



Local monitoring program for invasion of zebra mussel (*Dreissena polymorpha*) in the Dam lake Zhrebchevo, Bulgaria

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Abstract

Zebra mussels (*Dreissena polymorpha*) are bivalve mollusks approximately 1 to 5 cm long that live in freshwater lakes. They have invaded many Bulgarian freshwater ecosystems in recent decades. Because of their ability to settle on almost any substrate, zebra mussels cause severe damage to closed water systems, RAS and intensive fish farming systems. In order to assess the status of the mussel population in the lake in the area of the Forest group fish farm, the distribution, extent of colonization, abundance, biomass and size-frequency, structure of post-settlement stages were studied in 2012 and 2013. The purpose of this management plan is to identify the spread of zebra mussel colonies in the Zhrebchevo Dam Lake. Zebra mussels in Zhrebchevo Dam lake are category 2+, and 3+ according to the existing classification of abundance, population belongs to the class 6 and hydrochemical parameters have values close to the optimal development of invasive mussels.

Keywords: dam, invasion, zebra mussel, monitoring program

Introduction

The genus *Dreissena* includes 3 subgenera - *Dreissena*, *Pontodreissena* and *Carinodreissena* (Lovova and Starobogatov, 1982; Starobogatov and Andreeva, 1994; Marsden et al. 1996; Orlova, 2002; Therriault et al., 2004). It is acknowledged that the differentiation of *Dreissena* and *Pontodreissena* dates to 6-7 million years ago, and the separation of *Carinodreissena* from *Dreissena* – to 4.5 million years ago (Mio-Pliocene) (Banicki, 2003). The *Dreissena* subgenus – the zebra mussel *Dreissena polymorpha* Pallas, 1771 has invaded desalinated areas of Black Sea, Sea of Azov, Caspian Sea and Aral Sea, and from there it spread over the entire Europe territory and was brought to North America. The individuals inhabiting brackish waters of northern Caspian Sea are of smaller size and with more rounded umbo than freshwater forms.

In Bulgaria, the zebra mussel is prevalent from at least a century in Danube and the northern Black Sea coast (Shabla, Durankulak and Varna lakes). *D. polymorpha* was first reported in the country in the

Bulgarian section of Danube (Kreglinger, 1871). Later, Kobelt (1898), Wohlberedt (1911) and Hesse (1914) reported the species along the rivers Danube, Roussenski Lom and Devnya lakes. Drenski (1947), Petrbov (1947) and Valkanov (1957) have mentioned the Danube, the mouth of Kamchia and the Varna, Beloslav, Mandra and Burgas lakes.

The latest data of Roussev et al. (1994) and Angelov (2000) showed the presence of a typical invasion in new dams and water basin areas. During the last years, the *Dreissena* mussels have invaded for the first time the Maritsa river basin (Ovcharitsa and Zhrebchevo Dams), northwestern Bulgaria (Ogosta and Rabisha dams, Ogosta river, Vit river) and probably many other sites (Sopot Dam). The risk for the country is substantial, especially if dams used as drinking water reservoirs or strategic objects for industrial water supply and irrigation are affected, e.g. Mandra dam, Iskar dam, Yovkovtsi dam, Rozov Kladenets dam, dams along Dolna Arda etc. In general, economic losses could be unaffordable at a national scale if many strategic sites are affected in near

future. At present, there are no actions or programmes against *Dreissena* mussel invasion at a national, regional or local level, nor a program for monitoring or alert with respect to the spread of this species. It is believed that the novel invasion of mussels is resulting from the rapidly developing intensive fish farming in Bulgaria during the last decade. The contribution of people practicing recreational fishing is also possible.

Recently, *D. polymorpha* has appeared in the Ovcharitsa, Sopot, Rabisha and Zhrebchevo dams. *Dreissena polymorpha* (zebra mussel) was probably introduced in the Zhrebchevo Dam Lake in 1994, so its invasion in the dam continues more than 20 years. So far, the only counteraction measures consist in artificial stocking with the mullusk-eating species black carp (*Mylopharyngodon piceus*) – fry originating from Romania, increase in the population of plankton-eating fish – silver carp (*Hypophthalmichthys molitrix*, *Aristichthys nobilis*) and common carp (*Cyprinus carpio*). It is supposed that resulting from these measures, the mussel populations are generally suppressed but there are no systematic surveys and monitoring of the dam to outline clearly the specific trends. So far, there is no information whether other invasive species have also been introduced in the Zhrebchevo Dam Lake – for example the quagga mussel (*Dreissena bugensis*) with virtually the same features as *Dreissena polymorpha* except for its ability to inhabit deeper waters (up to 15-17 m) or the Asian clam (*Corbicula fluminea*) which has strongly invaded the Danube river during the last years.

The purpose of the study was to determine the zebra mussel invasion in the Zhrebchevo Dam lake through operational monitoring.

Table 1. Categories and numbers of zebra mussels in the Zhrebchevo Dam Lake

Group	Size	Number
I group	< 4 mm	–
II group	4 – 8 mm	3
III group	8 – 12 mm	12
IV group	12 – 18 mm	25
V group	18 – 22 mm	102
VI group	22 – 30 mm	271
VII group	> 30 mm	345

Material and Methods

The experiment was conducted at the Zhrebchevo Dam Lake in the areas of the Forest Group Co base. It began in the early May 2012 and

lasted one year. An operational monitoring of the dam was carried out with respect to the invasion of young and adult zebra mussels (*Dreissena polymorpha*): number, density, average size of individuals, development of populations with time, appearance of new and death of old individuals. The results were registered twice yearly during the reproduction period of the mussel (water temperature >10-12 °C). Quantitative data were obtained after placement of artificial substrates (plastic, cement or glass tiles, and stone ropes), of appropriate design which accounted the risk of storms, and drop in water level.

The ecological potential (species composition, age structure of the population) of piscifauna in the open part of the Zhrebchevo Dam Lake was followed out with sampling frequency one year through the accompanying industrial catch (periodic catches) of the Forest Group company. For this purpose, 4-5 representative catches or combinations of more catches within one-year period were selected for pisces fauna evaluation.

A direct correlation between some physico-chemical parameters and the development of zebra mussel populations was established: temperature of the surface water layer, °C; water electric conductivity, µS/cm; dissolved oxygen, mg/l; oxygen saturation, % and pH). The monitoring of parameters was done daily using routine standardised methods (BDS, ISO and CEN standards), most of them implemented in almost all physico-chemical labs in the country. The standard BDS 17.1.4.01 refers to measurement of sample water temperature with a mercury-in-glass thermometer, and standards BDS EN ISO 10523, BDS EN 25814 and BDS EN 27888 refer to an electrochemical method of determination of pH, dissolved oxygen and electrical conductivity of surface waters, respectively.

The key indicator for evaluation of water quality or dam's trophic status is the extent of growth of plants, plankton and benthos organisms in water. The zebra mussel (*Dreissena polymorpha*) consumes plankton algae and therefore, its invasion depends on their spread. The collection of plankton samples was performed once during the summer (August 2013). Plankton samples were stored in glass bottles in 2-4% formalin solution. Collected specimens were examined by light microscope Olympus CX31 at the Department of Biology and Aquaculture, Faculty of Agriculture, Trakia University.

Results and Discussion

The monitoring of young mussel population gives a more accurate idea about the potential of the

population. The colonisation of young mussels begins 3 to 6 weeks after the initial appearance of larvae, although it should be taken into consideration that the presence of larvae is not always followed by successful colonisation due to the high larval death rate.

Table 2. Colonisation density of zebra mussels in the Zhrebchevo Dam Lake

Class	Abundance	Numbers
Class 1	(single)	0-5 individuals
Class 2	(present)	6-20 individuals
Class 3	(weak to moderate)	21-100 individuals
Class 4	(moderate)	100–300 individuals
Class 5	(moderate to abundant)	300-500 individuals
Class 6	(abundant)	500-1000 individuals
Class 7	(very abundant)	> 1000 individuals

Quantitation of the dynamics of colonisation with adult *Dreissena polymorpha* mussels was made possible with placement of artificial substrates (cement tiles of a known area) or direct observation on solid surfaces in the Zhrebchevo Dam Lake (quays, fishnets etc.). A main requirement to substrates was to be at 2-3 m under the water level throughout the year and when possible, to be not exposed to a substantial human impact. The population density was determined by a standard rectangular frame, 15 cm x 15 cm. Within the frame, all mussels from the different age groups were counted and the abundance of each group was determined. Age groups were determined on representative samples according to shell length in mm by means of Vernier caliper or a ruler. Individual sizes of zebra mussels as measured during the study varied from <1 mm to 34 mm. Only mussels determined visually to be larger than 1 mm were measured. After being sorted, mussels were counted and measured. The results are presented in Table 1.

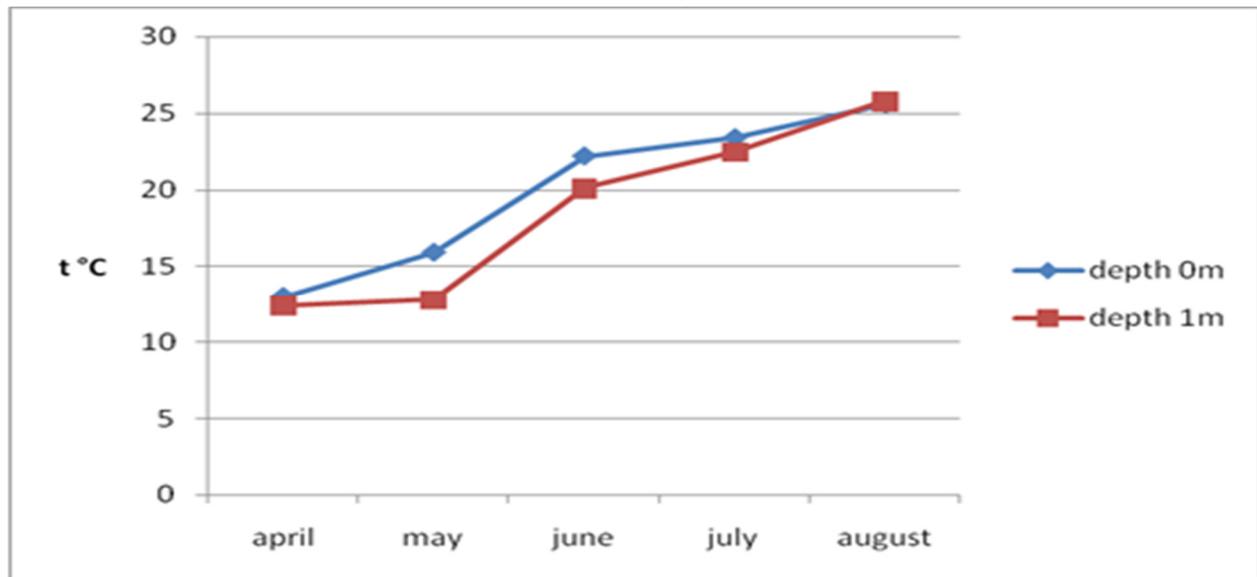


Figure 1. Water temperature (°C) in the Zhrebchevo Dam Lake at 0 m and 1 m depth

The data reflected the presence of mussels from at least three different age groups (1+, 2+ и 3+). Due to the very rapid development cycle, the division of zebra mussels into age groups is very difficult, but it could be conditionally presented as follows. The category 4–8 mm included mussels from the summer and autumn of 2013, classified as 1+. Zebra mussels larger than 10 mm were placed in group 2+ i.e individuals from the summer of 2012. After detailed examinations, the major part of individuals was of rather larger size: >22 mm (category 3+). The examination under strong magnifying glass revealed a

very worn shell. On the other hand, some mussels did not exhibit defense reaction on challenge (light, mechanical etc.). This allowed concluding that most of them should be dead. In conclusion, most of samples included individuals from 2+ and 3+ age groups. The lower number of individuals from age group 1+ was probably due to the lower water level and higher ambient air temperature.

The results permitted to determine the density as well. According to the current abundance classification (Table 2), the zebra mussel population in the Zhrebchevo Dam Lake was classified as belonging to class 6 with 746 mussels/m².

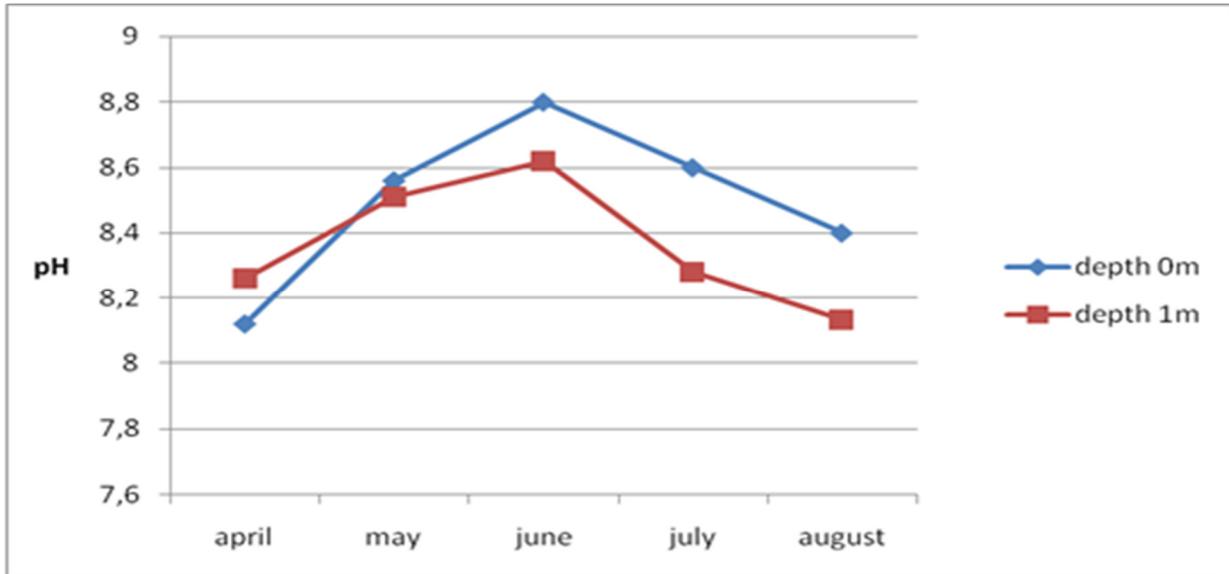


Figure 2. Water pH in the Zhebchevo Dam Lake at 0 m and 1 m depth

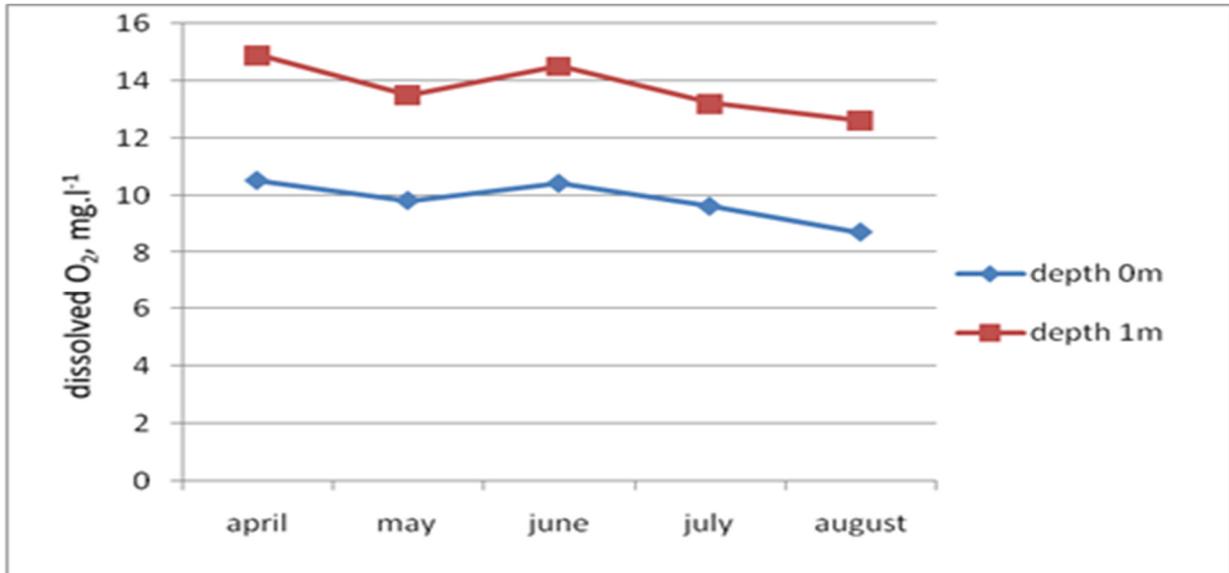


Figure 3. Dissolved oxygen (mg.l⁻¹) in the Zhebchevo Dam Lake at 0 m and 1 m depth

Table 3. Electrical conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$) of the water in the Zhebchevo Dam Lake at 0 m and 1 m depth

Parameter	Depth	24 April	21 May	27 June	21 August
Electrical conductivity, $\mu\text{S}\cdot\text{cm}^{-1}$	0 m	240	322	282	324
	1 m	310	350	323	366

Water chemical parameters during the experimental period were directly related to the development of zebra mussel population in Zhebchevo Dam Lake. According to the European Branch of the US Army Engineer Research and Development Center, the optimum temperature for appearance and development of zebra mussels

ranges between 17–25 °C, established in the current study (Figure 1).

As the water temperature decreased by the end of the summer, the filtration rate of mussels decreased. On the other hand, the drastic changes in the water level of the dam further reduced the populations of zebra mussels. The benthos lifestyle

was not able to compensate the sharp drop of water level and the population number went down.

The analysis of results (Figure 2) showed that water pH ranged between 8.12–8.80. It could be therefore concluded that it was within the range determined for invasion with this mussel species (7.45 – 8.5) as specified by the European Branch of the US Army Engineer Research and Development Center.

The water oxygen content needed for the prosperous development of zebra mussels is 8-10 mg.l⁻¹. The data from Figure 3 indicate that during most of the time, oxygen content varied from 8.77 to 14.9 mg.l⁻¹, and saturation – over 100%.

Water electrical conductivity corresponding to high extent of zebra mussel invasion is over 200 µS.cm⁻¹ (Table 3).

Table 4. Catch on fish with nets (16/65 mm) in the Zhrebchevo Dam Lake

Species	Total number	Total fish caught (kg)	Numbers in weight groups <100 g	Numbers in weight groups 100-200 g	Numbers in weight groups 200-400 g	Numbers in weight groups 400-1000 g
Roach (<i>R.rutilus</i>)	138	10.246	114	18	6	0
Eurasian perch (<i>P. fluviatilis</i>)	28	3.992	19	0	0	0
Malamida (<i>V. melanops</i>)	47	13.010	0	9	27	11
Zander (<i>S. lucioperca</i>)	7	1.407	3	1	3	0
Common bream (<i>A. brama</i>)	9	1.512	2	5	2	0
Bleak (<i>A. alburnus</i>)	48	2.016	48	0	0	0
Romanian barbel (<i>Barbus petenyi</i>)	2	0.216	1	1	0	0

With regard to the relative abundance of the phytoplankton in the present study, 89 species were established. Domination was observed for *Aphanizomenon flos-aquae* and *Microcystis aeruginosa*, which were mainly consumed by the studied mussel species. Other present species from the Cyanophyta division were *Anabaena* sp., *Nostoc paludosum*, *Oscillatoria agardhii*, *Pleurotaccium trabecala*, *Gomphosphaeria lacustris*, *Chroococcus* sp.

The species characteristics of zooplankton in the Zhrebchevo Dam Lake include 43 zooplankton taxa. The rotifers were predominating in the studied dam. The most prevalent species were *Asplanchna priodonta*, *Keratella quadrata*; *Brachionus* spp. and *Polyarthra* spp. From *Cladocera*, *Daphnia cucullata*, *Diaphanosoma lacustris* and *Bosmina* spp. are specific for the Zhrebchevo Dam Lake. The presence of high density of invasive mussel *D. polymorpha* larval forms in the dam was alarming. The presence of veliger larvae of zebra mussels in the zooplankton of the Zhrebchevo Dam Lake at winter temperatures under 4 °C could be interpreted as adaptation of this invasive species to the changing climate of Bulgaria.

The monitoring of the piscifauna contributes to evaluating the possibility of used fish societies as indicators of environmental changes and anthropogenic pressure.

The dominating species in the Zhrebchevo Dam Lake was the roach (*Rutilus rutilus*), but the subdominant was the malamida (*Vimba melanops*) (Table 4). The latter was represented by rather large subjects. Data about the abundance index showed relatively good reserves of dominating fish species in the main lake of the Zhrebchevo Dam, which are main food source of fish-eating birds. Thus, the calculated relative abundance indices of four dominating species as shown from the last catches in the coastal area with width of 100 m and depth up to 5 m demonstrated that roaches were the most numerous with nearly 4 fish/m within the period of stay of the net under the water, followed by malamidas with slightly more than 1 fish/m, bleaks – again nearly 1 fish/m, Eurasian perches – with average 0.5 fish/m, i.e. a good reserve of four ecologically important fish species serving as food both to waterfowl and predatory fish important for sport fishing and indirectly, for the species diversity of dam's pisces fauna.

Conclusions

The major part of the zebra mussel population at the Forest Group Ltd base in the Zhrebchevo Dam Lake were from age groups 2+ and 3+. According to the current abundance classification, the zebra mussel population at the Forest Group Ltd base in the Zhrebchevo Dam Lake was evaluated as belonging to class 6. The hydrological and water chemical parameters were close to the optimal values for development of invasive mussels. The highest prevalence was registered for the species *Aphanizomenon flos-aquae* and *Microcystis aeruginosa*, which were the main food for zebra mussels.

References

- Angelov, A., 2000. Catalogues faunae Bulgaria. 4. Mollusca: *Gastropoda et Bivalvia*, p.57.
- Banicki, J., 2003. Sea Grant research uses mussel DNA to differentiate species and Trace their origin. *Twine Line* 25 (5): 1-3.
- Drenski, P., 1947. Composition and distribution of freshwater Mollusca in Bulgaria. *Annual SU* 43 (3): 33-54.
- Hesse, P., 1914. Zur Kenntnis der Molluskenfauna von Ostrumelien III. *Nachrichtsblatt der Deutschen malakozoologischen Gesellschaft*, 46(2): 49-58.
- Kobelt, W., 1898. Studien zur Zoogeographie 1. Die Fauna der palaearktischen Region. *Wiesbaden*, 344.
- Kreglinger, C., 1871. Syst. Verzeichnis der in Deutschland leb. Binnemollusken Wiesbaden.
- Lovova, A., Starobogatov, J., 1982. A new species *Dreissena* (*Bivalvia*, *Dreissenida*) of Lake *Ohrid* *Zoolog. Journal* 61 (11): 1749-1752.
- Marsden, J., Spidle, A., Nay, B., 1996. Review of genetic studies of *Dreissena* spp. *American Zoologist* 36: 259-270.
- Orlova, M., 2002. *Dreissena polymorpha*: Evolutionary origin and biological peculiarities as prerequisites of invasion success. - In: Leppakoski, E., S. Gollasch, S. Olenin (eds.). *Invasive aquatic species of Europe. Distribution, impacts and management*. Dordrecht, *Kluwer Academic Publishers*, 127-134.
- Petrbok, J., 1947. The freshwater mollusca on Varneskoto and Gebedzhenskoto Lake. *Works in marine biology Varna* 13: 71-75.
- Roussev, B., Yaneva, I., Decheva, R., 1994. Invertebrates animals. *Limnology at Bulgarian Danube tributaries*, 130-186.
- Starobogatov, J., Andreeva, S., 1994. The area and history. *Dreissena polymorpha* (Pall.) (*Bivalvia*, *Dreissenidae*). In: species fauna of Russia and neighboring countries. *Nauka*, Moscow, 47-55.
- Therriault, T., Docker M., Orlova, M., 2004. Molecular resolution of the family *Dreissenidae* (Mollusca: *Bivalvia*) with emphasis on Ponto-Caspian species, including first report of *Mytilopsis leucophaeata* in the Black Sea basin. *Molecular phylogenetics and evolution* 30: 479-489.
- Valkanov, A., 1957. Catalogue of our Black Sea fauna. *Works in marine biology Varna* 19: 1-62.
- Wohlberedt, O., 1911. Zur Molluskenfauna von Bulgarien. *Abhandl. Naturforsch. Ges. Gorlitz*, 27: 167-234.