Research Article

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Ecological Status Assessment of a Hypersaline Lake: a Case Study of Atanasovsko Lake, Bulgaria

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Abstract: Atanasovsko Lake (7.200 ha, Southeast Bulgaria) incorporates diverse habitats and lies along the Europe's second largest migration route, *Via Pontica*. The dynamics of environmental conditions, factors controlling water quality and ecological status (water chemical parameters and biological quality elements) were studied for three years. The key factor for the lake is the salt production mechanism (salterns were created in 1906 and divided into two complexes of water bodies known as North Salterns and South Salterns, each being relatively autonomous as a salt production entity) and sea-freshwater exchange. The phytoplankton-based assessment in the North Salterns in 2015 showed progressive improvement. The same positive development was recorded in the South Salterns and Azmaka River. During this year, *Knipowitschia caucasica* was represented with small population only in the North Saltern as well as limited assemblages of *Ruppia maritima* were recorded and *Artemia salina* was registered. Thus, the freshwater supply in the northern part of the lake was recognized as a key mechanism for diminishing blooms and establishing of a more balanced environment. Nevertheless, the structure of benthic communities at North and South Salterns were disrupted, probably as a result of unstable bottom substrates, anthropogenic activities and predatory press by birds and fish. Baseline information contained in this study will be incorporated into future data analyses of the lake.

Key words: hypersaline lake, environmental conditions, biological quality elements

Introduction

Atanasovsko Lake is shallow (<1 m), alkaline and hypersaline. It is divided into numerous small ponds by a network of dikes and banks since the beginning of salt-production in 1906. The northern lake part has been a maintained reserve since 1999, with a management plan established in 2003, and the southern part is a protected area. The lake is located at the avian migratory route *Via Pontica* and is Wetland of International Importance, CORINE site and a part of the European Natura 2000 network. The whole lake is declared as a sanitation zone. As a result of this legal protection as well as the efforts of many scientists and nature conservation work, Atanasovsko Lake lagoon continues to be Bulgaria's richest bird site. Despite lake importance, only few studies focused on the aquatic ecosystem were published. The algal flora was reported to be comprised of 120 taxa (Management Plan of Atanasovsko Lake 2003). Aquatic macrophytes also have not been a subject of detailed research. *Zostera marina* L. was registered with limited distribution in the lake coastal zone (Kochev & Yordanov 1981). Vascular plants were inventoried within the management plan development (Management Plan of Atanasovsko Lake 2003).

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Three hundred and eleven species were registered in the period 2002-2005, among them 15 hydrophytes (Grozeva 2005). Thirty five benthic macroinvertebrate species were reported (Management Plan of Atanasovsko Lake 2003). Recently taxonomic diversity and some zoogeographical and ecological features of the invertebrates were analyzed (Hubenov *et al.* 2015). Previous studies showed that the lake was characterized by a remarkable low macrozoobenthos diversity and abundance, even lower than inherently usual for such environment (Casagranda *et al.* 2011). According to the Management plan of Atanasovsko Lake (2003), fish fauna was also not studied systematically probably due to the salt production. In total twenty-two fish species were reported.

The European Water Framework Directive (WFD) requires the determination of ecological status in European fresh and saline waters (European Commission 2000). Thus, we focused our study on the four key biological quality elements (BQEs) and their relationship with nutrient loads, extreme weather events and effects of salt production.

Materials and Methods

Study Area

Atanasovsko Lake (16.9 km^2) is situated in Southeast Bulgaria, on the Black Sea coast. According to the national typology, the lake belongs to the type L10: Black Sea hypersaline lakes with salinity >40‰, shallow (<1 m); polymictic, with eutrophic to polytrophic conditions. The closer common intercalibration type in Europe is NEA11 (in North Atlantic GIG): oligo- to euhaline (0-35‰), shallow (<30 m), partly or totally stratified water bodies (Water Framework Directive intercalibration technical report, Part 3: Coastal and Transitional waters, 2009).

The North site is characterized by an open, well-shaped littoral zone with emergent bank vegetation creating prerequisites for shelters and more stable conditions. The South site is divided into a number of pools by artificial barriers. Samples for water chemistry were collected from 3 sites (Fig. 1), as well as BQEs: North Salterns (N 42°34'16.4"; E 27°28'04.4"); South Salterns (N 42°31'42.4"; E 27°28'24.1") and Azmaka (N 42°34'44.9"; E 27°29'38.0").Fish fauna was studied also in additional sites due to the methodological requirement for a larger sampling area.

Sampling Techniques: Water Quality Parameters and Biology

Water chemical parameters were monitored 5 times per year. We measured *in situ* pH, conductivity, temperature and dissolved oxygen with Multi 3410 SET B TetraCON 952-3 (WTW). Phosphorus and nitrogen compounds were analyzed by pHotoFlex STD (WTW).

Phytoplankton was sampled twice in the period June-September. Ecological status assessment followed Belkinova *et al.* (2013). Aquatic macrophyte survey was carried out once during the main vegetation period within belt transects (Gecheva *et al*. 2013). Macroinvertebrates were collected ones a year during the period of low waters (end of July). An adapted version of the standardized (Regulation № H-4 2013) multi-habitat sampling method (Cheshmedjiev *et al.* 2011) was applied in accordance to the standard BDS EN ISO 10870:2012. Due to the varying salinity of the studied points a set of metrics that characterize both freshwater and coastal waters were used: Total number of taxa; adapted BioticIndex– BI (Clabby & Bowman 1979, Clabby 1989); Shannon-Weaver Diversity index calculated by the system for environmental assessment of coastal waters; AMBI (a biotic index based on species sensitivity/tolerance, with diversity and richness according the requirements of the WFD) and M-AMBI (a multimetric index for assessing the ecological status of marine and transitional waters, based on benthic macroinvertebrates, integrating AMBI), calculated with the software ambi_v 5.0 - AMBI: AZTI MARINE BIOTIC INDEX (AZTI-Tecnalia, www.azti.es).The metrics Total number of taxa and BI had a leading role in determining the ecological status of the third site (Fig. 1). The last two indices are published into national legislation (Regulation № H-4, 2013) and have more weight in defining the ecological status of North and South salterns.

Current European standards for fish monitoring are not applicable in Atanasovsko Lake, thus a new survey method was applied. The sampling technique was based on small bottom lying nets with exposition time up to 10 minutes and positioning over 10-15 m. The results were estimated as average catches per a unit area. A specific scale based on *Knipowitschia caucasica* (Berg, 1916) number for assessment of Atanasovsko Lake was developed within the current survey.

Results and Discussion

Environmental Conditions

Hydromorphological parameters of North and South salterns vary greatly. Alkaline pH was constant during the three years, more pronounced at the North Saltern (Table 1). Dissolved oxygen levels were reduced during each autumn. In South Saltern,

Table 1. Water chemical parameters: minimum, maximum and average values in 2013-2015

North Saltern	**2013**			**2014**			**2015**		
parameter	min	max	average	min	max	average	min	max	average
T °C	17.2	25.1	21.1	7.8	29.3	19.18	10.0	29.8	19.6
pH	8.36	8.7	8.6	8.4	9.4	8.8	8.4	8.9	8.6
DO (mg L^{-1})	7.5	15.4	11.1	10.0	14.9	11.9	8.1	14.8	10.5
Oxygen saturation (%)	89.6	158.9	124.8	116.1	147.2	127.6	97.3	129.0	109.8
C (mS cm^{-1})	45.9	78.3	65.3	60.9	71.7	66.1	11.4	60.3	31.6
Salinity ‰	30.1	54.3	43.6	39.9	49.3	44.5	6.3	40.2	19.8
N–NO2 (mg L^{-1})	0.11	0.21	0.15	0.11	0.61	0.26	0.08	0.17	0.12
N–NO3 (mg L^{-1})	□ 0.2			<0.1	0.78	n.a.	<0.1	0.21	n.a.
N–NH4 (mg L^{-1})	0.13	□ 1.5	n.a.	0.14	>1.5	n.a.	0.05	0.74	0.33
P-PO4 (mg L^{-1})	0.60	4.5	1.97	0.74	1.8	1.3	0.15	0.85	0.43
South Saltern	**2013**			**2014**			**2015**		
parameter	**min**	**max**	**average**	**min**	**max**	**average**	**min**	**max**	**average**
T °C	19.7	28	22.9	9.1	31	20.4	10.1	29.8	18.9
pH	8.3	8.8	8.5	8.5	8.7	8.6	8.3	8.7	8.5
DO (mg L^{-1})	8.4	15.7	11.5	4.3	13.1	8.9	5.9	9.9	8.1
Oxygen saturation (%)	112.2	172.5	133.1	50.9	176.5	103.8	59	100.3	86.7
C (mS cm^{-1})	37.9	40.4	38.9	29.1	36.3	32.1	9.9	52	26
Salinity ‰	23.9	30.1	26.3	17.5	23	19.9	5.7	33.7	16.6
N–NO2 (mg L^{-1})	0.041	0.057	0.048	0.03	0.07	0.048	0.043	0.128	0.064
N–NO3 (mg L^{-1})	<0.1			<0.1			<0.1	10.9	n.a.
N–NH4 (mg L^{-1})	□0.02	0.07	n.a.	□ 0.2	0.11	n.a.	0.06	0.13	0.088
P-PO4 (mg L^{-1})	0.12	1.54	0.957	0.11	1.08	0.552	<0.05	0.09	n.a.
Azmaka	**2013**			**2014**			**2015**		
parameter	**min**	**max**	**aver-age**	**min**	**max**	**average**	**min**	**max**	**average**
T °C	16.2	25.4	20.8	12.2	23.4	20.4	9.7	28.7	16.9
pH	7.9	8.6	8.2	7.8	8.2	8.0	7.5	8.0	7.8
DO (mg L^{-1})	6.4	14.9	10.6	5.7	9.8	7.1	5.0	7.6	6.5
Oxygen saturation (%)	76.1	142.1	109.1	64.0	92.1	74.8	46.3	80.9	65.9
C (mS cm^{-1})	1.2	1.8	1.5	1.3	2.4	1.6	1.0	1.4	1.2
Salinity ‰	0.60	0.90	0.75	0.60	1.2	0.78	0.40	0.60	0.50
N–NO2 (mg L^{-1})	0.04	0.06	0.05	0.03	0.04	0.04	0.04	0.05	0.05
N–NO3 (mg L^{-1})	<0.1			<0.1			<0.1		
N–NH4 (mg L^{-1})	0.02	0.08	0.05	<0.02	0.02	n.a.	□ 0.02	0.32	n.a.
P-PO4 (mg L^{-1})	0.92	2.2	1.6	1.3	5.4	3.3	0.13	0.79	0.54

the decreases were close to the minimum level of 5 mg x L^{-1} recommended for saltwater fish in warm waters. During the first two years, North Saltern was traditionally with highest salinity, while in 2015 salinity in the whole lagoon was up to 30‰ by the end of July. Phosphorus and nitrogen compounds, which were traditionally higher at the northern part of the lake, were reduced in the last year up to 3 times as a result of freshwater supplied by heavy rainfalls. It could be suggested that registered nutrient seasonal fluctuations were connected with water-sediment interaction in the end of summer, stimulated by higher temperatures and low water level. Due to the possibility ammonium nitrogen and other N- and P-compounds could be released from the lake bottom, sediment analysis was recommended.

Phytoplankton

Low number of species (n=11-14) was character for the North Saltern with representatives of 5 taxonomic groups: cyanobacteria, green algae, diatoms, dinoflagellates and cryptomonads. Picocyanobacteria (d<1.5 µm) and picoeukaryotic algal species (size: 2-2.5 µm): *Oocystis parva* W. & G. S. West, *Cocconeis placentula* Ehr. and *Nitzschia closterium* (Ehr.) W. Sm. were constant. Picoplankton species

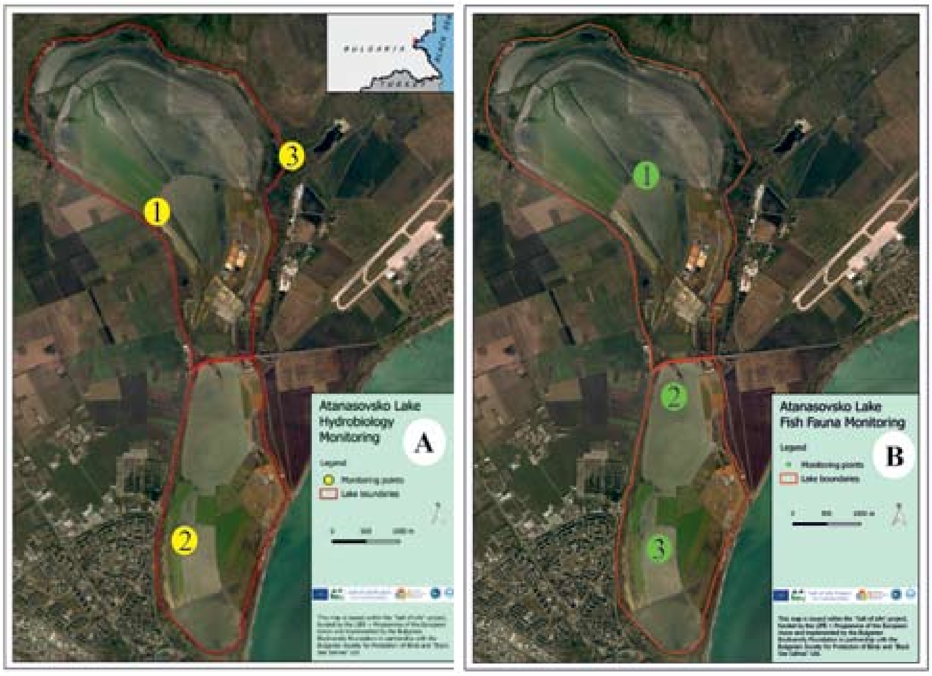

Fig. 1. Sites' location. A.1: North Saltern, 2: South Saltern, 3: Azmaka. B: Fish monitoring sites

dominated, within bloom status in the first two summer seasons. The photoautotrophic picoplankton was assumed to have selective advantage in shallow saline lakes, which are under light limitation (Somogyl *et al.* 2011, Borics *et al.* 2012). Euryhaline species were recorded in North Saltern together with declined salinity in 2015 (*Merismopedia punctata* Meyen, *Phormidium chlorinum* (Kütz. ex Gom.) Anagn., *Pseudanabaena minima* (G.S. An) Anagn.). Quantitatively, phytoplankton development (biovolume 0.7 mm^3 L^{-1}) was 15 times decreased in comparison with 2014 (10.7 mm^3 L^{-1}), respectively above 25 times towards 2013 (17.8 mm^3 L^{-1}).

Similar small number of species was registered at the South Saltern (n=8–16): cyanobacteria, green algae, diatoms, dinoflagellates and euglenoids. Common species for the three years were *Amphidinium* cf. *ovum*, *N. closterium* and *Eutreptia globulifera* van Goor. These species are characteristic for the Black Sea and brackish waters. As a result of the similar salinity levels in 2015, the North and South Salterns had several common mezohaline species: *P. chlorinum*, *P. minima* and picoplankton species. Dinoflagellates were dominants in the total biovolume (above than 70%) during the three years (*Amphidinium*, *Gymnodinium*). Quantitatively, development in 2015 was seriously decreased regarding previous years. The average total biovolume was 1.3 mm^3x L^{-1}, which is about 19 times lower than in 2014 (19.3 mm^3x L^{-1}), 4 times respectively than in 2013 (5 mm^3x L^{-1}).

The highest species number was recorded in Azmaka site (varying from 23 to 57). Diatoms, cyanobacteria, green, euglenoids and chrysophyte algae dominated. The phytoplankton had underlined freshwater character. Typical limnoplankton species (*Ankyra lanceolata* (Korsh.) Fott, *Euglena limnophila* Lemm.) as well as potamoplankton species (*Planothidium frequentissimum* (Lange-Bert.) Lange-Bert., *Navicula gregaria* Donk., *Nitzschia capitellata* Hust.) were registered. The average biovolume for the last year (5.1 mm^3x L^{-1}) was about two times lower than its value during previous 2014 (11.7 mm^3x L^{-1}) and comparable to the biovolume measured at 2013 (4.14 mm^3x L^{-1}) and corresponded to the nutrients measured (Table 1).

All three sites showed improvement of the ecological potential at the end of the study. The overall assessment in the North Saltern in 2015 was maximum potential, showing progressive improvement through the three years. Seasonal mean total biovolume of 0.7 mm^3x L^{-1}reflected lower quantitatively development. Moreover, biovolume was more equally distributed between taxonomic groups in comparison with the previous two years, and without blooming species. The main reason was the diminished salinity in the spring of 2015, which, together with lower nutrient concentrations, was recognized as a barrier towards intensive development of halophile picoplankton species. The same positive development was recorded in the South Saltern and Azmaka (both sites assessed at the boundary between maxi-

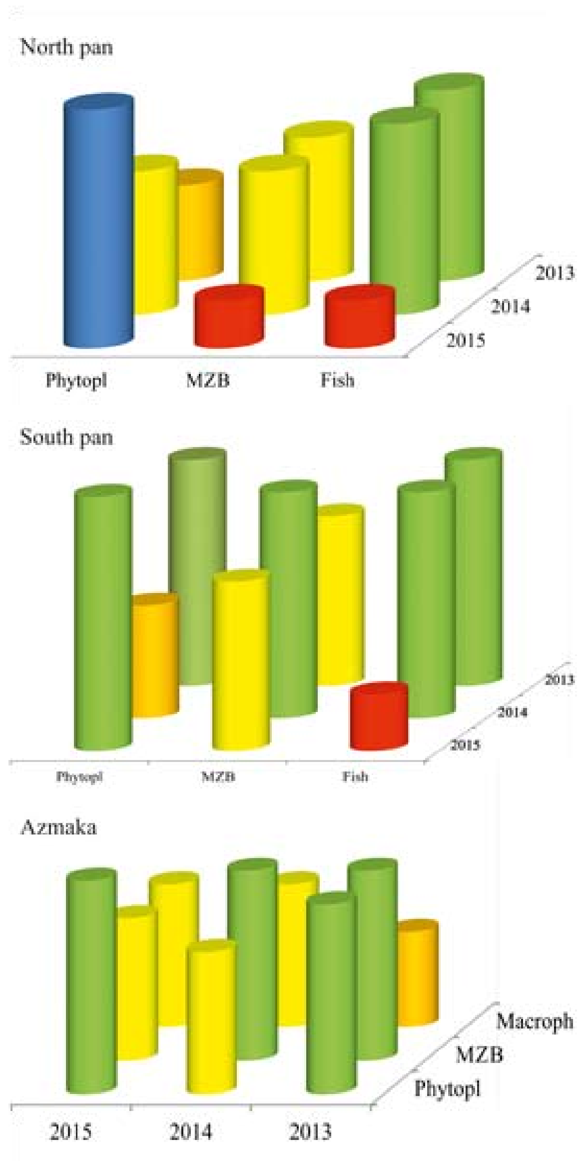

Fig. 2. Assessed ecological status/potential in 2013-2015

mum and good potential in the last year). The results showed that freshwater supply is probably the key mechanism for diminishing the blooms and establishing a more balanced distribution between the taxonomic groups.

Macrophytes

No aquatic macrophytes were observed both in North and South salterns during the study. High summer temperatures, dissolved oxygen limitation and extreme turbidity were recognized as main barriers for macrophyte development. A transect study in the reserve lake area recorded a community dominated by *Phragmites australis* (Cav.) Trin. ex Steud. (80% cover) in conditions similar to marsh habitat (up 10 cm water level) and silt bottom. *Sparganium erectum* L., *Typha angustifolia* L., *Lemna minor* L. and *Cladophora* spp. were also abundant.

Ruppia maritima L. was registered in 2015 with assemblages restricted to two small ponds at the Northern lake part, with high transparency. *Ruppia* development with alternative metrics as density and cover of the populations should be further monitored. Macrophyte-based assessment of Azmaka site, where 5 aquatic species were registered, resulted in poor and moderate status.

Macroinvertebrates

A total of 29 taxa were identified during 2015, together with a trend of decreasing taxonomic richness. The greatest reduction (almost 60%) was registered at the South Saltern and in particular at Azmaka site (50%). If registered during the study trend of decline in biodiversity persists in the coming years, it could be interpreted as a dramatic loss of habitat preferred by benthic macroinvertebrates.

Despite hydromorphological differences, North and South salterns had relatively similar bottom communities. Over the past two years in both studied sites, chironomid larvae, characterized by permanent presence in 2013, were not found. Even typical for Atanasovsko Lake brackish species *Chironomus salinarius* (Kiefer, 1915), tolerant to extreme changes of salinity, was not registered. For the first time in 2015, with single specimens halobiontic brine shrimp *Artemia salina* (L., 1758) was recorded. The ostracods *Cyprideis torosa* (Jones, 1850) was registered at the northern lake part in high density. As Hubenov (2015) noticed, the species is typical of hypersaline lagoon lakes. In contrast, another mass marine species, *Abra segmentum* (Récluz, 1843), was established only with single specimens. Of interest are the changes registered in the composition of oligochaete fauna, in particular euribiontic *Tubifex tubifex* (Muller, 1774) and Ponto-Caspian relict *Potamothrix hammoniensis* (Michaelsen, 1901). Although with low density, the two species were recorded in 2013 while, in 2015, only the second worm was found. Such a change was described in Vaia Lake (Georgieva *et al.* 2014).

The assessment based on BI for the first year varies between good for Azmaka site and moderate for North and South salterns. Nevertheless, the metric Total number of taxa resulted between high and moderate status, while the Shannon-Weaver index characterized northern and southern part as being in bad status. The above indicated that the cenotic structure of North and South salterns was disrupted probably as a result of unstable bottom substrates and anthropogenic activities, including registered values of nutrients and ongoing mainly diffuse inflow of pollutants. Negative impact also has the predatory (birds and fish) press. Only Azmaka site was assessed in good status both by Shannon-Weaver index and BI. In 2013, the ecological status based on M-AMBI overlapped with that based on the values of BI.

The final evaluation during 2013 was determined as moderate in North and South salterns, and good for Azmaka (Figure 2). Data from 2014 showed that the ecological status of North Saltern was moderate, while of South Saltern and Azmaka was good. One degree decrease in the status was observed at all sites during the last year (2015). For comparison, the study by Subeva *et al.* (2015) showed that the ecological status according BI was mostly moderate in freshwater and predominately good in the brackish areas.

Fish

Three species with importance for the conservation were recorded, among them the most common was *Knipowitschia caucasica*. Previously reported *Syngnathus abaster* was not registered in the current study. Due to the inapplicability of the existed fish-based indices, a newly developed method for assessment was applied. The classification system was based on the number of *K. caucasica* and maximum potential was assigned if more than 9 specimens per m^2 were recorded, while bad potential was accepted when the number was less than 0.5 specimens per m^2. According to the above, the potential was assessed as good for the first two years, while in 2015 it declined to bad.

The South Saltern population of *Knipowitschia caucasica* disappeared in 2015 and the population in the north part of the lake was diminished more than 3 times. Factors that provoked this extinction are still unknown. It could be suggested that the decline in salinity started in the end of 2014 till the mid of 2015 may resulted into gobies extinction and reproduction prevention. Nevertheless, there is no clear answer why part of the population survived in a lake basin, where the salinity was the same as in the other basins. The results showed that optimum salinity for the gobies is about 20-27‰; they experienced a reduction (<0.5 per m^2) at 30-40‰ and became extinct at 50‰. The lower limit probably is 5‰, but further studies are needed. Positive relationship between populations of *Knipowitschia caucasica* and *Gammarus* was observed.

During our study, the invasive species *Gambusia affinis* thrived at over 36‰.

Integrated assessment

The applied metrics and methods for assessment are still under development for the national L10 lake type. Thus the integrated assessment represented the average value of scoring assessment both of applied metrics (phytoplankton and benthic invertebrates) and of the four key BQEs. Relative improvement throughout the three years could be recognized for the site located at the North Saltern (Fig. 2), with moderate to good potential in the last year. Both South Saltern and Azmaka were in moderate potential.

The recorded changes in environmental conditions, after the flood events in 2015, were more pronounced at the North Saltern and were reflected by its improved ecological potential. In the period March-June 2015, the salinity was up to 30‰. During the summer and autumn, both salterns had similar salinity. The obtained results showed that optimum salinity for gobies is between 20-27‰. The same dilution was recorded for nutrients (reduction >5 times for ammonium nitrogen, 3 times for phosphates).

Conclusion

The temperature and pH were relatively constant over the three years. There was a clear salinity gradient along studied sites in the period 2013-2014. Hyperhaline environment in the North Saltern turned into euhaline in the South Saltern, while Azmaka can be classified as an oligohaline water body. The above gradient had seasonal character over the whole lake due to extreme weather events in 2015. The assessment based on the key BQE phytoplankton resulted in maximum potential in 2015. In the same year, *Knipowitschia caucasica* was presented in small quantity only in the North Saltern as well as limited assemblages of *Ruppia maritima* were recorded and *Artemia salina* was registered.

Nevertheless, benthic macroinvertebrate-based assessment indicated deterioration at all studied sites during the three years. Macrozoobenthic community structure showed a reduction in species richness and abundance at all three sites, despite diverse microenvironments with complex combination of physicochemical and hydromorphological parameters. The salinity fluctuations as well as hydrological conditions had no prior influence on macroinvertebrates. Adverse impacts on bottom communities were due to the unstable bottom substrates, high salinity and ongoing direct and diffuse inflow of pollutants. That probably causes the load of sediments (basis habitat of benthic communities) and was reflected by the unfavourable assessment of ecological status based on macrozoobenthos.

Atanasovsko Lake is a very specific ecosystem. Temperature, salinity and nutrients fluctuate even on a daily basis and reach more frequently extreme values than they do at other surface waters. From a biotic point of view, the lake is highly productive and of particular significance for birds. Being both under protection of national and European legislation, and under pronounced anthropogenic pressure, it is obvious that further hydrobiological monitoring is crucial for long-term preservation of the lake. Maintaining the stability and functioning of the aquatic ecosystem as well as optimal environmental conditions for the development of aquatic flora and fauna require adequate measures to halt direct discharges and sources of diffuse loads.

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