilis*, *C. hepseti*, *Sphaerospora dicentrarchi*, *S. mugilis*, *Alataspora solomoni*, *Kudoa nova*, and *K. quadratum*). The rest 12 species (*Sinuolinea rebae*, *Ortholinea orientalis*, *Zschokkella iskovi*, *Ceratomyxa elegans*, *C. informis*, *Sphaerospora caudata*, *S. elegans*, *Myxobilatus medius*, *M. gasterostei*, *M. platessae*, *Chloromyxum ovatum*, and *Ch. schulmani*) still have not been found in the Mediterranean Sea, mainly due to very few studies on this group of parasites. Meanwhile, identification of most of the species mentioned above in close regions of the World Ocean allows suggesting that they penetrated the Black Sea only through the Mediterranean Sea. As for *Ceratomyxa elegans* from *Scorpaena porcus*, as well as for *Myxobolus parvus*, *M. episquamalis*, *M. exiguus*, and *M. spinacurvatura* from mullets, they can also become parasites for Pacific Ocean fishes. Taking into consideration that at the end of Neogenic period of Cainozoan era, the Atlantic and the Pacific oceans were connected by a wide strait for some time [44], we can assume presence of each of these parasites in a common large areal in the past which then was split due to powerful geological transformations on our planet. To our opinion, this also applies to widely spread brackish-water species *Myxobilatus medius*, *M. gasterostei*, and *Sphaerospora elegans* having marine origin that penetrated the Ponto-Caspian basin from the Mediterranean Sea many years ago. Possibly in the future, *Zschokkella sturionis* – a parasite for sturgeons whose parasite fauna in the Black Sea has not been studied well – will also be found in the Black Sea.

Of 46 myxosporean species of freshwater origin found in the Black Sea, most species (29) inhabit freshwater fish in the region of Dnieper Delta, as well as Dnieper and Dnieper-Bug estuaries with water salinity of 0.08–0.5 to 3–4 ‰ (less often of 6–8 ‰) within the main territory. Freshwater hosts are mostly carps, bass, and pike. There are also euryhaline hosts: Black Sea salmon (of freshwater origin); sea herring and mullet; brackish-water Gasterosteidae (of marine origin). Parasites for euryhaline fish are also registered in other full-salt sea parts, where pure marine myxosporean species also survive. Brackish-water forms (*Sphaerospora caspialosae* and *S. elegans*) are represented in the gulfs. *S. caspialosae* is also registered in the Kerch Strait – a region with salinity of 10–14 ‰ to depth of 50 m.

The fauna of Sea of Azov fish parasites includes 42 myxosporean species [3, 9, 36, 37]; 32 of them are found in the Black Sea, 20 – in the Caspian Sea. Totally 19 species (*Henneguya schizura*, *Hoferellus conifer*, *Myxidium lieberkühni*, *M. pfeifferi*, *Myxobolus bliccae*, *M. cyprini*, *M. dispar*, *M. dogieli*, *M. ellipsoides*, *M. kubanicus*, *M. macrocapsularis*, *M. magnus*, *M. musculi*, *M. oviformis*, *M. pseudodispar*, *M. rutili*, *M. sandrae*, *Thelohanellus pyriformis*, and *Zschokkella striata*) are freshwater species, and they are parasitic only on freshwater fish species. Ten species are of freshwater origin but can be registered in marine fish species (mostly in mullets – *Myxobolus adeli*, *M. bramae*, *M. episquamalis*, *M. exiguus*, *M. ichkeulensis*, *M. muelleri*, *M. parvus*, *M. spinacurvatura*, and *Zschokkella nova*; one in gobies – *M. najdenovae*). Of the freshwater species, *Myxobolus najdenovae* is Ponto-Azov endemic. Totally 13 myxosporean species are marine ones: 2 species (*Myxodavisia cornuta* and *Myxidium bentophili*) are Sea of Azov endemics; 3 species (*Shaeromyxa sevastopoli*, *Myxidium melanostomi*, and *Ortholinea gobiusi*) are Ponto-Azov endemics; 8 species (*Ceratomyxa hepseti*, *Kudoa nova*, *Myxidium gadi*, *Myxobilatus medius*, *Sphaerospora caudata*, *S. dicentrarchi*, *S. mugilis*, and *Zschokkella sturionis*) are Mediterranean invaders.

Number of myxosporean species common for the Sea of Azov and the Black Sea is 32. Totally 12 are freshwater parasites on freshwater fish; 9 are parasites on euryhaline mullets; 1 is gobies parasite found in full-salt waters; 10 are marine species (2 of them are brackish-water forms).

The Caspian Sea fauna is well described in works of A. A. Gazimagomedov [10, 11] and Sh. R. Ibragimov [13, 14, 15, etc.]. On its myxosporean fauna, the Caspian Sea is very similar to the Black Sea. In the fauna composition, there are 68 myxosporean species with 8 having marine origin (1 (*Zschokkella sturionis*) is ancient marine species; 1 (*Sinuolinea sakinachanumae*) is Ponto-Caspian endemic; 6 (*Sphaerospora caspailosae*, *S. donecae*, *S. elegans*, *Ceratomyxa caspia*, *Myxobilatus gasterostei*, and *M. medius*) are brackish-water myxosporeans) and 60 being freshwater species. The researchers mentioned above reported 5 Caspian Sea endemics, with *Ceratomyxa caspia* and *Shaerospora donecae* being of marine origin and *Myxobolus saidovi*, *M. alievi*, and *Henneguya chaibulaevi* – of freshwater origin.

Common fauna of such different water basins as the Black Sea and the Caspian Sea can be explained by the fact that connection between them exists mostly due to freshwater forms (27 species). Among marine species, only 5 are common (4 of them are of brackish-water origin).

The Caspian Sea and the Sea of Azov have 18 common freshwater parasites and 2 marine parasites (one of them is of brackish-water type). Unlike the Caspian Sea, the Sea of Azov is characterized by presence of Mediterranean invaders who could penetrate it from the Black Sea after the separation of the Caspian Sea, which became an independent basin.

Conclusion. When moving from west to east (from the Black Sea to the Caspian Sea), a gradual impoverishment of marine myxosporean fauna and its replacement by freshwater species are observed. In the Black Sea, correlation of marine and freshwater forms is approximately equal. In the Sea of Azov, elements of freshwater fauna begin to dominate. They reach considerable priority in the Caspian Sea where marine species are represented mainly by freshwater forms. As for impoverishment of abundance of myxosporean species directed from the Black Sea to the Caspian Sea (it was marked by Z. Donets), it does occur.

Part of this work conducted by V. M. Yurakhno was carried out within the framework of government research assignment of IBSS no. AAAA-A18-118020890074-2.

REFERENCES

1. Arkhangelsky A. D. Hydrogen sulfide contamination of some Tertiary sea basins of the Crimea and Caucasus region and its connection with the origin of petroleum. *Neftyanoe khozyaistvo*, 1926, vol. 10, iss. 4, pp. 483–486. (in Russ.)
2. Arkhangelsky A. D., Strakhov N. M. *Geological History of the Black Sea.* Moscow ; Leningrad : Izd-vo AN SSSR, 1938, 226 p. (in Russ.)
3. Bortnikov E. S., Strizhakova T. V., Boiko N. E., Ruzhinskaya L. P., Shevkoplyasova N. N. Myxobolosis of the flathead grey mullet *Mugil cephalus* in the Russian waters of the Black Sea. In: *Contemporary Problems of Theoretical and Marine Parasitology : Coll. of Sci. Papers* / K. V. Galaktionov, A. V. Gaevskaya (Eds). Sevastopol : Izd-l' Bondarenko N. Yu., 2016, pp. 211–213. (in Russ.)

4. Campbell N. The myxosporean parasitofauna of the Atlantic horse mackerel, *Trachurus trachurus* (L.) in the North-East Atlantic Ocean and Mediterranean Sea. *Acta Parasitologica*, 2005, vol. 50, no. 2, pp. 97–101.
5. Dogel V. A. *General Parasitology*. Leningrad : Izd-vo Leningr. un-ta, 1962, 464 p. (in Russ.)
6. Donets Z. S. Zoogeographical analysis of myxosporeans in the USSR southern water reservoirs. *Trudy Zoologicheskogo instituta AN SSSR*, 1979, vol. 87, pp. 65–90. (in Russ.)
7. Donets Z. S. Formation of myxosporeans fauna in the USSR southern seas. *Trudy Zoologicheskogo instituta AN SSSR*, 1981, vol. 107, pp. 74–79. (in Russ.)
8. Donets Z. S. *Myxosporeans of the USSR Southern Rivers Basins* : avtoref. diss. … d-ra biol. nauk. Leningrad, 1982, 43 p. (in Russ.)
9. Gaevskaya A. V., Gusev A. V., Delyamure S. L., Donets Z. S., Iskova N. I., Kornyushin V. V., Kovaleva A. A., Margaritov N. M., Markevich A. P., Mordvinova T. N., Naidenova N. N., Nikolaeva V. M., Parukhin A. M., Pogoreltseva T. P., Smogorzhevskaya L. A., Solonchenko A. I., Shteyn G. A., Schulman S. S. (Contrs). *Opredelitel' parazitov pozvonochnykh Chernogo i Azovskogo morei. Paraziticheskie bespozvonochnye ryb, ryboyadnykh ptits i morskikh mlekopitayushchikh.* Kiev : Naukova dumka, 1975, 552 p. (in Russ.)
10. Gazimagomedov A. A. To the study of parasitic Protozoa of the Caspian Sea fish. In: *Sbornik nauchno-issledovatelskikh rabot Dagestanskogo nauchno-issledovatel'skogo veterinarnogo instituta.* Makhachkala, 1970, vol. 4, pp. 291–293. (in Russ.)
11. Gazimagomedov A. A. New species of mucous sporozoans of the Caspian Sea fish. In: *Voprosy fiziologii, biokhimii, zoologii, parazitologii* : sbornik nauchnykh soobshchenii kafedry fiziologii cheloveka i zhivotnykh, zoologii i biokhimii. Makhachkala, 1970, pp. 72–76. (in Russ.)
12. Gürkanlı C., Okkay S., Çiftçi Y., Yurakhno V., Özer A. Morphology and molecular phylogeny of *Ortholinea mullusi* sp. nov. (Myxozoa) in *Mullus barbatus* from the Black Sea. *Diseases of Aquatic Organisms*, 2018, vol. 127, pp. 117–124. https://doi.org/10.3354/dao03192
13. Ibragimov Sh. R. *Caspian Sea Fish Parasites (Species Composition, Ecology, Origin and Fauna Formation)* : avtoref. dis. … d-ra biol. nauk. Baku, 1991, 47 p. (in Russ.)
14. Ibragimov Sh. R. Zoogeographical status of Caspian Sea with consideration of the data on fish parasites. In: *Sovremennye problemy biologicheskikh resursov Kaspiiskogo morya* : materialy mezhdunar. konf., posvyashch. 90-letiyu Azerb. NIIRKh, 15–17 Sept., 2003. Baku, 2003, pp. 224–228. (in Russ.)
15. Ibragimov Sh. R. Parasite fauna of the Caspian Sea for long period of observations. In: *Problemy ikhtiopatologii v nachale XXI veka* : materialy mezhdunar. nauch. konf. Saint Petersburg, 2009, pp. 65–78. (in Russ.)
16. Iskov M. P. Myxosporeans (Myxosporea). In: *Fauna Ukrainy*. Kiev : Naukova dumka, 1989, vol. 37, iss. 4, 210 p. (in Russ.)
17. Kessler K. F. Fish inhabiting and occurring in Aral-Caspian-Pontian ichthyological region. *Trudy Aralo-Kaspiyskoi ekspeditsii,* 1877, iss. 4, 360 p. (in Russ.)
18. Kolesnikova M. G., Donets Z. S. Fauna of myxosporeans in the Crimean coast fish. In: *Parazitologiya i patologiya morskikh organizmov* : tez. dokl. 14-go Vsesoyuzn. simp. Kaliningrad, 1987, pp. 89–90. (in Russ.)
19. Lubat V., Radujkovic B., Marques A., Bouix G. Parasites des poissons marins du Montenegro: Myxosporidies. *Acta Adriatica*, 1989, vol. 30, no. 1/2, pp. 31–50.
20. Moshu A. Ja., Trombitsky I. D. New parasites (Apicomplexa, Cnidospora) of some Clupeidae fishes from the Danube and Dniester basins. In: *Academician Leo Berg – 130 : Coll. of Sci. Articles.* Bendery : Eco-TIRAS ; Tipogr. "ELAN POLIGRAF", 2006, pp. 95–103.
21. Naidenova N. N. *Parazitofauna ryb semeistva bychkovykh Chernogo i Azovskogo morei*. Kiev : Naukova dumka, 1974, 182 p. (in Russ.)
22. Osmanov S. U. Materials for the Black Sea fish parasitic fauna. *Uchenye zapiski Leningradskogo pedinstituta im. Gertsena*, 1940, vol. 30, pp. 187–265. (in Russ.)
23. Ovcharenko M., Yurakhno V., Tytar V. Review of microparasites of mullets in the Mediterranean basin and the Sea of Japan. *Revista Română de Parazitoilogie*, 2007, vol. 17 (suppl.), pp. 142–143.

24. Özer A., Okkay S., Gürkanlı C. T., Çiftçi Y., Yurakhno V. Two novel myxosporean parasites in Black Sea fishes: *Kudoa niluferi* sp. nov. and *Kudoa anatolica* sp. nov. (Cnidaria: Myxosporea). *Diseases of Aquatic Organisms*, 2018, vol. 128, no. 3, pp. 225–233. https://doi.org/10.3354/dao03227
25. Özer A., Özkan H., Yurakhno V. New host and geographical records for *Ortholinea orientalis* (Schulman et Schulman-Albova, 1953) (Myxozoa, Myxosporea). *Acta Zoologica Bulgarica*, 2015, vol. 67, no. 4, pp. 595–597.
26. Özer A., Özkan H., Güneydağ S., Yurakhno V. First report of several myxosporean (Myxozoa) and monogenean parasites from fish species collected from Sinop coasts of the Black Sea. *Turkish Journal of Fisheries and Aquatic Sciences*, 2015, vol. 15, pp. 741–749. https://doi.org/10.4194/1303-2712-v15_3_18
27. Özer A., Özkan H., Gürkanlı C. T., Yurakhno V., Çiftçi Y. Morphology, histology and phylogeny of *Henneguya sinova* sp. nov. (Myxobolidae: Myxozoa) infecting gills of *Parablennius tentacularis* in the Black Sea, Turkey. *Diseases of Aquatic Organisms*, 2016, vol. 118, no. 3, pp. 207–215. https://doi.org/10.3354/dao02968
28. Özer A., Öztürk T., Özkan H., Çam A. First report of *Enteromyxum leei* (Myxozoa) in the Black Sea in a potential reservoir host *Chromis chromis*. *Fish Pathology*, 2014, vol. 49, iss. 2, pp. 57–60. https://doi.org/10.3147/jsfp.49.57
29. Özer A., Yurakhno V. Parasite fauna of garfish *Belone belone* collected from Sinop coasts of the Black Sea, Turkey. *Bulletin of the European Association of Fish Pathology*, 2013, vol. 33, no. 6, pp. 171–180.
30. Reshetnikova A. V. *Parasitic Fauna of Some Food Fish Species of the Black Sea* : avtoref. dis. … kand. biol. nauk. Leningrad, 1954, 14 p. (in Russ.)
31. Şimşek E. First molecular data on *Myxobolus episquamalis* (Myxozoa: Myxosporea) infecting the scale of grey mullet (*Mugil cephalus*) off the Turkish Black Sea coasts. *Türkiye Parazitoloji Dergisi = Turkish Journal of Parasitology*, 2019, vol. 43, iss. 3, pp. 135–142. (in Turkish). https://doi.org/10.4274/tpd.galenos.2019.6294
32. Strakhov N. M. *Istoriya geologii.* 2^{nd} edition, revised. Moscow : Gosuchpedizdat, 1938, 501 p. (in Russ.)
33. Strakhov N. M. *Osnovy istoricheskoi geologii.* In 2 parts. Moscow : Gosgeolizdat, 1948, pt. 1, 253 p. ; pt. 2, 396 p. (in Russ.)
34. Yurakhno V. M. New data of the fauna of myxosporidians from fishes of the Black Sea. *Parazitologiya*, 1993, vol. 27, no. 4, pp. 320–326. (in Russ.)
35. Yurakhno V. M. *Myxosporeans of the Black Sea Fish: Systematics, Fauna, Ecology, Zoogeography.* [dissertation]. Sevastopol, 1994, 297 p. (in Russ.)
36. Yurakhno V. M. To fish myxosporean fauna (Myxozoa, Myxosporea) in Taganrog Gulf (Sea of Azov). *Vestnik zoologii*, 2005, suppl. 19, pt. 2, pp. 369–370. (in Russ.)
37. Yurakhno V. M. The origin of the Black Sea fish myxosporean (Myxozoa, Myxosporea) fauna. *Vestnik zoologii*, 2009, suppl. 23, pp. 199–207. (in Russ.)
38. Yurakhno V. M., Naidenova N. N. *Davisia cornuta* sp. n. (Myxosporea: Sinuolineidae), the parasite of *Neogobius fluviatilis* Pallas in the Azov Sea. *Ekologiya morya*, 2000, iss. 51, pp. 78–80. (in Russ.)
39. Yurakhno V. M., Ovcharenko N. A. Myxosporeans of the World Ocean mullets. In: *Parazitologiya v XXI veke – problemy, metody, resheniya* : materialy IV Vseros. kongr. Parazitologicheskogo obshchestva Rossiiskoi akademii nauk, 20–25 Oct., 2008. Saint Petersburg : “Lema”, 2008, pp. 231–234. (in Russ.)
40. Yurakhno V. M., Ovcharenko M. O. Study of Myxosporea (Myxozoa), infecting worldwide mullets with description of a new species. *Parasitology Research*, 2014, vol. 113, iss. 10, pp. 3661–3674. https://doi.org/10.1007/s00436-014-4031-5
41. Yurakhno V. M., Özer A. N. Fauna of myxosporeans of the Ponto-Caspian basin fishes (origin, comparative analysis). In: *Sovremennye problemy parazitologii i ekologii. Chteniya, posvyaschennye pamyati S. S. Shulmana* : sbornik trudov Vserossiiskoi nauchnoi konferentsii s mezhdunarodnym uchastiem, Tolyatti, 15–17 May, 2018. Tolyatti : Poliar, 2018, pp. 334–345. (in Russ.)
42. Zenkevich L. A. *Fauna i biologicheskaya produktivnost’ morya.* Moscow : Sovetskaya nauka, 1947, 588 p. (Morya SSSR ; vol. 2). (in Russ.)
43. Zenkevich L. A. *Biologiya morei SSSR.* Moscow : Izd-vo AN SSSR, 1963, 740 p. (in Russ.)
44. *Zhizn’ zhivotnykh* : in 7 vols / Polyanskiy Yu. I. (Ed.). Moscow : Prosveshchenie, 1987, vol. 1, 448 p. (in Russ.)

ИСТОРИЯ ФОРМИРОВАНИЯ И ОСОБЕННОСТИ ФАУНЫ МИКСОСПОРИДИЙ РЫБ ПОНТО-КАСПИЙСКОГО БАССЕЙНА

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Рассмотрена история становления фауны миксоспоридий рыб Понто-Каспийского бассейна. Работа основана на оригинальном материале по миксоспоридиям рыб Чёрного моря (собран в 1987–2018 гг.) и Азовского моря (1997–2016). Всего исследованы 15 368 экз. рыб 87 видов (14 297 экз. рыб 80 видов в Чёрном море и 1071 экз. рыб 19 видов в Азовском). Материал собран методом неполных паразитологических вскрытий и обработан по общепринятым методикам. Также проанализированы все доступные литературные источники по миксоспоридиям черноморских, азовских и каспийских рыб (в библиографическом списке указаны только наиболее значимые публикации). Проведён сравнительный анализ фауны миксоспоридий рыб Чёрного, Азовского и Каспийского морей. Указано, что всего в Чёрном море известно 108 видов этих паразитов, в Азовском море — 42, в Каспийском море — 68. Общими для фауны миксоспоридий рыб Азовского и Чёрного морей являются 32 вида, Каспийского и Чёрного — 32, Азовского и Каспийского — 20. Во всех трёх морях встречаются 16 видов миксоспоридий. К настоящему времени из 108 видов миксоспоридий в Чёрном море 29 обнаружены исключительно в пресноводных рыбах, обитающих в устьях рек и лиманах со значительным опреснением. Для 79 видов хозяевами служат морские виды рыб, встречающиеся в основном в полносолёной части моря. Из них 17 имеют пресноводное происхождение: 7 видов встречаются в пресноводных рыбах и эвригалинных кефалях, 1 является паразитом пресноводных и морских лососевых рыб, а 9 имеют хозяевами только кефалей или других видов морских рыб. Морские формы представлены эндемиками: понтическими (22 вида), понто-азовскими (3) и понто-каспийскими (2), а также средиземноморскими вселенцами (35 видов). Среди пресноводных миксоспоридий только 1 вид эндемичен для Чёрного моря и 1 является понто-азовским эндемиком; большинство остальных видов широко представлено в пресных водоёмах. Среди средиземноморских вселенцев следует отметить 23 вида, найденных в Средиземном море; 12 видов пока не встречены в Средиземном море (вероятно, главным образом из-за слабой изученности в нём этой группы паразитов). Фауна миксоспоридий рыб Азовского моря насчитывает 42 вида миксоспоридий, 32 из которых найдены в Чёрном море, 20 — в Каспийском. К пресноводным видам, паразитирующим исключительно в пресноводных видах рыб, относятся 19. Ещё 10 видов имеют пресноводное происхождение, но встречаются и в морских видах рыб (преимущественно в кефалевых, а один вид — в бычковых). Из пресноводных видов 1 является понто-азовским эндемиком. К морским относятся 13 видов миксоспоридий: 2 азовских эндемика, 3 понто-азовских эндемика, 8 средиземноморских вселенцев. Из 68 видов миксоспоридий рыб Каспийского моря 8 являются морскими (1 древнеморской вид, 1 понто-каспийский эндемик, 6 солоноватоводных миксоспоридий), 60 — пресноводными. Эндемиков Каспийского моря известно 5 (2 вида морского происхождения и 3 — пресноводного). Установлено, что при продвижении с запада на восток (от Чёрного моря к Каспийскому) наблюдается постепенное обеднение фауны морских миксоспоридий и замещение её фауной пресноводных видов. Отмечено также обеднение видового состава миксоспоридий рыб Азовского и Каспийского морей по сравнению с таковым Чёрного моря.

Ключевые слова: фауна, миксоспоридии, рыбы, Чёрное море, Азовское море, Каспийское море

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Морской биологический журнал, 2020, том 5, № 1, с. 111–113
Marine Biological Journal, 2020, vol. 5, no. 1, pp. 111–113
https://mbj.marine-research.org
ISSN 2499-9768 print / ISSN 2499-9776 online

CHRONICLE AND INFORMATION

TO THE MEMORY OF YUVENALI ZAITSEV
(18.04.1924 – 08.01.2020)

An outstanding hydrobiologist, Academician of the National Academy of Sciences of Ukraine, D. Sc., and Professor Yuvenali Zaitsev passed away. It was he who discovered marine neuston and formulated the concept of contour biotopes of the sea. Yu. P. Zaitsev is the author and co-author of more than 350 scientific publications, as well as of a number of popular science books. Under his scientific supervision, 6 doctoral and 24 master's dissertations were completed and defended.

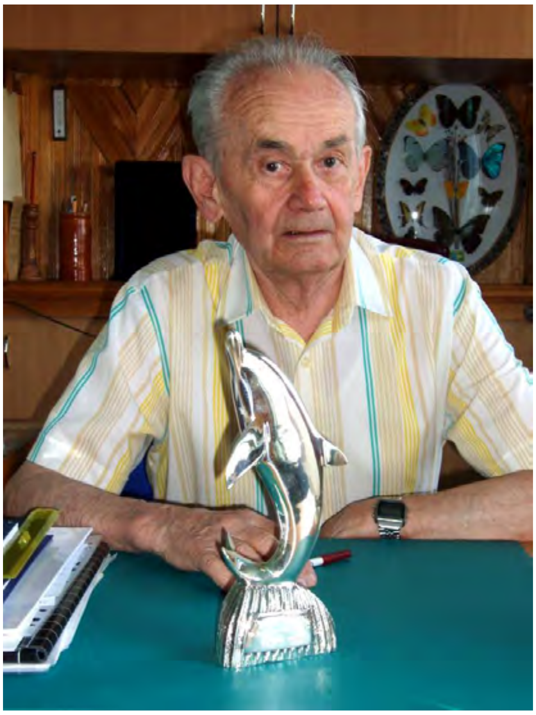
On January 8, 2020, in Odessa, after a long illness, an outstanding hydrobiologist, Academician of the National Academy of Sciences of Ukraine, D. Sc., Professor, Honored Worker of Science and Technology of Ukraine, and Laureate of the State Prize of Ukraine Yuvenali Petrovich Zaitsev passed away.

He was born in the village of Bayramcha, Akkerman county of Bessarabia (now the village of Nikolaevka-Novorossiysk, Saratsky district, Odessa region of Ukraine) in the family of a teacher. He spent his childhood and youth in the village of Bolshaya Balabanovka (now the village of Nikolaevka, Belgorod-Dniester district, Odessa region).

In 1949, he graduated from the Biology Department of Odessa I. I. Mechnikov State University (OSU; now Odessa National University). From 1950 to 1956 he worked at the Hydrobiological Station of OSU as a laboratory assistant. Under the scientific supervision of professor I. I. Puzanov, Yu. P. Zaitsev completed and defended his dissertation "Reproduction of fish with pelagic eggs in the Gulf of Odessa" at OSU in 1956. For the first time in science, the specific weight of pelagic eggs of various Black Sea fish species was determined, and this parameter made it possible to reveal the depth of eggs location in the anisotropic pelagic zone of desalinated northwestern part of the Black Sea.

In 1956, Yuvenali Petrovich as a junior researcher of the Odessa Biology Station of the Institute of Hydrobiology of the Academy of Sciences of the Ukrainian SSR began complex studies of the unknown to science community of organisms discovered by him in the Black Sea – marine neuston (primarily its lower tier – hyponeuston). The use of semi-submerged networks of the original design helped to collect a large amount of new scientific information concerning various marine organisms adapted to life in the upper water layer (0…–5 cm). The study of the conditions of the evolutionary formation of the marine neuston showed the universal nature of the presence of the near-surface pelagic community, and over time it was confirmed by studies throughout the World Ocean.

The discovery aroused the interest among western scientists and contributed to the start of marine neuston studies in France, Italy, Germany, the UK, Algeria, and other countries. Yu. P. Zaitsev's monograph "Marine Neustonology", published in 1970 in Kyiv, was released in English in the USA and Israel the following year. In 1974, "Soviet Life" magazine included the discovery of the marine neuston among the most important scientific achievements in the USSR. Using a network of Zaitsev's designs, foreign colleagues found and described diverse neuston in various oceans. In 1964, the scientist G. G. Polikarpov defined the marine neuston as the ecological target of the radioecological factor and the most "critical" biocenosis on the globe, since the highest concentrations of radionuclides are observed precisely in the surface water film and in the foam.



By invitation of foreign governments, Yuvenali Petrovich gave scientific reports and a course of lectures to students and teachers of France, the USA, Canada, Republic of South Africa, Turkey, and Japan.

In 1964, Yu. P. Zaitsev defended his dissertation "Hyponeuston of the Black Sea and its significance" at OSU. By a resolution of the Presidium of the Academy of Sciences of the Ukrainian SSR, the Hyponeuston Department was created in the Odessa Branch of IBSS. The staff studied bacteria, unicellular algae, invertebrates, and fish larvae at early stages of ontogenesis in a specific layer of neustal.

In 1968, Yuvenali Petrovich was approved in the rank of Professor by the Supreme Attestation Commission of the USSR. In 1969, he was elected a corresponding member of the Academy of Sciences of the Ukrainian SSR.

Yu. P. Zaitsev formulated the concept of contour biotopes of the sea inhabited by communities of contourobionts. The external contours of the pelagic zone at its borders with atmosphere, coast, bottom, and rivers were identified: aerocontour (pelagic – atmospheric border), psammocontour (pelagic – sandy coast and bottom border), lithocontour (pelagic – rocky coast and bottom border), pelocontour (pelagic – silty coast and bottom border), and potamocontour (border between sea and river water masses). Each contour biotope is characterized by groups of organisms adapted to its specific conditions, mainly consisting of individuals at the early stages of ontogenesis.

An exception to this rule was the deep-sea pelocontour of the Black Sea in the hydrogen sulfide area, where the existence of oxybionts was considered *a priori* impossible. However, integrated researches initiated by Yu. P. Zaitsev and G. G. Polikarpov allowed finding viable stages of oxybiontic development from the upper Black Sea layers in bottom sediments at the depths up to 2200 m, from which heterotrophic bacteria, fungi, and microalgae cultures were bred in laboratory conditions.

As an international expert on biological diversity and marine ecology, Yuvenali Petrovich took part in the work of the Black Sea Ecological Programme (BSEP). His monographs and articles on international Black Sea issues were published in New York at the UN publishing house.

He participated in the work of the UN Joint Group of Experts on the Scientific Aspects of Marine Protection (GESAMP). At the meeting on the topical issue "The Sea-surface Microlayer and Its Role in Global Change" in the USA in 1994, Yu. P. Zaitsev made a report on the topic "Neuston of the seas and oceans".

The final document of this forum, GESAMP Reports and Studies (no. 59, 1995), proposed the organization of the monitoring system “Neuston Watch” to monitor the state of the neuston, which affected the process of mass-energy exchange between the ocean and the atmosphere, in various parts of the World Ocean.

Yuvenali Petrovich is the author and co-author of more than 350 scientific publications, including 18 monographs, published in 20 countries. He also wrote a number of popular science books on environmental education and education of young readers. Thus, “Through the glass of undersea mask”, “This amazing sea”, “Your friend the sea”, “Life of the sea surface”, “World of delta”, “Introduction to the Black Sea ecology”, and “Wild nature in the city” served as a guidance for young people in choosing professions of biologist and environmentalist.

Under the scientific supervision of Yu. P. Zaitsev, 6 doctoral and 24 master’s dissertations were completed and defended.

For 17 years (1972–1989), Yuvenali Petrovich headed Institute of Marine Biology (in the past, Institute of Biology of the Southern Seas) of the National Academy of Sciences of Ukraine. In the summer of 2019, IMB celebrated the 95th anniversary of Yu. P. Zaitsev’s birth, dedicating a large scientific conference to this date, in which the jubilee himself participated actively.

Despite the illness, Yuvenali Petrovich was engaged in scientific work until his last days. His health seriously deteriorated after the tragic events in his institute – the December fire that claimed the lives of his student and successor in the director position, Corresponding Member of the NAS of Ukraine Boris Alexandrov and the secretary Galina Ivanovich.

Yu. P. Zaitsev lived a long, fruitful, and glorious life – the life of a genuine Scientist and Intelligent. He was distinguished by constant goodwill, friendliness, and optimism. The bright memory of Yuvenali Petrovich will forever remain in our hearts.

Colleagues from IBSS RAS and IMB NASU

ПАМЯТИ ЮВЕНАЛИЯ ПЕТРОВИЧА ЗАЙЦЕВА
(18.04.1924 – 08.01.2020)

Ушёл из жизни всемирно известный учёный-гидробиолог, академик НАН Украины, доктор биологических наук, профессор Ювеналий Петрович Зайцев. Ему принадлежат открытие морского нейстона и формулирование концепции контурных биотопов моря. Ю. П. Зайцев — автор и соавтор более чем 350 научных работ, а также ряда научно-популярных книг. Под его руководством выполнено и защищено 6 докторских и 24 кандидатских диссертации.

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ИнБЮМ – IBSS

Морской биологический журнал, 2020, том 5, № 1, с. 114
Marine Biological Journal, 2020, vol. 5, no. 1, pp. 114
https://mbj.marine-research.org
ISSN 2499-9768 print / ISSN 2499-9776 online

TO THE MEMORY OF YURI BURCHENKO
(09.05.1945 – 22.02.2020)

> I haven't been able to think of anybody
> whom I'd care to send out to look
> for horses in his stead.
> – *J. D. Salinger, "Raise High the Roof Beam, Carpenters"*

Yuri Mikhailovich Burchenko passed away. He worked in IBSS for about 30 years, directing the departments of information, patents, and international relationships. He was an impeccable linguist.

It is of great sadness that Yuri Mikhailovich Burchenko passed away. It is hard to believe, because you cannot imagine someone who would be more life loving.

Yu. M. Burchenko spent all his long labor life in two academic institutions – Institute of Microbiology and Virology in Kyiv and Institute of Biology of the Southern Seas in Sevastopol, where he directed the departments of information, patents, and international relationships. Yuri Mikhailovich worked in IBSS for about 30 years. He met many foreign colleagues, was a member of international marine expeditions and meetings, and actively contributed to the integration of IBSS researches in the world science.

He tenderly loved Sevastopol. He could reproduce historical events in much detail and "mold" a perfectly accurate outline of developments, surprising even professional historians. Yu. M. Burchenko amazed by originality of judgments and encyclopedic mindset, for which there were no limits. His unique mind kept everything that he carefully "put" there throughout his life. And it is really hurtful, that he did not leave it for us: he could not stand all kinds of paper records, and he did not really like electronic media too. Yuri Mikhailovich was an impeccable linguist, and, regardless of the language he had to write in, his work with every text, whether it was an article or a monograph, was kind of an art of understanding the very meaning of words.

It is sorry to say that Yu. M. Burchenko will no longer look with love at every corner of his hometown. He has left our time to enter into eternity, and the only consolation is that he could not get into the company of boring people.

ПАМЯТИ ЮРИЯ МИХАЙЛОВИЧА БУРЧЕНКО
(09.05.1945 – 22.02.2020)

Ушёл из жизни Юрий Михайлович Бурченко. Более 30 лет он проработал в ИнБЮМ, где в разное время руководил отделами информации, патентоведения и международных связей. Ю. М. Бурченко был неординарным лингвистом.

ISSN 2499-9768

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Институт биологии южных морей
имени А. О. Ковалевского РАН,
Зоологический институт РАН

издают
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- Подписной индекс в каталоге «Пресса России» — Е38872. Цена свободная.

Заказать «Морской биологический журнал»

можно в научно-информационном отделе ИнБЮМ.
Адрес: ФГБУН ФИЦ «Институт биологии южных морей имени А. О. Ковалевского РАН», пр. Нахимова, 2, г. Севастополь, 299011, Российская Федерация.
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