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Using phytoplankton as a tool for evaluating changes in the ecological status of two Bulgarian reservoirs (2020–2021)

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Abstract

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The aim of the present research is an attempt to assess the environmental status of two Bulgarian reservoirs by using the assemblage Q index based on the analysis of the data collected from the phytoplankton during 2020-2021.A total of 86 algae belonging to 20 functional groups were identified in Aheloy Reservoir and 61 from 22 groups in Ovcharitsa Reservoir. We found a change in the dominant phytoplankton functional groups compared to previous data published for the same reservoirs. In the Aheloy Reservoir these changes can be ordered as follows from the codons: S1 – H1 – P, trough L_0 – W2 – J to D – E – J, and in Ovcharitsa Reservoir from P trough X2 – L_0 – M to F – MP – P.The implementation of the assemblage Q index also provides an explanation of these changes by reporting an improvement in the ecological status of the studied reservoirs compared to an earlier publication.The ecological status of Aheloy Reservoir has changed from „poor"to „tolerable", and of the Ovcharitsa Reservoir from „moderate" to „good"according to the results obtained from the calculation of the Q index. We recognize as controversial the question of the application of the values for the *F* factor and the possibility of mistakes in establishing ecological status, related to the lack of reliable statistical database concerning the individual characteristics of highly modified water bodies, such as reservoirs. The ecological status assessments obtained using the Q index of these two reservoirs in the period 2020-2021 are relatively close compared to the evaluation based on our unpublished average values for phytoplankton biomass.

Keywords: algae; assemblage index; dominant functional group; *F* factor; Q index

Introduction

According to European regulations (WFD:2000/60/EC), phytoplankton is one of the biological elements required to evaluate the ecological status of surface waters.Following the same regulations, the assessment of phytoplankton includes: species composition,species abundance, as well as frequency and intensity of algal blooms.The phytoplankton plays a major role in the functioning of aquatic ecosystems. In most freshwater water bodies, algae are the main primary producers and significantly influence other communities in the foodchain. The use of phytoplankton for assessing water quality and especially eutrophication are nowadays very important (Pasztaleniec & Poniewozik 2010). The coexistence of algal species with similar requirements and ecological needs of the environment they inhabit in freshwaters is called a functional group (Reynolds et al., 2002). Today, the functional groups approach based on physiological, morphological and ecological characteristics of species is proving to be more effective compared to the analysis of seasonal changes in phytoplankton biomass (Becker et al., 2010).

In Bulgaria the application of the functional groups approach and the perspectives for the implementation of this concept have been published by Stoyneva et al. (2013), Stoyneva (2014), Belkinova et al. (2014),Dochin &Iliev (2019), Dochin (2019), Dochin et al. (2020) etc. Padisak et al. (2006) developed assemblage index (Q index) using the functional group approach. The index expresses the weight of each functional group to the total biomass with a factor number (*F*) for each group related to the type of water body. According to the creators of the index, it is a reliable tool for assessing the ecological status of water bodies in different climatic regions (Padisak et al., 2006).However, there are not many studies related to the assessment of the ecological status based on the research of phytoplankton in inland waters of the country (Cheshmedjiev et al., 2010;Gecheva et al., 2013; Stoyneva et al., 2013; Belkinova et al., 2014; Stoyneva et al., 2015).So far, the application of the assemblage Q index to assess the ecological status of reservoirs in Bulgaria has only been published in a few papers (Belkinova et al., 2014; Stoyneva 2014). The presented article is an attempt to evaluate the ecological status of Aheloy and Ovcharitsa reservoirs by using the assemblage Q index based on the data obtained from the phytoplankton survey during 2020 and 2021.

Material and Methods

Study area and sampling

The study was conducted in two lowland reservoirs in the Southeastern part of the country: Aheloy IBW 3032 and Ovcharitsa IBW 2317 (Michev & Stoyneva 2007) (Figure 1).

These water bodies are used as important water resources in irrigation, recreational purposes and for aquaculture. In the Aheloy Reservoir is cultivated mainly common carp (*Cyprinus carpio* Lineus), and in Ovcharitsa Reservoir common carp, grass carp (*Ctenopharyngodon idella* Valenciennes), bighead carp (*Hypophthalmichthys nobilis* Richardson) and channel catfish (*Ictalurus punctatus* Rafinesque). Forty-eight

Fig. 1. Location of the studied reservoirs

water samples for phytoplankton analysis were collected by Niskin-Type water sampler 5 L model (Hydro-Bios Apparatebau GmbH, Germany). The phytoplankton samples were collected and processed by standard methods of fixation with formalin to final concentration 4% and further sedimentation (ISO5667-1: 2006/ AC:2007; ISO5667-3: 2003/AC: 2007). Microscope work has been done on Bürker chamber. The species composition was determined by light microscope (Carl Zeiss, Axioscope 2 plus) with magnification 400x using standard taxonomic literature with critical use of AlgaeBase (Guiry & Guiry, 2022). Diatoms were identified according to Cox (1996). The main counting unit was the cell and the biomass was estimated by the method of stereometrical approximations (Rott, 1981; Deisinger, 1984). Counting is carried out individually (cell, filament or colony). The total biomass of each sample was assessed and it was defined as the amount of biomass of all species summarized in separate taxonomic groups. Phytoplankton taxa were classified into phytoplankton functional groups (Reynolds et al., 2002; Padisak et al., 2009). Functional classifications were defined for species that contributed at least 5% of the mean phytoplankton biomass.

The assemblage index (Q)

Using the concept of functional groups proposed by Reynolds et al. (2002), in the work of Padisak et al. (2006) was developed an assemblage Q index to classify and assess the ecological status of different lake types. The index calculates the relative contribution of functional groups to the total biomass, and a factor number (*F*) assigned to each functional group. The Q index ranges in values between 0 and 5 and classified the ecological status into five scales: 0-1: poor, 1-2: tolerable, 2-3: moderate, 3-4: good and 4-5: excellent. Following the steps of Padisak et al. (2006) in applying the Q index, the *F* factor was adopted for each functional group that was encountered, with type 4 being the relatively closest for Ovcharitsa Reservoir and type 5 for Aheloy Reservoir.

$$Q = \sum piF$$

where $pi = ni/N$; ni – biomass of the i-th functional group; N – total biomass of functional groups in total biomass and a factor number (F) created by the i-th functional group.

Results

Phytoplankton species composition and functional groups

The species composition and functional classification of phytoplankton are presented in Table 1. During the study period, 86 taxa were found in Aheloy Reservoir. Members of 8 algal groups have been identified: Cyanoprokaryota (20), Chlorophyta (38), Streptophyta (4), Euglenophyta (6), Pyrrhophyta (6) and Ochrophyta – 12 (Chrysophyceae – 1, Synurophyceae – 1 and Bacillariophyceae – 10). For the same period 61 taxa from 8 divisions were found in Ovcharitsa Reservoir: Cyanoprokaryota (10), Chlorophyta (26), Streptophyta (2), Euglenophyta (1), Pyrrhophyta (3) and Ochrophyta– 19 (Raphidophyceae – 1, Synurophyceae – 1 and Bacillariophyceae).In 2020, twenty functional groups were identified in Aheloy Reservoir (Table 1).

The most common group in the reservoir are L_0, W2 and J.The biomass of *Peridinium bipes* F.Stein from L_0 codon, increasing to 73% in July, and that of *Peridiniopsis* sp.to 41.3% in October.In early summer in June the biomass of *Trachelomonas planctonica* Svirenko from W2 group increases to 27.4%, and the green algae *Coelastrum microporum* Nägeli(58.8%) and *Desmodesmus communis* (E.Hegewald)

Table 1. List of identified phytoplankton taxa

Taxa	Aheloy Reservoir	Ovcharitsa Reservoir	FG
Cyanoprokaryota			
Aphanizomenon cf. *gracile* Lemmermann		*	H1
Anabaena sp.	*		H1
Anabaenopsis sp.	*		H1
Aphanocapsa sp.		*	K
Aphanothece sp.	*	*	K
Chroococcus turgidus (Kützing) Nägeli		*	Lo
Cuspidothrix issatschenkoi (Usachev) P.Rajaniemi, Komárek, R.Willame, P. Hrouzek, K.Kastovská, L.Hoffmann & K.Sivonen	*		H1
Dolichospermum lemmermannii (Richter) P.Wacklin, L.Hoffmann & J.Komárek		*	H2
Dolichospermum flos-aquae (Bornet & Flahault) P.Wacklin, L.Hoffmann & Komárek	*	*	H1
Dolichospermum planctonicum (Brunnthaler) Wacklin, L.Hoffmann & Komárek	*		H1
Limnothrix redekei (Goor) Meffert	*	*	S1
Merismopedia sp.	*		Lo
Merismopedia tenuissima Lemmermann	*		Lo
Microcystis aeruginosa (Kützing) Kützing		*	M
Microcystis sp.	*		M
Microcystis wesenbergii (Komárek) Komárek ex Komárek	*		M
Planktolyngbya limnetica (Lemmermann) Komárková-Legnerová & Cronberg	*		S1
Planktolyngbya sp.	*	*	S1
Planktothrix agardhii (Gomont) Anagnostidis & Komárek	*	*	S1
Pseudanabaena catenata Lauterborn	*		MP
Pseudanabaena mucicola (Naumann & Huber-Pestalozzi) Schwabe	*		MP
Pseudanabaena sp.	*		S1
Raphidiopsis mediterranea Skuja	*		SN
Raphidiopsis raciborskii (Woloszynska) Aguilera & al. 2018	*		SN
Romeria sp.	*		K
Chlorophyta			
Actinastrum hantzschii Lagerheim	*	*	J
Ankistrodesmus fusiformis Corda	*		X1
Ankyra ancora (G.M.Smith) Fott		*	X1
Ankyra judayi (G.M.Smith) Fott		*	X1
Characium sp.		*	
Chlamydomonas cf. *reihardtii*	*	*	X2
Chlamydomonas sp.	*	*	X2
Coelastrum astroideum De Notaris	*		J
Coelastrum microporum Nägeli	*	*	J
Crucigenia quadrata	*		X1

Crucigenia tetrapedia (Kirchner) Kuntze	*		X1
Crucigeniella sp.	*	*	X1
Desmodesmus bicaudatus (Dedusenko) P.M.Tsarenko	*		J
Desmodesmus communis (E.Hegewald) E.Hegewald	*	*	J
Desmodesmus denticulatus (Lagerheim) S.S.An, T.Friedl & E.Hegewald	*		J
Desmodesmus spinosus (Chodat) E.Hegewald	*		J
Dictyosphaerium simplex Korshikov	*		F
Golenkinia radiata Chodat	*		X1
Hariotina polychorda (Korshikov) E.Hegewald	*		J
Hyaloraphidium contortum Pascher & Korshikov	*		X1
Korshikoviella limnetica (Lemmermann) P.C.Silva	*		X1
Lagerheimia ciliata (Lagerheim) Chodat		*	X1
Lagerheimia genevensis (Chodat) Chodat	*		X1
Micractinium pusillum Fresenius	*		F
Micractinium quadrisetum (Lemmermann) G.M.Smith		*	F
Monoraphidium contortum	*	*	F
Mucidosphaerium pulchellum (H.C.Wood) C.Bock, Proschold & Krienitz	*	*	F
Oocystidium planoconvexum(Hindák) Stenclová & Pazoutová		*	
Oocystis lacustris Chodat	*	*	F
Oocystis sp.		*	F
Oocystis borgei J.W.Snow	*	*	F
Pandorina morum (O.F.Müller) Bory	*	*	G
Pandorina sp.	*		G
Pediastrum duplex Meyen	*	*	J
Pediastrum simplex Meyen	*		J
Scenedesmus arcuatus (Lemmermann) Lemmermann	*		J
Scenedesmus ecornis (Ehrenberg) Chodat	*	*	J
Schroederia spiralis (Printz) Korshikov	*	*	X1
Sphaerocystis planctonica (Korshikov) Bourrelly	*	*	F
Sphaerocystis sp.	*		F
Tetradesmus lagerheimii M.J.Wynne & Guiry	*	*	J
Tetradesmus obliquus (Turpin) M.J.Wynne	*		J
Tetraedron caudatum (Corda) Hansgirg	*		X1
Tetraedron minimum (A.Braun) Hansgirg	*		X1
Tetraedron triangulare Korshikov	*		X1
Tetrastrum sp.		*	X1
Treubaria planctonica (G.M.Smith) Korshikov		*	X1
Treubaria sp.		*	X1
Streptophyta			
Closterium aciculare T.West		*	P
Cosmarium sp.	*	*	N
Closterium sp.	*		P
Elakatothrix gelatinosa Wille	*		F
Staurastrum sp.	*		N
Euglenophyta			
Euglena sp.	*		W1
Lepocinclis sp.	*		W1
Trachelomonas hispida (Perty) F.Stein	*		W2
Trachelomonas planctonica Svirenko	*		W2

Trachelomonas sp.	*	*	W2
Trachelomonas volvocina (Ehrenberg) Ehrenberg	*		W2
Pyrrhophyta			
Ceratium furcoides (Levander) Langhans	*	*	Lo
Glenodinium sp.		*	Y
Peridiniopsis cf. *kevei*	*		Lo
Peridiniopsis sp.	*		Lo
Peridinium bipes	*		Lo
Peridinium cf. *cinctum*	*		Lo
Peridinium sp.	*	*	Lo
Raphidophyceae			
Gonyostomum sp.		*	Q
Chrysophyceae			
Dinobryon divergens O.E.Imhof	*		E
Synurophyceae			
Mallomonas sp.	*	*	E
Bacillariophyceae			
Amphora sp.		*	MP
Aulacoseira granulata (Ehrenberg) Simonsen	*	*	P
Cocconeis pediculus		*	MP
Cocconeis sp.		*	MP
Cyclotella cf. *commensis*		*	A
Cyclotella glomerata H.Bachmann		*	A
Cyclotella meneghiniana Kützing	*	*	C
Cyclotella sp.	*	*	B
Cymbella sp.	*	*	MP
Discostella cf. *stelligera*	*	*	B
Fragilaria crotonensis Kitton	*	*	P
Gomphonema sp.		*	MP
Lindavia bodanica (Eulenstein ex Grunow) T.Nakov, Guillory, Julius, Theriot & Alverson	*	*	B
Navicula sp.	*	*	MP
Nitzschia sp.	*	*	MP
Stephanodiscus hantzschii Grunow		*	D
Stephanodiscus sp.		*	D
Ulnaria acus (Kützing) Aboal	*		D

E.Hegewald(32%) members from J codon. In 2021, nineteen functional groups were identified. The most common groups are W2, D, J, L_0 and E.Among the most abundant species in biomass are again W2 member *T. planctonica* with biomass values up to 43.6% in May, *Ulnaria acus* (Kützing) Aboal from D group (49.6%) in June, and J codon members *Pediastrum duplex* Meyen (26.5%) and *Coelastrum microporum* (13.9%) in September. At the same time significantly increase their biomass species *Dinobryon divergens* O.E.Imhof(32.2%) from codon E and L_0 group species *Peridiniopsis* sp. (11.1%).

In Ovcharitsa reservoir in 2020 were found twenty-two functional groups (Table 1).Most common in the reservoir are the functional codons X2, L_0, G, P, A and M. The taxa from codon X2 *Chlamydomonas* cf. *reinhardtii*(38.8%), L_0 *Peridinium* sp. (64.6%), G *Pandorina morum* (O.F.Müller) Bory (34.1%), P *Aulacoseira granulata* (Ehrenberg) Simonsen (95.6%), A *Cyclotella* sp. (74.2%) and M *Microcystis aeruginosa* (Kützing) Kützing (41.2%) are among the most abundant species recorded in 2020 at all sites studied in the reservoir. In 2021, 16 functional groups were identified. In the reservoir the most common are the functional groups A, F P, MP, J, B, X2 and H1. The codon J species *Sphaerocystis* cf. *planctonica*, have a variable contribution to the biomass from May to June (56.7 to 77.8%), *Oocystis lacustris* Chodat (27.3%) in July and P group member *Closterium aciculare*

T.West (47.9%) in June. In May among the dominants is also found *Cymbella cymbiforfis* C.Agardh (59.3%) from MP group, G codon member *P. duplex*(25.9%) and A member *Cyclotella* sp.(34.2%) in June. Among the most abundant in July are identified X2 member *Chlamydomonas* cf. *reinhardtii* (40%)*, Dolichospermum flos-aquae* (Bornet & Flahault) P.Wacklin, L.Hoffmann & Komárek (31.9%) from H1 functional group. In September ubiquitously dominated P codon member *A. granulata* (93.2%).

The assemblage index (Q)

In 2020, the highest Q index levels in Aheloy Reservoir were recorded on the wall in late summer where the phytoplankton biomass were dominated by members of the codon W2 (3.0-3.3, Figure 2). In the same year, the Q index values ranged from 1 (tolerable) and 3.3 („good"). In 2020, the worst („tolerable") and best („good") environmental status of the reservoir was found at the reservoir wall in July when members of functional groups J and L_0 are the most abundant in biomass. In 2020, the average values for the Q index determine „moderate" (2.19) environmental status at the site near the reservoir wall. In the middle part of the reservoir in the dominance of species from the codons J and L_0 the index values range from 1.32 to 2.8. The average values of the indicator show „tolerable" (1.87) status. In the tail of the reservoir, the levels change from 1.87 to 2.25, and the average values of the index indicate „moderate" (2.25) status with most common again J and L_0. In 2021, the Q index levels range from 0.99 (with the most abundant again J and L_0) in the tail to 3.034 in the area of the reservoir wall. Average assemblage index values in 2021 at the same station indicate „tolerable" (1.82) status. At the station in the middle part of the reservoir the values are also evidence of „tolerable" (1.80) status under the domination of groups W2, J, D and E. In the same year, the worst ecological status was determined by the average assemblage index values („tolerable",1.64) measured in the tail of the reservoir for the most common codons W2, J and L_0. The average Q index values during the two years of the study determine the „tolerable" (1.93) ecological status of Aheloy reservoir.

In the Ovcharitsa Reservoir, the assemblage Q index values in 2020 varied widely from 0.25 („poor") to („excellent") 5 (Figure 3). In the same year, the average values of the index are indicative of a „good" ecological status (3.61) of the sites near the wall with the most common functional groups during the period X2, P and B. At the same time, in the middle part of the reservoir, the latter indicator also defines a „good" (3.44) status, where the most abundant in biomass are members of the codons L_0, P and J. In the area of the tail of the reservoir the index levels show „moderate" (2.56) ecological status. In 2020 the tail of the reservoir is dominated by groups W1, P and M. In 2021, the assemblage

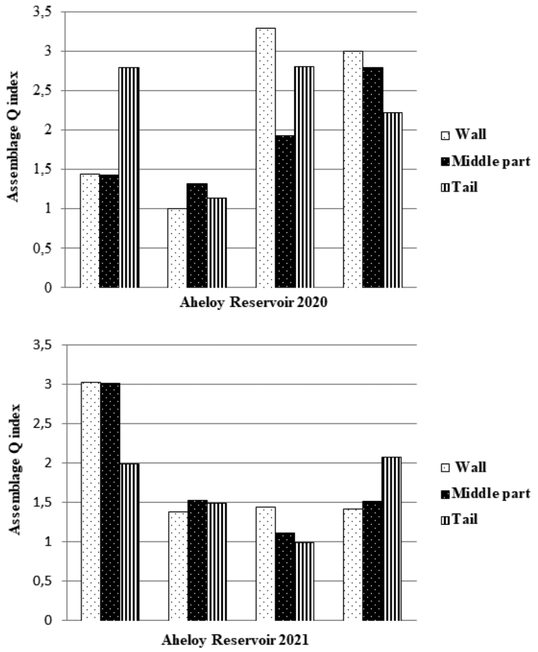

Fig. 2. Assemblage Q index values in Aheloy Reservoir (2020-2021)

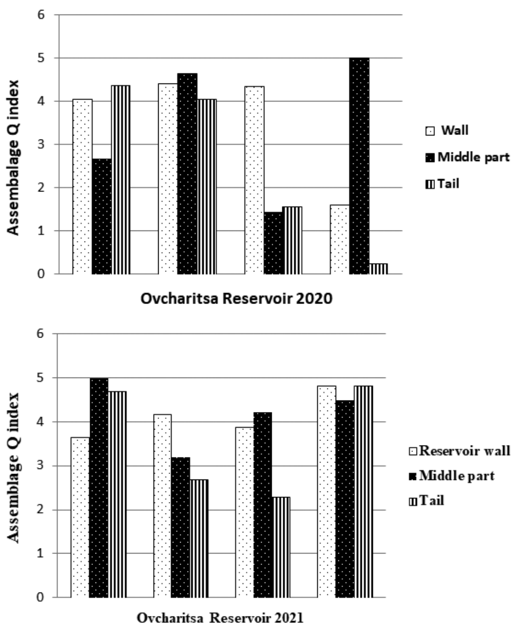

Fig. 3. Assemblage Q index values in Ovcharitsa Reservoir (2020-2021)

index ranges within 2.28 („moderate") to („excellent") 5. On the wall, the index values during the year show „excellent" (4.13) ecological status, at the most abundant members of the codons F and P. Under the domination of groups MP, J, F and P the analysis of the Q index in the middle part have also determines an „excellent" (4.23) ecological status of the study site.While at the tail of the reservoir site the average assemblage index levels in 2021 are indicative of „good" (3.62) status, with the highest biomass being members of the codons F, B, X2 and P.Overall in 2020 and 2021, the average values for the assemblage index (3.6) are indicative of „good" ecological status of Ovcharitsa Reservoir.

Discussion

According to the study of Belkinova et al. (2014) in the Aheloy Reservoir dominated cyanoprokaryotes from functional group S1 and H1. The same authors report that members of codon S1 determine 80% of the phytoplankton biomass. S1 functional group members occurs predominantly in turbulent and turbid waters (Padisák et al., 2009). Compared to Belkinova et al. (2014) data in the period 2020-2021 in the Aheloy Reservoir the largest contribution to the phytoplankton biomass is represented by species from the codons L_0, W2 and J. *Aphanizomenon flos-aquae*, a member of the H1 group was found in the mountain Vacha Reservoir, lowland Aheloy and Ovcharitsa reservoirs (Belkinova et al., 2014; Stoyneva et al., 2017), as well as in the high-mountain Dospat (Dochin & Stoyneva, 2014; Dochin &Stoyneva, 2016), Tsankov Kamak (Dochin &Ivanova, 2017) and Batak Reservoir (Dochin et al., 2018).

Based on the calculation of the Q index, the same study defines the ecological status of the Aheloy Reservoir as „bad" (Belkinova et al., 2014). In the last publication as the most abundant species are reported *Limnothrix redekei* (Goor) Meffert (64.3%), *Pseudanabaena limnetica* (Lemmermann) Komárek (19.7%) and *Aphanizomenon flos-aquae* Ralfs ex Bornet & Flahault (5.7%). On the other hand, a change in the dominant species in the reservoir was found in 2020. Aheloy Reservoir is a shallow water body with an average depth of 13 meters, in 2020 very low water levels were recorded as a result of its use for irrigation as well as technical difficulties during the operation of the facility. In our opinion, this is one of the probable reasons for the changes in phytoplankton structure in the reservoir in 2020 and 2021.

Unfortunately, we do not have more data on water levels of the reservoir in previous years to compare the results obtained for phytoplankton at different water levels. In 2020 the most abundant are members of the functional groups L_0, W2 and J, as with the highest biomass are *Peridinium bipes* (73.%), *Trachelomonas planctonica* (27.4%) and *Coelastrum microporum*(58.9%). The group Lo is considered a common functional group of the summer epilimnion of mesotrophic lakes (Reynolds et al., 2002). The members of W2 functional group are found in shallow waters, but sometimes they also occur in open waters. The colonial green algae from the functional group J usually occurs in shallow lakes and reservoirs in the summer (Reynolds et al., 2002; Sarmento & Descy, 2008; Becker et al., 2009). In 2021, we again found a change in the dominant algal species with the most common in the Aheloy Reservoir are D group member *Ulnaria acus*, *Dinobryon divergens* from codon E and *Pediastrum duplex* from J. According to the assemblage Q index data from this study, the ecological status of the reservoir in 2020 is defined as „moderate" and in 2021 as „tolerable". Compared to the data published by Belkinova et al. (2014) found an improvement in the ecological status (from „poor" to „tolerable") of the Aheloy Reservoir, according to the data for the dominant taxa and the assembage Q index values that we obtained.

Belkinova et al. (2014) report that the ecological status of Ovcharitsa Reservoir as „moderate" mainly due to blooms of P codon member colonial diatom *A. granulata*. In 2020 among the most common are the functional groups X2, L_0 and M (*Chlamydomonas* cf. *reinhardtii, Ceratium furcoides* (Levander) Langhans, *Peridinium* sp. and *M. aeruginosa*). Codon X2 is found in the mixing layers of mesoeutrophic shallow lakes (Reynolds et al., 2002). The member of X2 functional group (*Chlamydomonas* sp.) is identified as the first dominant in the Poroj Reservoir (Belkinova et al., 2014). In 2021 the most abundant are members of codons F, P and MP (*Sphaerocystis* cf. *planctonica*, *A. granulata* and *C. cymbiforfis*). Similar to our data from 2020 the codon L_0 member *C. furcoides* together with *M. aeruginosa* of group M dominate the Ovcharitsa Reservoir (Belkinova et al., 2014). According to Padisak et al. (2009), the dominant functional group M inhabits small to medium-sized eutrophic ponds. The member of the functional group Lo *C. furcoides* is registered among the dominants in the reservoirs Kurdzhali and Koprinka (Gecheva et al., 2020). In Aheloy Reservoir the same species caused blooms in coexistence with cyanoprokaryotes (Dochin, 2021). Compared to data from 2014 of Belkinova et al. (2014) in 2020, no significant change was found in the most abundant species by biomass in Ovcharitsa Reservoir. In 2021, however, with the exception of P codon member *A. granulata* there has been a change in the dominant species in the reservoir with members of groups F and MP are among the first dominant.These changes in the dominant species in 2021 may be among the reasons for the change in the ecological status. According Belkinova et al. (2014) members of the codon MP are typical in oligotrophic semi-montain reservoirs. The same authors consider that the good condition recorded in some of them indicates that this group may be indicative of good ecological status. The members of P functional group inhabit eutrophic waters (Reynolds et al., 2002; Padisak et al., 2009; Belkinova et al., 2014). The recent authors report that the massive presence of the P-species is an indication for a change in the ecological status. Group F species increase their biomass during the summer months. This group contains large, colonial species which prefers mesotrophic waters and are tolerant of low nutrient levels (Reynolds et al., 2002). Members of codon F are specific to many water bodies, but are most commonly found in deep oligotrophic lakes which are in good condition (Belkinova et al., 2014; Dochin 2019). Other authors such as, Sarmento & Descy (2008) classified F group as dominant in mesotrophic waters. The taxa classified in functional group F dominate some large reservoirs in the country as Studen Kladenets, Kardzhali, and Ivailovgrad (Belkinova et al., 2014; Dochin & Iliev, 2019).Our reported average values of the Q index in 2020 and 2021 indicate a „good" ecological status of the Ovcharitsa Reservoir. The results from the same period indicate an increase in the ecological status from „moderate" to „good" based on phytoplankton composition and assemblage Q index data collected.

The determination of the factor numbers (*F*) is the most important part in the assessment of the ecological status based on the assemblage Q index (Padisak et al., 2006; Crossetti & Bicudo, 2008; Belkinova et al., 2014).The factor *F* needs to be specified for each water body type, but lack of data can lead to confusion (Belkinova et al., 2014). The same authors report that, its application is possible for lowland small and medium-sized lakes and also mountain and semi-mountain lakes.The reservoirs that are the subject of this study, as defined by Belkinova et al. (2014) most similar to type 7 of the typology proposed by the Padisak et al. (2006). In 2014, the ecological status of Aheloy Reservoir was assessed as „poor"and that of the Ovcharitsa Reservoir as „moderate" (Belkinova et al., 2014). When we use values of the *F* factor close to the characteristics of type 7 of the Hungarian lake typology, the ecological status of the two studied reservoirs is evaluated as significantly better than the real one. Based on our unpublished results on phytoplankton biomass, the ecological status is assessed as significantly poorer compared to the data obtained from the calculation of the assemblage index corresponding to type 7 of the same typology. In our opinion, the data that we propose are analysed by classifying Ovcharitsa Reservoir close or relatively close to type 4 and for the Aheloy Reservoir to type 5 similar to the Hungarian lakes typology described by Padisak et al. (2006), as the average levels for the Q index show „good" status for the first and „tolerable" for the second reservoir. One of the possible reasons for the differences in the assessment of the ecological status of the studied reservoirs, as it became clear from the text mentioned above, is their classification into different types according to the Hungarian lake typology. The duration of the survey and the number of samples also has a significant impact on the final results of the evaluation. However, according to our results, calculating the index based on type 7 leads to an ecological assessment different from the real one. The latter is not comparable to the assessment based on the average phytoplankton biomass values. This fact, once again confirms the difficulties encountered by experts in determining the *F* factor. We believe that great caution is necessary in selecting the values for the *F* factor for the index Q calculation, consistent with the type and individual geographic and hydrological characteristics of the water bodies, especially when applied to reservoirs. Furthermore, the determination of *F* factor for different water body types remains a debated issue, depending on a multitude of factors, and requirements that need to be implemented such as a uniform classification system, the lack of data on the functional groups of some species, and many others. Therefore, the application of the Q index in highly modified water bodies such as reservoirs, mostly in large, deep ones is very difficult and may lead to mistakes in the determination of the ecological status, especially when a large database and statistically valid values are not available. The use of multiple metrics in ecological assessment based on phytoplankton leads to dilution of results and divergence from the real condition. In our view, simplifying the procedure by using phytoplankton biomass, and especially focusing on cyanoprokaryotes and the presence of potential toxic species is sufficient for an objective assessment of the ecological status of reservoirs.

Conclussion

During the research, 86 phytoplankton taxa were identified in Aheloy Reservoir and 61 in Ovcharitsa Reservoir, belonging to 20 functional groups in the first and 22 in the second. Changes in the dominant functional groups of phytoplankton were detected in 2020 and 2021 compared to previous data from 2014, especially in Aheloy Reservoir. While in 2014 the most common are members of the codons S1 and H1, in 2020 L_0, W2 and J, and in 2021 D, E and J. At the Ovcharitsa Reservoir the changes in the dominant functional groups are as follows: in 2014 group P dominates, in 2020 the codons X2, L_0 and M, and in 2021 F, MP and P.When applying the assemblage Q index for the assessment of the ecological status the average values for the period 2020-2021 indicates a „tolerable" status in Aheloy Reservoir and a „good" status in Ovcharitsa Reservoir, as well as improving the condition compared to previous studies. Finally, the developed Q index is based on a widely known and broadly used concept with a solid scientific foundation. The critical point in its use is the correct selection of the water body typology and the choice of an *F* factor values tailored to the their individual characteristics. In our view, the application of the assemblage index Q in monitoring programmes is justified only after careful expert evaluation and selection of the *F* factor values based on a typology adapted to the individual characteristics of the different water bodies.

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