Long-term dynamics of the water quality in the Rybinsk reservoir according to biotesting

Roza A. Lozhkina, Irina I. Tomilina*, Maria V. Gapeeva

¹I.D. Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences, Borok 109, Nekouzsky District, Yaroslavl Region, 152742 Russia

Received: 23.03.2020
Accepted: 02.05.2020
Published online: 12.08.2020
DOI: 10.23859/estr-200323
UDC 504.4.064.36:574'405'
ISSN 2619-094X Print
ISSN 2619-0931 Online

Introduction

It is necessary to control the quality of natural waters in order to solve the problem of rational exploitation of biological resources of the water bodies successfully and to supply locals with drinking water. Nowadays, the income of pollutants into aquatic ecosystems still takes place, despite the improvement of technological processes and a tendency to decrease the anthropogenic load on the environment, emerged in recent decades (Hrabik and Watras, 2002; Moiseenko, 2009; Moiseenko and Gashkina, 2016).

The Rybinsk Reservoir is one of the largest freshwater artificial reservoirs in Russia, formed in the Mologa-Sheksna lowland after the construction of a hydroelectric complex upstream Rybinsk city on the Sheksna and Volga rivers (Butorin et al., 1975). In the reservoir, four reaches were distinguished (Volga, Mologa, Sheksna, and Central) according to the morphological features of the riverbed, the bottom contour and gradations of water colour in the reservoir (Fortunatov, 1959; Rybinskoé..., 1972).

The Rybinsk Reservoir is one of the major sources of drinking water supply, strongly affected by industrial, municipal and agricultural wastewater. Nowadays, over a hundred enterprises discharge wastewater into the reservoir. The most significant water users are municipal unitary enterprise “Vodokanal” and the enterprises of the Cherepovets industrial hub (PJSC “Severstal”, OJSC “Severstal-Metiz”, and OJSC “PhosAgro-Cherepovets”). Wastewater from these enterprises, as well as agricultural wastewater, enters the reservoir with the waters of the rivers Koshta, Sheksna, Yagorba, and some others (Tikhanovskaya and Mashikhina, 2016); these enterprises have a pronounced technogenic effect on the ecosystem of the reservoir, in particular, on the Sheksna Reach. The annual intake of fresh water from the rivers of the Rybinsk Reservoir basin is about 550 million m³, and the discharge of wastewater is 470 million m³. Despite the presence of treatment facilities, the volume of normatively treated wastewater is less than 20% of the amount of polluted wastewater requiring treatment (Doklad..., 2019).

Pollution by toxic substances, which may disrupt bioproduction processes and lead to profound changes in the structural and functional organization of the biotic component of ecosystems, is the main and the most dangerous type of anthropogenic influence.
on all components of the natural environment (Krainyukova, 2009). First of all, these substances include heavy metals, organochlorine pesticides, phenols, oil products, synthetic surfactants, and a number of other pollutants that are usual components of wastewater (Ecologicheskie..., 2001; Flerov, 1990; Kolpakova et al., 1996).

Hydrochemical research methods have their advantages, but the most effective results are achieved when they are used in combination with biotesting methods. The last should be understood as integral approaches that combine the total effect of all biologically hazardous substances present in the test sample on aquatic organisms, while hydrochemical methods, on the contrary, break down the sum of the present pollutants into their constituent parts and allow to identify the source of toxicity. Consequently, the combination of these groups of methods in environmental monitoring is a necessary condition for obtaining complete information for taking further measures to prevent negative anthropogenic impact (Zhmur, 2018). Biotesting gives a holistic assessment of the aquatic toxicity, this significantly reduces the effort for chemical and analytical work and makes it possible to control the pollution of water body more quickly (Braginskii, 1985).

The study aims to assess the long-term changes in the aquatic toxicity of the Rybinsk Reservoir using the biotesting method and to search for the factors influencing this parameter.

Materials and methods

Integral water samples (394 samples in total) were taken with the Elgmork bathometer (Korneva, 2015) sequentially from each 1-m water layer from surface to bottom in different parts of the Rybinsk Reservoir (Fig. 1) from 1994 to 2019 (Lazareva, 2018). Water was filtered through ash-free filter (white ribbon filter) and then poured into food grade plastic bottles (0.5 l) under a tightly screwed cap to exclude the ingress of oxygen. Prior to biotesting, the samples were stored in a refrigerator at a temperature of 2–4 °C for 7–14 days.

Biotesting of water samples was carried out on a laboratory reared Ceriodaphnia dubia Richard, 1894 (Opredelitel'..., 2010) in accordance with the standard procedure (Metodika..., 2007; Mount and Norberg, 1984). On the first day after hatching, genetically homogeneous crustaceans were placed individually in the cups filled with 15 ml of the test water; the observations were performed until each female spawned three times. During the experiment, the animals were fed once every two days with green algae Chlorella vulgaris Beij., 1890 at a concentration of 250-300 thousand cells/ml at the time of water changing (Metodika..., 2007). Optimal conditions were maintained throughout the entire experiment, particularly, water temperature of 21 ± 3 °C, pH 7.5–8.0, dissolved oxygen at saturation level, light regime 16 h light: 8 h darkness (white light lamp). The control group of crustaceans was kept under similar conditions in equilibrated artesian water. The death of at least 50% of crustaceans within 48 h in the test water served as a criterion for acute toxicity, provided that their death in the control did not exceed 10%. The death of 20% or more test organisms and a significant change in fecundity (the number of survived offspring compared to the control) were the criteria for chronic toxicity (Metodika..., 2007). An increase in the fecundity of crustaceans by more than 30% was considered as a manifestation of a chronic toxic effect (Aleksandrova, 2009; Ol’kova and Dabakh, 2014; Zhmur, 2018).

The toxicity index (TI) was calculated in order to obtain comparable results of biotesting; TI was a value expressed in fractions of a unit for each measured indicator according to the equation:

\[
TI = \frac{TPV_e}{TPV_c},
\]

where \(TPV_e\) is the value of the test parameter in the experiment, \(TPV_c\) in the control.

The concentrations of pollutants in the water bodies, measured in different years, were converted into a water pollution factor (WPC) for each toxic pollutant (TP), calculated using the equation (Kriterii..., 2011):

\[
WPC = \frac{C_i}{MPC_i},
\]

where \(C_i\) is the concentration of the \(i\)-th component of the pollution complex, mg/l; \(MPC_i\) is the maximum permissible concentration of the \(i\)-th component of the pollution complex, mg/l (Perechen’..., 1999).

The total water pollution coefficient \(WPC_{sum}\) for six TP was calculated using the equation:

\[
\sum WPC = \sum \left( \frac{C_i}{MPC_i} \right).
\]

Original and published data were used for WPC calculating (Gapeeva, 1993, 2013; Gapeeva and Tsel’movich, 1989, 1990; Tomilina et al., 2018b).

Data are presented as mean values and their errors (\(m \pm \text{SE}\)). The significance of differences was assessed by analysis of variance (ANOVA, LSD test) at a significance level \(p < 0.05\) (Sokal and Rohlf, 1995). The stepwise linear regression was used to search for the quantitative relationship of biotesting parameters with hydrological indicators and pollutant concentration in water. The studied parameters characterized by asymmetric distribution (Shapiro-Wilk test) were analyzed using the Spearman’s rank correlation coefficient (\(r_s\), \(p < 0.05\)).

Results

The survival rate of the test objects is the main test parameter in determining the aquatic toxicity in natural reservoirs. According to the results of biotesting of water samples from the Rybinsk Reservoir using the cladocerans Ceriodaphnia dubia no acute toxicity was reported for the observation period from 1994 to 2019, except in 2019 on the Mologa Reach at Ves’egonsk station and the Sebla
Fig. 1. Schematic map of sampling stations in the Rybinsk Reservoir. Volga Reach (I): 1 – Krutets, 2 – Myshkin opposite the Yukhot’ River, 3 – Eremeitsevo, 4 – Koprino, 5 – Kamenniki; Mologa Reach (II): 6 – Protiv’e, 7 – Ves’egonsk; Central Reach (III): 8 – the mouth of the Sebla River, 9 – Pervomaika, 10 – the mouth of the Sit’ River, 11 – Breitovo, 12 – Mologa, 13 – Volkovo, 14 – Milyushino, 15 – Vsekhsvyatskoe, 16 – the mouth of the Ukhra River, 17 – the mouth of the Sogozha River, 18 – Tsentral’ný Dvor, 19 – Sredni Dvor, 20 – Gorodok, 21 – Izmailovo, 22 – Navolok, 23 – Yagorba; Sheksna Reach (IV): 24 – Myaksa, 25 – Lysubets, 26 – Kargach Island, 27 – Vaganikha Island, 28 – Torovo, 29 – the Suda River at the railway bridge, 30 – the mouth of the Koshtia River, 31 – the Koshtia River at the car bridge, 32 – the mouth of the Serovka River, 33 – Kabachino.
River mouth, where crustacean mortality reached 50 and 60%, respectively. Chronic toxicity in terms of survival (the mortality of crustaceans in the experiment exceeded that observed in the control for 7–10 days of exposure, by 20%) was noted on certain dates of observations in the Mologa Reach at stations Ves’egonsk (Plenishnik Bay, Renya River), Central (Yagorba, Vsekhsyvatskoe, Volkovo, Tsentral’nyi Cape, Mologa, Navolok, Izmailovo, Pervomaiska, the mouths of the Ukhra, Sebla, Sit’, and Terekh’ River), Volga (Myshkin town opposite the Yukhot’ River, Koprino, Glebovo), and the Sheksna reaches (Myaksa, Lyubets, Vaganikha, Roshchino, the mouths of the rivers Koshta, Suda, Solntsevka, Pacha, and Kondosha).

The fecundity rate preconditions the maintenance of the population size and allows assessing the chronic toxic effect of water. The average fecundity in the reaches in most cases did not reach the control values (Fig. 2). The exceptions were 2011, 2015, and 2016, when the fecundity of crustaceans in the experiment was significantly higher by 30% or more compared to the control. In addition, in 2009 for the Volga Reach, an excessive fecundity was also noted, but it was statistically insignificant (25% higher than the control values).

The average values of the crustacean fecundity (expressed as TI) for all reaches were below the control values for the entire observation period. The exception was 2011, 2013, 2015, 2016, and 2019, for which TI exceeded 1.0 (Table 1). When comparing TI between the reaches, this parameter was characterized by the evenness, i.e. the average number of juveniles obtained from one female did not differ significantly. The lowest values of TI were recorded in 1996, in particular, 0.25 in the Mologa Reach, 0.51, the Central Reach, and 0.40 at the Volga Reach (Table 1). In the Sheksna Reach, the minimum TI value (0.40) was recorded in 1994. The maximum TI values were recorded in 2011 in the Mologa Reach (1.70), in 2015, in the Central Reach (1.61), in 2016, in the Volga (1.56) and Sheksna (1.52) reaches.

When comparing the proportion of stations with a chronic toxic effect (CTE) of water on the fecundity of *Ceriodaphnia* in the Volga and Mologa reaches, these values were uniform and did not exceed 10% in most cases (Fig. 3). In the Volga Reach, an excess of more than 10% was observed in 1994, 1997, 2015, and 2016; in the Mologa Reach, the year of 2019 was the only exception, when the share of CTE stations was 17%.

The share of CTE stations in the Sheksna and Central reaches was more variable; these stations were characterized by the maximum CTE values. In the Central Reach, in 1994-2008, there was a decrease in the share of the CTE station, and then it increased gradually until 2015. In 1998, 2014, and 2018, no CTE stations were noted, when the water had an effect on the crustacean fecundity. In the Sheksna Reach, the maximum number of CTE stations was recorded in 1996 (42%), 1997 (70%), and 2014 (42%) (Fig. 3). The average share of CTE stations, calculated by the number of sampling stations in the reach, but not by the total number of stations throughout the entire water area, in most cases exceeded 50%.

The maximum total WPC for six elements was observed in 1987 after the accident at the Cherepovets metallurgical plant, the minimum WPC, in 2010 (Table 2). Significant differences were registered between the Central and Sheksna reaches for WPC of cadmium in 1987, copper and lead, in 2010.

It was not always possible to find a direct relationship between the concentration of TP in water and water suitability for living organisms. Searching for the relationships (dependencies) between the studied variables was possible using the correlation analysis. The correlations between biotesting parameters and hydrological and/or hydrobiological indicators, as well as with the concentration of chemical elements in water are presented in Table 3. Obviously, the reproductive indices of *Ceriodaphnia* correlated positively with the air temperature and water conductivity (Table 3). Significant negative correlation was found between the concentrations of chemical elements in water and the

![Fig. 2. Dynamics of chronic aquatic toxicity from different reaches of the Rybinsk Reservoir by fecundity of *Ceriodaphnia dubia* (average number of juveniles per female for 7-day period, % of control).](image-url)
reproductive parameters of crustaceans (Table 3). The closest relationship was observed for the metal ions of Mg, Co, Cd, and Fe ($r_s > 0.7$).

The main variables that significantly affected the mortality of cladocerans were the concentrations of ammonium nitrogen and ions of Mg, Al, Pb, and Cu ($p < 0.05$). The regression model of the mortality parameters for *Ceriodaphnia* may be described by the linear equations:

$$\text{Mortality, } 48 \text{ h} = 9.117 - 69.074 \times \text{COD} + 8.629 \times [\text{NH}_4] - 981.959 \times [\text{Cd}], \quad (1)$$

$$\text{Mortality, } 10 \text{ days} = 3.682 + 0.000 \times [\text{Mg}] - 0.004 \times [\text{Al}] - 0.004 \times [\text{Ca}] - 0.004 \times [\text{Pb}] + 0.008 \times [\text{Cu}] - 0.057 \times [\text{Cd}] - 0.240 \times [\text{total REE}] + 0.001 \times [\text{Zn}] \quad (2)$$

It should be noted that the obtained correlations were conditional, since the literature data were used to compile the general matrix in addition to the original dataset; the published data referred to the average values of the TP concentration without specifying the sampling stations. In addition to the originally studied dependences, the final result could be influenced by other TP, and the data on their concentrations were not in the open access at the moment of this study.

**Discussion**

Currently, more than a hundred standards of the quality of water used for the household and drinking water supply are controlled (SanPiN..., 2001). A comprehensive analysis of all the environmental indicators does not allow to assess their complex impact. The current system for monitoring the pollution of the water bodies, based on the analyses of certain toxic substances by analytical methods, does not ensure the environmental safety of water bodies due to the incompleteness of data on the concentration of pollutants, the heterogeneous nature of the interaction of individual components in the mixture, and re-formed compounds, which may be more toxic than the original ones. Therefore, chemical analysis is only a statement of the presence or absence of any chemical element in a sample, and it does not reflect the properties of chemical elements in the natural environment and their effect on living objects, both direct and indirect (Bakaeva et al., 2009). Therefore, the use of integral methods for assessing the toxicity of natural environments, including biotesting, is of particular importance (Ol'kova, 2014).

| Year | Reach       | Mologa | Central | Volga | Sheksna | Average |
|------|-------------|--------|---------|-------|---------|---------|
| 1993 | –           | 0.93 (4) | 0.88 (9) | –     | 0.89 (13) |
| 1994 | –           | 0.61 (9) | 0.50 (3) | 0.40 (1) | 0.57 (13) |
| 1996 | 0.25 (1)    | 0.51 (9) | 0.40 (2) | 0.48 (14) | 0.47 (26) |
| 1997 | –           | 0.71 (1) | 0.54 (2) | 0.53 (10) | 0.55 (13) |
| 1998 | –           | 0.94 (8) | 0.81 (3) | 0.77 (4) | 0.87 (15) |
| 2008 | 0.59 (2)    | 0.87 (24) | 0.82 (3) | 0.84 (15) | 0.84 (44) |
| 2009 | 0.79 (1)    | 0.95 (10) | 1.24 (2) | 0.86 (10) | 0.93 (23) |
| 2010 | 0.78 (1)    | 0.83 (8) | 0.65 (2) | 0.77 (5) | 0.78 (16) |
| 2011 | 1.70 (2)    | 0.97 (8) | 1.47 (4) | 1.33 (5) | 1.25 (19) |
| 2012 | 0.85 (1)    | 0.82 (10) | 1.00 (2) | 0.79 (2) | 0.84 (15) |
| 2013 | 0.57 (1)    | 1.10 (17) | 0.87 (2) | 0.70 (2) | 1.02 (22) |
| 2014 | –           | 1.00 (2) | –       | 0.97 (10) | 0.98 (12) |
| 2015 | –           | 1.61 (9) | 1.47 (5) | –     | 1.56 (14) |
| 2016 | 1.37 (1)    | 1.13 (18) | 1.56 (4) | 1.52 (11) | 1.31 (34) |
| 2017 | –           | 0.74 (4) | 0.95 (5) | 0.69 (9) | 0.78 (18) |
| 2018 | 0.93 (7)    | 0.93 (14) | 0.87 (7) | 0.93 (22) | 0.93 (22) |
| 2019 | 1.13 (11)   | 1.09 (18) | 1.08 (5) | 1.08 (13) | 1.10 (46) |
| Average | 0.90 ± 0.13 (28) | 0.93 ± 0.06 (173) | 0.94 ± 0.09 (60) | 0.84 ± 0.08 (133) | 0.92 ± 0.07 (394) |

**Table 1.** Index of aquatic toxicity in various reaches of the Rybinsk Reservoir by the average fecundity of *Ceriodaphnia dubia* (average number of juveniles per female). The number of samples is indicated in brackets.
Some researchers argue that the assessment of the hazard of pollutants at the ecosystem level requires the use of several test objects; however, the meta-analysis of numerous literature data evidenced that the results of such testing did not differ much from those obtained at the level of an individual organism (Slooff et al., 1986). Using Ceriodaphnia as a test object seems reasonable, since these hydrobionts are filter feeders, which makes them highly sensitive to substances dissolved in water, moreover, they are easily reared in laboratory. In addition, given the importance of chronic experiments for final conclusions about the hazards of the studied media, the use of Ceriodaphnia as a model object provides a significant advantage in the study duration (Zhmur, 2018).

The absence of acute toxicity of the samples and the low mortality of crustaceans over the exposure period of 7–10 days give grounds to assert that most of the studied water samples are safe in terms of crustacean survival over the entire observation period. However, the informative value of biotests is generally low if considering the mortality rates. This is quite understandable and reasonable in terms of toxicology, since the death of test organisms is a manifestation of an extreme degree of toxic effect, which does not give an estimate of the resistance and tolerance of the population to the toxicant. The experiments aimed on studying the changes in motor activity, nutrition activity, reproduction rate, etc., are much more indicative when searching for a chronic toxic effect. Reproductive rate is among the most sensitive, allowing to detect low levels of pollution (Of’kova and Makhanova, 2018).

The fecundity of a laboratory culture of cladocerans depends on the season and the degree of aquatic toxicity and serves as an adequate indicator of the water quality (Filenko et al., 2013). In our experiments, the fecundity of crustaceans (average for the reaches) in most cases did not reach the control values. In 2011, 2013, 2015 and 2016, a stimulating effect was noted, when the reproductive indices in the experiment were significantly higher by 30% or more when comparing to the control. Such a phenomenon does not necessarily serve as a response to toxicity, since it may be a response to the factors that increase metabolic processes, namely, the presence of oxidizable organic and biogenic substances, vitamins, hormones, and biological stimulants in the studied medium (Zhmur, 2018). In our case, the stimulation of the fecundity of test organisms is possibly associated with the intake of organic substances of non-anthropogenic origin into the reservoir, which is indirectly evidenced by the high correlation coefficients of biological parameters and biochemical oxygen consumption for 5 days (BOD₅) (Table 3).

Moreover, the outcome of a toxicant effects on the organism depends on the interaction of destructive and compensatory processes. When the organism is exposed to toxic substances in low concentrations, which are typical for surface waters, both full compensation and the increase in the activity of body functions are possible. At long-lasting exposure to toxicant, destructive processes begin to prevail, which may lead to malfunctioning of biochemical and physiological processes. In turn, this may affect both the reproductive abilities of the organism and its survival.

No significant interannual differences in Ceriodaphnia fecundity was found both in the reaches and in the entire reservoir. In general, the lowest toxicity indices
### Table 2. Water pollution coefficient (WPC) of the Rybinsk Reservoir, mean ± error of the mean. * – statistically significant difference between indicators (Gapeeva, 1993; Gapeeva, 2013; Tomilina et al., 2018b). Values exceeding MPCs for fish aquaculture are highlighted in bold (Perechen’..., 1999).

| Reach         | 1987       | 2009       | 2010       | 2014       |
|---------------|------------|------------|------------|------------|
|               | WPC$_{Cr}$ | WPC$_{Ni}$ | WPC$_{Cu}$ | WPC$_{Zn}$ | WPC$_{Cd}$ | WPC$_{Pb}$ | Total WPC | Average WPC |
| Central (5)   | 0.02 ± 0.01| 0.56 ± 0.01| 5.88 ± 1.34| 2.90 ± 0.44| 0.24 ± 0.06*| 0.73 ± 0.08| 10.32 ± 1.57| 1.72 ± 0.26 |
| Sheksna (22)  | 0.02 ± 0.00| 0.43 ± 0.05| 7.36 ± 0.89| 2.49 ± 0.19| 0.14 ± 0.01*| 0.58 ± 0.023| 11.01 ± 0.92| 1.84 ± 0.15 |
| Mean          | 0.02 ± 0.00| 0.45 ± 0.05| 7.13 ± 0.78| 2.55 ± 0.17| 0.16 ± 0.01| 0.60 ± 0.03| 10.91 ± 0.81| 1.82 ± 0.13 |
| Mologa (1)    | 0.00 ± 0.00| 0.07 ± 0.00| 5.69 ± 0.00| 0.53 ± 0.00| 0.03 ± 0.00| 0.63 ± 0.00| 6.95 ± 0.00| 1.16 ± 0.00 |
| Central (10)  | 0.01 ± 0.01| 0.06 ± 0.01| 4.26 ± 0.81| 0.24 ± 0.06| 0.02 ± 0.00| 0.42 ± 0.25| 5.01 ± 1.00| 0.84 ± 0.17 |
| Volga (2)     | 0.00 ± 0.00| 0.07 ± 0.07| 3.75 ± 3.74| 0.22 ± 0.22| 0.05 ± 0.05| 0.13 ± 0.13| 4.21 ± 4.21| 0.70 ± 0.70 |
| Sheksna (10)  | 0.01 ± 0.01| 0.09 ± 0.02| 5.73 ± 1.83| 1.27 ± 0.72| 0.02 ± 0.01| 0.71 ± 0.39| 7.83 ± 2.40| 1.31 ± 0.40 |
| Mean          | 0.01 ± 0.00| 0.07 ± 0.01| 4.92 ± 0.89| 0.70 ± 0.32| 0.02 ± 0.00| 0.53 ± 0.20| 6.25 ± 1.17| 1.04 ± 0.20 |
| Central (8)   | 0.02 ± 0.02| 0.01 ± 0.00| 1.12 ± 0.09*| 0.73 ± 0.15| 0.06 ± 0.01| 0.00 ± 0.00*| 1.93 ± 0.21| 0.32 ± 0.04 |
| Volga (2)     | 0.05 ± 0.04| 0.01 ± 0.00| 1.48 ± 0.13| 0.94 ± 0.20| 0.07 ± 0.01| 0.03 ± 0.02*| 2.57 ± 0.15| 0.43 ± 0.02 |
| Sheksna (5)   | 0.01 ± 0.00| 0.01 ± 0.00| 1.53 ± 0.22*| 0.82 ± 0.14| 0.06 ± 0.01| 0.01 ± 0.00*| 2.43 ± 0.35| 0.40 ± 0.06 |
| Mean          | 0.02 ± 0.01| 0.01 ± 0.00| 1.30 ± 0.10| 0.79 ± 0.09| 0.06 ± 0.00| 0.01 ± 0.00| 2.18 ± 0.18| 0.36 ± 0.03 |
| Central (2)   | 0.06 ± 0.01| 0.05 ± 0.01| 3.45 ± 0.08| 1.57 ± 0.05| 0.00 ± 0.00| 0.13 ± 0.00| 5.26 ± 0.12| 0.88 ± 0.02 |
| Sheksna (10)  | 0.06 ± 0.00| 0.18 ± 0.04| 4.68 ± 0.34| 2.73 ± 0.26| 0.00 ± 0.00| 0.19 ± 0.02| 7.83 ± 0.60| 1.31 ± 0.10 |
| Mean          | 0.06 ± 0.00| 0.16 ± 0.04| 4.48 ± 0.31| 2.54 ± 0.25| 0.00 ± 0.00| 0.18 ± 0.02| 7.41 ± 0.58| 1.23 ± 0.10 |
for the reservoir were recorded in 1994–1997, the minimum Ti, in Mologa Reach in 1996 (Table 1). The lowest average long-term value was recorded in the Sheksna Reach (despite the year of observation); however, this value did not differ significantly from the other means in terms of Ti (Table 1).

The natural chemical composition of water of the Rybinsk Reservoir is characterized by a low concentration of dissolved salts (calcium bicarbonate dominates), by low concentrations of mineral forms of nitrogen and phosphorus, and by a high concentration of organic matter of a humic origin and, as a consequence, a high water colour index (Ekologicheskie..., 2001). Due to the ability to bind metal ions, humic compounds may both increase the mobility of trace elements in the environment (through complexation reactions) and reduce their migration ability due to the tendency of humic substances to adsorb on mineral surfaces (Linnik and Nabivanets, 1986; Mercier et al., 2001). This, in turn, may affect the degree of manifestation of the toxic properties of pollutants in various water bodies (Stravinskene, 2012).

In the Rybinsk Reservoir, the water masses are affected by the anthropogenic load the most in the zone of influence of the Cherepovets industrial hub. There is an excess of the established standards (MPC) for such components as ammonium nitrogen, copper, manganese, iron, oil products, BOD₅ (Grigor'eva et al., 2011a). In the Koshta River, where wastewater from PJSC “Severstal” is discharged, high concentrations of sulfates (154.0–191.6 mg/dm³) and chlorides (50.3–107.2 mg/dm³) were also noted in the water (Grigor’eva et al., 2011b).

The concentration of heavy metals in the water of the Rybinsk Reservoir undergoes significant spatial and temporal changes. Recent studies have revealed a high concentration of Cu and Zn in water, as evidenced by the high contamination factors of these elements (Table 2). According to data obtained in 2014, the concentrations of Cu and Zn at all studied stations of the Sheksna Reach were higher than the MPC values established for fishery. At the same time, at stations located outside the city of Cherepovets, the concentration of Zn exceeded the MPC value by 2.2 times on average, and Cu, by 40.6 times; for the stations within the city, by 3.5 and 49.6 times, respectively (Tomilina et al., 2018a). It is known that the copper concentration in natural waters, as a rule, is higher than MPC, since it is presented mostly by the metal-organic ligand complexes. It is possible that the high concentration of Cu and Zn in the water of the Rybinsk Reservoir is also due to the natural geochemical features of this region (Gapeeva, 2019; Tomilina et al., 2018b). According to correlation analysis, the concentrations of Al, Fe, Ni, Cu, Co, Zn, Mo, and Cd in the water affected both mortality rate of Ceriodaphnia and its reproductive indices. Almost all metals, except cadmium (its biological role is currently unclear), actively participate in biological processes, they are part of many enzymes and are necessary for the organism in trace amounts. However, they become biologically hazardous in concentrations exceeding MPC. For ecological toxicology, the most interesting metals are those that pollute water bodies the most due to their significant use in industrial activities and that are dangerous due to their biological activity and toxic properties; namely, these are cobalt, nickel, copper, molybdenum, and cadmium (Moore and Ramamoorthy, 1984). Cadmium is considered one of the most toxic elements, along with copper and mercury. Despite the low concentrations of cadmium in the water of the Rybinsk Reservoir, a close relationship was found between Cd concentration and the fecundity of crustaceans (rₛ > 0.75). In comparison with other animals, aquatic organisms are most sensitive to the toxic effects of molybdenum. The calcium and phosphate metabolism malfunctions are pronounced the most at the excess of this metal in organism. The LC₅₀ for minnow in soft water (pH 7.0) is 70 μg/L, in hard water, 370 μg/L, due to the presence of calcium and magnesium bicarbonates (Moore and Ramamoorthy, 1984). Zinc is relatively little toxic to endotherms, but it is dangerous to aquatic organisms in high concentrations: Zn concentration of 0.4 mg/l causes the death of sticklebacks and Daphnia, 0.05 mg/l, of salmonids (Moore and Ramamoorthy, 1984).

The presence of rare earth elements (REE) in water enhances the death of crustaceans and reduces their fecundity, as shown both in this study (Table 3) and in earlier works (Lozhkina and Tomilina, 2016; Michael and Barry, 2000). The bioavailability of REE is one of the main factors that determines its environmental hazard. Cladocerans absorb most of REE via carapace. The mechanism of absorption of lanthanum and some other REE in biological systems is similar to that for calcium; this may lead to abnormal molting in crustaceans. They actively absorb calcium during each molt cycle, which contributes to the penetration of REE into the internal environment, causing the toxic effects for the organism and, subsequently, affecting the crustacean fecundity (Das et al., 1988).

Toxicity can also be influenced by water-soluble organic compounds of anthropogenic origin, which is indirectly evidenced by the correlations of biological parameters of Ceriodaphnia and chemical oxygen demand (COD) observed in our study (Table 3). The ecosystem of the Rybinsk Reservoir contains persistent organic compounds; the Cherepovets industrial hub is their local source (Chuiko et al., 2010). Toxicity of water and of bottom sediments was repeatedly observed during 2008–2017 at the stations located in the Sheksna Reach in the zone of influence of the Cherepovets industrial hub (Tomilina et al., 2018b).
A significant effect of ammonium nitrogen and ions of Mg, Ca and Cu is noted under the regression equation (2). Copper ions at a concentration of 0.03 mg/l (fishery MPC for copper is 0.1 mg/l) caused the death of *Daphnia magna* Straus, 1820, and reduced their reproductive parameters and filtration activity (Shilova et al., 2010). As the water hardness increases, the mortality of invertebrates (*D. magna* and *Diaptomus forbesi* Light, 1996) and the algae *Chlorella vulgaris* Beij, 1890 (Dutta and Kavitaj, 2001; Santore et al., 2001) decreases even at high concentrations of cadmium and copper. Since ammonium nitrogen, magnesium, and copper are biogenic elements, the different degree of their influence on the vital functions of cladocerans probably depends on the nature of the effect on the biochemical processes taking place in aquatic organisms.

In addition to pollutants, the biological parameters of crustaceans may be influenced by intra-population interactions, as well as meteorological and phenological factors, such as atmospheric pressure, solar activity, the changes in the Earth’s magnetic field and in the daylight period (Galkovskaya and Morozov, 1981; Krylov, 2008). The correlations between the reproductive indices of *Ceriodaphnia* and zooplankton abundance indirectly testify to this (Table 3). Thus, the maximum abundance of zooplankton was observed in 2005 and 2009–2011 (Lazareva and Sokolova, 2018). Meantime, during the biotesting of water samples in 2011, the fecundity of *Ceriodaphnia* exceeded the control values by 33–70% (Table 1).

Therefore, numerous data indicate persistent long-term pollution of the water of the Rybinsk Reservoir with heavy metals, organic substances, oil products, biogenic elements, and other pollutants. According to the analysis of pollution factors, based on the data averaged for the reaches, the Sheksna Reach is exposed the most to pollution by heavy metals. The average WPC values for six major metals are higher here than those for other reaches (Table 2).

An objective assessment of the level of toxic pollution of aquatic ecosystems under anthropogenic load is possible only with a complex approach combining both chemical and biological research methods. The first one allows identifying and quantifying the pollutants in the aquatic environment, the second one, assessing both the degree of environmental hazard in general and the total effect of pollutants on aquatic organisms.

### Table 3. Correlations between the concentration of chemical elements, hydrological parameters and biological parameters of *Ceriodaphnia dubia* during biotesting in the water of the Rybinsk Reservoir (1Stepanova and Bikbulatova, 2018a; 2Stepanova and Bikbulatova, 2018b; 3Lazarev and Sokolova, 2018).

| Parameter | Hydrological parameters (Spearman’s rank correlation coefficient, p < 0.05) | Element concentration (Spearman’s rank correlation coefficient, p < 0.05) |
|-----------|---------------------------------------------------------------------------------|------------------------------------------------------------------------|
| Mortality (%), 48 h | colour of water (0.260), COD (−0.421)2 | P<sub>tota</sub> (0.465), ammonium nitrogen (0.525), Mn (0.261), Cd (−0.342) |
| Mortality (%), 10 days | concentration O<sub>2</sub> in water (−0.285), water transparency (0.339), ammonium nitrogen (0.525) | P<sub>min</sub> (−0.333), nitrites (−0.669), Mg (0.481), Al (0.547) |
| Average number of spawns per female | air temperature (0.397), nitrites (−0.370), concentration O<sub>2</sub> in water (−0.576), surface water temperature (−0.576) | P<sub>tota</sub> (0.758), ammonium nitrogen (0.731), nitrites (0.823), nitrites (−0.823) |
| Average number of juveniles per female | air temperature (0.410), electrical conductivity of water (0.256), total water salt concentration (−0.586) | P<sub>tota</sub> (0.410), electrical conductivity of water (−0.749), COD (−0.775), nitrites (−0.370) |

1Stepanova and Bikbulatova, 2018a; 2Stepanova and Bikbulatova, 2018b; 3Lazarev and Sokolova, 2018.
the functioning of aquatic organisms. The long-term studies of the toxicity of the water of the Rybinsk Reservoir indicate that general toxic pollution of surface waters persists, as well as the unevenly distributed aquatic toxicity. The long-term studies of the toxicity of the water of the Rybinsk Reservoir indicate that general toxic pollution of surface waters persists, as well as the unevenly distributed aquatic toxicity. However, the assessment of toxicity does not fully reflect the negative consequences of pollution of natural water bodies, since the compounds capable of causing long-term effects (e.g. mutagens and carcinogens), which are practically not monitored, are the greatest danger to the health of present and future generations.

Conclusions
According to long-term biotesting, the Volga and Sheksna reaches are among the most polluted areas of the Rybinsk Reservoir, which are exposed to household and industrial wastewater, as well as to diffuse runoff from agricultural lands and major highways.

In general, the lowest toxicity indices in the reservoir were recorded in 1994–1997.

The use of biotesting methods does not replace physical and chemical control; however, biotests significantly supplement its results by assessing the complex effect of toxicants presented in water. Correlation analysis evidences that the concentration of Al, Fe, Ni, Cu, Co, Zn, Mo, Cd, and REE in water influences the mortality rate and reproductive indices of *Ceriodaphnia*. The data obtained prove the feasibility of comprehensive monitoring of the water bodies, based on both physicochemical and biological methods for assessing water quality.

Acknowledgments
The authors are grateful to G.M. Chuiko, V.V. Yurchenko, V.V. Zakonov, A.I. Tsytov, and Yu.G. Udodenko for the invaluable help in water sampling.

The study was performed within the framework of the State Task of the Ministry of Science and Higher Education of the Russian Federation, theme no. AAAA-A18-118012690123-4 (“Physiological, biochemical and immunological reactions of aquatic organisms under the influence of biotic and abiotic environmental factors”).

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