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Association Between the Environmental Parameters and Biological Indicators in Reservoirs with Intensive Net-Cage Aquaculture: A Case Study in Kardzhali Reservoir, Bulgaria

Ivan Iliev[1*], *Kostadin Dochin*[2], *Mariana Marhova*[1], *Angelina Ivanova*[2], *Sonya Kostadinova*[1]

1 - Plovdiv University "Paisii Hilendarski", Faculty of Biology, Department of Biochemistry and Microbiology, 24 Tzar Assen St., 4000 Plovdiv, BULGARIA
2 - Institute of Fisheries and Aquaculture, 248 Vasil Levski St., 4003 Plovdiv, BULGARIA
*Corresponding author: iziliev79@gmail.com

Abstract. The present study analyzed the interactions between the environmental parameters, microbiological water quality indicators, and phytoplankton in Kardzhali reservoir, which is one of the largest and economically significant water bodies in Bulgaria, with highly developed cage-aquaculture. Data sets of eighteen parameters from 5 monitoring sites during 2016–2018 were used for analysis. We have applied multivariate methods, aiming to identify the key parameters affecting the communities, including the impact of the net-cage farms. The ANOSIM (analysis of similarities), showed significant differences in the values of physicochemical factors between the control site and the area for aquaculture, with higher nitrate, total nitrogen, and COD (chemical oxygen demand) content near the net-cages. The results were confirmed by the high *R*-value ($R=0.87$; $p<0.01$). The conducted PCA (principal component analysis) showed that only three principal components (PCs) are need to group the physicochemical parameters, explaining 90.5% of the total data variation. PC1 was formed by nitrogen forms and COD, PC2 represents the physical source of the variability (pH and dissolved oxygen) and PC3 was loaded with total phosphorus (0.537) and ammonium nitrogen (0.764) concertation. The parameters with the highest impact on the abundance of heterotrophic bacteria (TVC) include temperature, TN, and COD, while the phytoplankton community was negatively correlated with Secchi depth and COD. The redundancy analysis confirmed that the location of the sampling station significantly affects the studied variables and that net-cage aquaculture is a major anthropogenic factor in Kardzhali reservoir.

Key words: Microbiological indicators, environmental factors, PCA, RDA, phytoplankton, Kardzhali Reservoir.

Introduction

Freshwater resources are an important component of the biosphere, ensuring the sustenance of aquatic beings (Jackson *et al.*, 2001). Worldwide they constitute only 3% of the total water resources. The latest Eurostat information on water statistics shows that a significant proportion of freshwater in Bulgaria is received as an external surface water inflow. In fact, Bulgaria had the highest volume of water received (85.1 billion m^3) among the EU Member States, forming 84% of the total available annual water resources (Eurostat, 2017). This makes ensuring the best water quality of lakes, rivers, and reservoirs a sensitive issue and a great environmental concern.

The economically significant reservoirs in Bulgaria are multipurpose water bodies. They are often used for irrigation, power production, aquaculture, recreational activities, etc. The agricultural, urban and industrial activities are considered to be major factors for eutrophication as a result of pollution with excess nutrients and toxic chemicals (ISCEN *et al*., 2008; LENART-BORON *et al*., 2016). The large Bulgarian reservoirs are often used for intensive aquaculture production. The accession of the country to the European Union in 2006, lead to an increase of the aquaculture production and construction of new farms (HADJINIKOLOVA, 2010). These processes can result in significant changes in major environmental factors. Such changes in the trophic state of the water bodies can lead to a number of problems such as algal blooms, anoxia, fish mortalities and reduction of the biodiversity (OUYANG *et al*., 2007). More so these waters support microbiological communities including pathogenic microorganisms (HONG *et al*., 2009). They are an indispensable component of the nutrient cycles and they are extremely sensitive to changes in the physicochemical parameters of the environment.

The effective program for the prevention of water pollution is based on the physical, chemical and biological parameters in order to sustain a balanced ecosystem (ISCEN *et al*., 2008; MISHRA, 2010). One of the major problems in the analysis of surface water quality has always been the complexity of the system itself and the uncertainty what environmental parameters to measure. This makes it harder to establish suitable indicators (YU *et al*., 1998). The data sets usually contain vast information regarding the studied environmental indicators and the classification of the data is crucial for the correct interpretation of the results. The problem can be approached through the application of multivariate statistical analysis cited in a number of papers (ISCEN *et al*., 2008; MISHRA, 2010; GU *et al*., 2016; LENART-BOROŃ *et al*., 2016).

We have conducted the present study in the Kardzhali reservoir which is one of the oldest in Bulgaria. It has been an object of a number of previous papers focused mainly on the composition of the phytoplankton (BESHKOVA *et al*., 2008; DOCHIN & STOYNEVA, 2014) and the physicochemical properties of the waters (TRAYKOV, 2005; ILIEV, 2015). However, there is no sufficient data concerning the lasting effect of the intensive net-cage aquaculture on ecosystem stability. The object of the present study was to assess the connection between the environmental factors and main biological indicators and to identify the key parameters affecting the communities in the reservoir.

Materials and Methods

Site description and sampling

The research was carried out in the aquatory of the Kardzhali reservoir for the period June 2016 – October 2018. The reservoir is situated in the central part of Southern Bulgaria. It was designed as a heavily modified water body and was classified as a large, deep reservoir (L11) according to the national classification of lakes and reservoirs (Regulation H-4/2012 on characterization of surface waters. Ministry of Environment and Water, Bulgaria. State Gazette, 22). Kardzhali reservoir is fed by the Arda River and has one outlet, where it drains into the same river. It is used for intensive aquaculture for over 30 years. At present 6 net-cage farms operate in the aquatory of the reservoir, used primarily for sturgeon farming and are concentrated in the area of 300 ha with allowed capacity 2610 t (East aegean river basin directorate, Fishfarming report, 2016).

Sampling was conducted at five sampling stations monthly from April to October each year, with the exception of 2016 (the study started in June), in order to assess the period of active feeding in the aquaculture farms and the active tourist season. The stations include one control site and four net-cage farms (NC), situated in the aquatory of the reservoir as follows: Control site (41.648541 N, 25.291899 E), NC1 (41.641229 N, 25.310090 E); NC2 (41.642802 N, 25.314325 E); NC3(41.639326 N, 25.319163 E); NC4(41.641198 N, 25.320919 E). The control site was situated upstream from the area for aquaculture in the open aquatory equidistant from the net-cages and the riverine part of the reservoir, while the NC-sites were positioned in close vicinity of the four largest net-cage farms.

Water samples with an analytical volume of 500 ml for each site for microbiological analysis were collected with MICROS water sampler (Hydro-Bios Apparatebau GmbH, Germany) from the superficial water layer (0.5-1.0 m). The samples were kept in the dark at 4°C until analysis for no longer than 24 h (ISO 5667-1, ISO 5667-2, ISO 5667-3.). For the phytoplankton and physicochemical analysis mixed water, samples with an analytical volume of one liter were collected with Niskin-Type water sampler (Hydro-Bios Apparatebau GmbH, Germany) from the water column at each station. The sampling depths were calculated based on the Secchi depth. The samples were processed by the standard method of fixation with formalin to final concentration 4% and further sedimentation (ISO 5667-1:2006/AC:2007; ISO 5667-3:2003/AC:2007). For the physicochemical analysis, samples were stored in dark (4°C) until laboratory processing no longer than 24 h. Ninety-five samples for each of the analyzed parameters were collected in the period of study.

Physicochemical analysis

Temperature and dissolved oxygen were measured in situ with WTW oxygen meter (OXY 1970i). Transparency was established by the Secchi disk method. The water pH was measured in the laboratory using WTW model pH-meter (315/SET). Chemical analyses, including Manganese III COD, ammonium (NH4-N) and nitrate (NO3-N) nitrogen, nitrite (NO2-N) nitrogen, and total nitrogen, were performed using standard analytical methods (ISO 8467:1993; ISO 5664:1984; ISO 26777:1984; ISO 7890-1:1986). Total phosphorus concentration (TP) was determined by Phosphate Cell Test (114543, Merck Millipore) on Spectroquant Nova 400 photometer (Merck Millipore). Chlorophyll-*a* concentration was determined spectrophotometrically (ISO 10260:1992).

Microbiological analysis

Microbiological indicators were analyzed by standard analytical procedures for waters. The enumeration of culturable microorganisms (total viable count) was conducted by colony count after inoculation in a nutrient agar culture medium (ISO 6222:2002). *E. coli* and fecal coliforms (FC) were enumerated by membrane filtration method for waters (ISO 9308-1:2014) after supplementation of the nutrient medium with novobiocin (5 $mg.L^{-1}$) for the suppression of the bacterial background flora. Detection and enumeration of intestinal enterococci (FS) were done according to ISO 7899-2:2000.

Phytoplankton analysis

The species composition was determined by light microscopy on Axioscope 2 plus (Carl Zeiss) with magnification 200x and 400x using standard taxonomic literature with the critical use of AlgaeBase (Guiry & Guiry, 2018). Diatoms were identified according to the methodology of Cox (1996). The main counting unit was the cell and biomass was estimated by the method of stereometric approximations (Rott, 1981, Deisinger, 1984). The counting was carried out individually (cell, filament or colony). Assessed by the total biomass of each sample, defined as the amount of biomass of all species, summarized in separate taxonomic groups.

Statistics

Statistical analyses were completed using Primer 6 (Primer-E, Ltd) and Microsoft Excel with the XLSTAT software package (Addinsoft). All parameters were standardized prior to the analysis. Principle Component Analysis (PCA) was used to detect environmental parameters variations between samples. Permutational multivariate analysis of variance (perMANOVA) and analysis of similarities (ANOSIM) was used to test spatial and temporal variations of physicochemical and biological parameters. Redundancy analysis was applied to establish the relationships between biological communities, and environmental factors. The plots were constructed to visualize the similarity in the biological communities between the different sites. Correlation analysis (Pearson) was used to analyze the correlation between parameters. The significance of all statistical tests was assessed at $\alpha = 0.05$.

Results and Discussion

The average values for the analyzed environmental parameters are presented in Table 1. The results indicate seasonal changes in the water status with the highest water temperature, transparency and nutrient (NO3-N, TN, and COD) loadings in summer and lowest in the spring period. The ANOSIM, based on the physicochemical parameters, categorized the control site (Ctr) and the net-cage area (NC) into two distinct groups, which were confirmed by the high R-value ($R=0.87$; $p<0.01$). Both, sampling year, the inter-annual sampling and site location affected the rate of change in water quality ($p < 0.05$). Permutational MANOVA design test was applied to further evaluate the spatial and temporal variations and to confirm the differences between the Ctr and NC sites. It substantiated the significant seasonal and spatial variation of the parameters established by the ANOSIM. After 5 permutations of random subsampling each the control site was consistently found to be significantly distinct from NC4 and NC5. In general, there were no differences in water temperature ($F = 1.0287$; $p = 0.612$), transparency ($F = 2.0865$; $p = 0.136$), pH ($F = 0.699$; $p = 0.502$) and phosphorus concentration ($F = 0.3651$; $p = 0.6961$). However, the nitrate ($F = 6.0623$; $p = 0.0045$), nitrite ($F = 72.6801$; $p = 0.0000$), total nitrogen ($F = 14.8769$; $p = 0.00001$), and COD ($F = 20.1018$; $p = 0.00000$) content were higher around the net-cages compared to the control station, and varied significantly between the three years of study ($p > 0.001$). A Principal Component Analysis (PCA) was used to reveal the association between different variables and to establish a new basis of factors. The initial set of 11 parameters was reduced to a smaller number of factors summarizing the correlating factors and separating the non-correlating parameters. In the present study, a scree plot is used to detect the number of principal components (Fig. 1A). It shows a pronounced change of slope after the third eigenvalue, so only three principal components (PCs) are need to group the water samples, explaining 90.5% of the total data variation. The adequacy of the model was confirmed by Kaiser-Meyer-Olkin and Bartlett tests. The factor biplot is shown in Fig. 1B.

The quantitative analysis of the microbiological indicators for water quality and phytoplankton communities revealed seasonal and annual variation patterns following the variation in the environmental parameters. The total number of heterotrophic microorganisms was used as an indicator of the trophic status of a body of water. The spatial analysis demonstrated evident variation between the control site and the net-cage areas (ANOSIM $R=0.325$, $p=0.003$), with significant elevation in near the largest farms NC4 and NC5 (Fig. 2A). The abundance of heterotrophic bacteria in the aquaculture area remained high for the whole period, coinciding with intensive nutrition. This suggests that intensive aquaculture, accompanied by the release of untreated food and feces released by fish, is a major driver for bacterial communities in Kardzhali reservoir. Sanitary state indicators including fecal coliforms (FC), *E. coli* and fecal enterococci (FS) ware analyzed in order to separate the environmental pressure from the cages and from domestic wastewater discharges. For the whole period, the values for FC do not exceed 1000 CFU.100 ml^{-1}, and for *E. coli* and FS - 100 CFU.100 ml^{-1}. The highest values for all three years were established in samples from the NC1, NC2 and NC3 ($p < 0.05$). The persistent detection of *E. coli* and FC (albeit low in number) and their absence in the control zone in the reservoir indicates the presence of secondary sources of pollution other than cage aquaculture, possibly in the form of permanent discharge or fecal filtration - domestic wastewater in this area. Despite that, during the three-year monitoring campaigns, the reservoir was in excellent sanitary condition in terms of indicators (Fig. 2B), based on the criteria written in the sub-program for conducting research/own monitoring in connection with the assessment of the pressure and impact of intensive cage fish farming of the East Aegean RBMP 2017-2021. The results are evident for annual dynamics, with high levels of variation especially in 2017, indicative of a need for the development of a monitoring program.

The lowest phytoplankton numbers (0.09×10^6 cells.L^{-1}) and biomass (0.004 mg.L^{-1}) in the three years of study were established in the early spring period followed by an increase in the abundance, reaching its peak in the summer of 2016 and 2018, and in the autumn in 2017. In the peak period, the PHn and PHb averaged 33.3×10^6 cells.L^{-1} and 2.85 mg.L^{-1}, which is 10-15 times higher than in the other months (Fig. 2C and Fig. 2D). The 2017 results stand out as outliers, compared to the other samples, reaching values of 291×10^6 cells.L^{-1} and 4.16 mg.L^{-1} respectively for abundance and biomass decrease along the longitudinal axis of the reservoir in the direction of the water current, with a secondary increase near the net-cage areas NC3 and NC5. However, the study found no significant differences between stations (Fig. 2C and 2D).

Pearson correlation matrix was constructed for the assessment of the relationship between microbiological indicators and phytoplankton communities in the studied aquatory. Only a few parameters exhibited significant correlation relationships (Table 2). The parameters with the highest impact on the abundance of heterotrophic bacteria (TVC) include *Temp*, TN, and organic matter content (COD). A negative correlation was established for all factors with the exception of *Temp*. Both phytoplankton abundance and biomass are negatively correlated with Secchi depth and COD. Redundancy analysis was applied to visualize the correlations of environmental factors with biological parameters in the control site and net-cage area. The resulting model could explain 59.97% of the total variation of biological parameters (Fig. 3). The findings highlighted the differences between reservoir sampling sites. The first RDA axis (explaining 38.79%) was significant ($p < 0.001$) and strongly correlated with the developed intensive cage-aquaculture area (NC2, NC3, and NC4), situated in close proximity to Glavatartsi village. This gradient separated the zone with high anthropogenic pleasure from the lacustrine zone of the reservoir. The results indicated that differences in microbiological indicators were significantly correlated with water quality parameters. The first axis was negatively correlated with Secchi depth, pH, NO3-N, COD and dissolved oxygen, but positively correlated with TN concentration. The second RDA axis (explaining 21.18%) was also significant ($p <0.001$) and was related to Ctr and NC1 sites. It was positively correlated with water pH, NH4-N and COD.

Table 1. Physicochemical parameters (±SD) in the Kardzhali reservoir for the studied period (2016-2018). Legend: *Ctr – control station; *NC– average values for the net-cage area.

Station*	Secchi (m)	pH	Temp (°C)	Oxi (mg.L^{-1})	NH4-N (µg.L^{-1})	NO3-N (µg.L^{-1})	NO2-N (µg.L^{-1})	TN (µg.L^{-1})	TP (µg.L^{-1})	COD (mg.L^{-1})
Ctr_{2016}**	3.3±1.4	8.2±0.6	23.3±3.3	7.9±0.6	66±11	240±30	55±23	362±23	100±30	4.69±0.1
NC_{2016}	3.2±1.2	8.1±0.5	23.5±3.1	6.9±0.8	74±12	268±56	63±61	418±46	123±51	4.66±0.2
Ctr_{2017}	3.8±1.5	8.0±0.5	20.4±4.1	9.1±2.3	83±10	380±102	78±9	518±140	87±61	4.30±2.1
NC_{2017}	3.5±1.4	8.0±0.5	21.2±5.2	8.9±2.0	84±10	365±153	84±20	533±153	115±42	3.98±1.8
Ctr_{2018}	3.0±0.6	8.3±0.8	23.5±2.5	9.1±4.22	70±9	280±166	10±0	351±165	116±45	1.67±0.2
NC_{2018}	2.7±0.7	8.2±0.7	23.8±2.3	7.9±2.35	66±17	240±113	2.5±0	310±105	99±30	2.09±0.6

***Number of samples for each parameter*: Ctr_{2016} (n=5); NC_{2016} (n=20); Ctr_{2017} (n=7); NC_{2017} (n=28); Ctr_{2018} (n=7); NC_{2018} (n=28)

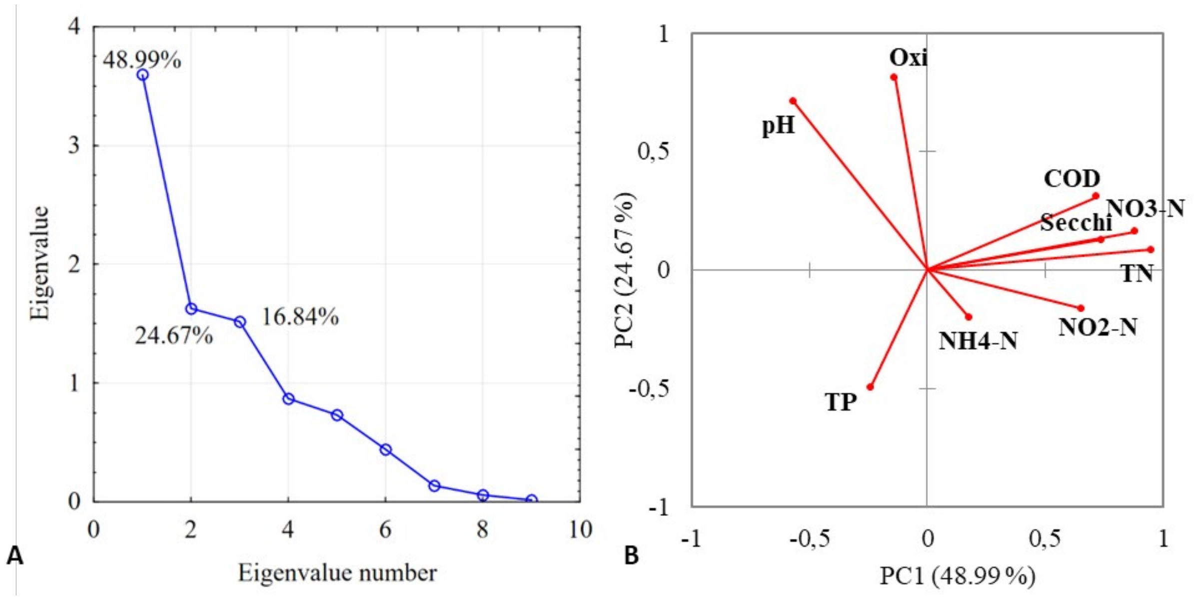

Fig. 1. Scree plot of the eigenvalues (A) and PCA biplot (B), based on the environmental parameters sampled from the control station and the four net-cage farms in the aquatory of Kardzhali reservoir.

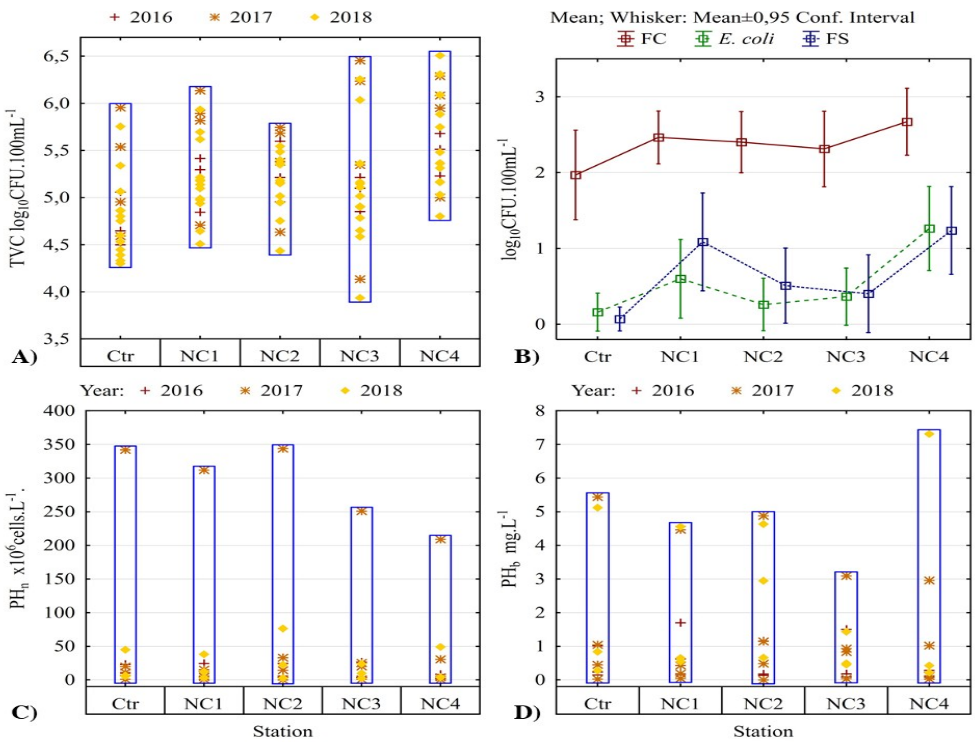

Fig. 2. Variability plots, based on the annual data for TVC (A), sanitary indicators (B) phytoplankton abundance (C) and phytoplankton biomass (D).

Kardzhali reservoir is one of the most extensively studied water bodies in Bulgaria. The interest in its ecological status is driven by the high economic interest, as it harbors the largest net-cage farms in the country. The present study is focused on the changes in the physicochemical parameters and their association with some of the biological indicators in conditions of intensive aquaculture during the period of active feeding. The results from the physicochemical analysis point out that the water quality of the reservoir has not changed significantly over the last 18 years period of intensive study in 2001-2018. The established temperature regime and water transparency have been observed in earlier studies of the reservoir (TRAYKOV, 2005; ILIEV, 2015; DOCHIN, 2015). The correlation analysis (Pearson) showed no relationship between temperature and dissolved oxygen, which was generally close to saturation in summer and spring. This could also be due to the high level of PHn and PHb during these periods (BRAHIM ERRAHMANI *et al*., 2015). A principal component analysis was applied to further elucidate the intricate relationships between the environmental parameters. The PCA allows transforming the original dataset into new, unrelated to each indicator (WU *et al*., 2014). Based on the correlation analysis, factors that were most significantly impacted by the cage aquaculture are Secchi depth and nutrients concentrations (NO3-N, TN, COD), while pH and dissolved oxygen concertation were not affected. During that period a large amount of uneaten feed remains in the water column (WESTON *et al*., 1996; BUREAU & CHO, 1999), which affects the water transparency and nutrient content in close vicinity of the farms (PEREIRA *et al*., 2004; GORLACH-LIRA *et al*., 2012). The uneaten feed and fish feces are a major source of phosphorus and nitrogen load in the intensive aquaculture, which explains the positive correlation between the parameters (PODEMSKI & BLANCHFIELD, 2006). The negative correlation between pH and COD is a result of the high organic load, which in combination with a high water temperature in the summer period in terms elevate the concentration of ammonia and organic acids (SHRESTHA & KAZAMA, 2007). This makes pH a suitable indicator of the nutrient load in the water (PARINET *et al*., 2004).

Table 2. Pearson correlation matrix of the environmental parameters and biological indicators in Kardzhali Reservoir 2016-2018. *correlations are significant at α= 0.05 (2-tailed).

	SD	Chl-a	pH	Temp	Oxi	NH_4^+	NO_3^-	NO_2^-	TN	TP	COD	Phn	Phb	TVC	FC	*E. coli*	FS
SD	**1**																
Chl-a	**-0,669**	**1**															
pH	-0,476	**0,544**	**1**														
Temp	**-0,640**	**0,677**	**0,577**	**1**													
Oxi	0,100	0,058	**0,678**	0,200	**1**												
NH_4^+	-0,019	0,130	-0,114	-0,019	0,099	**1**											
NO_3^-	**0,610**	-0,623	-0,416	-0,767	-0,052	-0,047	**1**										
NO_2^-	0,310	-0,305	-0,286	-0,426	0,000	**0,523**	0,347	**1**									
TN	**0,607**	-0,577	-0,436	-0,766	-0,044	0,188	**0,939**	**0,598**	**1**								
TP	-0,206	-0,106	-0,028	0,124	-0,092	0,053	-0,313	0,220	-0,218	**1**							
COD	0,423	-0,261	**-0,771**	-0,511	0,001	-0,061	**0,565**	**0,549**	**0,643**	-0,244	**1**						
Phn	**-0,227**	0,114	-0,408	-0,391	-0,329	0,352	-0,010	0,299	0,099	0,274	**-0,265**	**1**					
Phb	**-0,386**	**0,206**	-0,169	0,054	-0,192	0,454	-0,247	-0,127	-0,213	-0,007	**-0,497**	**0,874**	**1**				
TVC	-0,141	-0,017	-0,040	**0,491**	0,127	0,179	-0,287	0,217	**-0,398**	**0,544**	**-0,427**	**0,272**	0,066	**1**			
FC	-0,202	0,298	0,050	0,096	-0,250	0,138	-0,085	0,181	-0,005	0,445	0,152	0,019	-0,045	-0,047	**1**		
E. coli	-0,173	-0,032	-0,235	-0,086	-0,287	-0,020	0,003	-0,141	0,000	0,143	-0,168	0,137	0,164	0,082	**0,651**	**1**	
FS	-0,147	0,074	-0,216	-0,016	-0,298	-0,013	-0,069	-0,211	-0,076	0,063	-0,136	0,015	0,147	-0,012	**0,833**	**0,693**	**1**

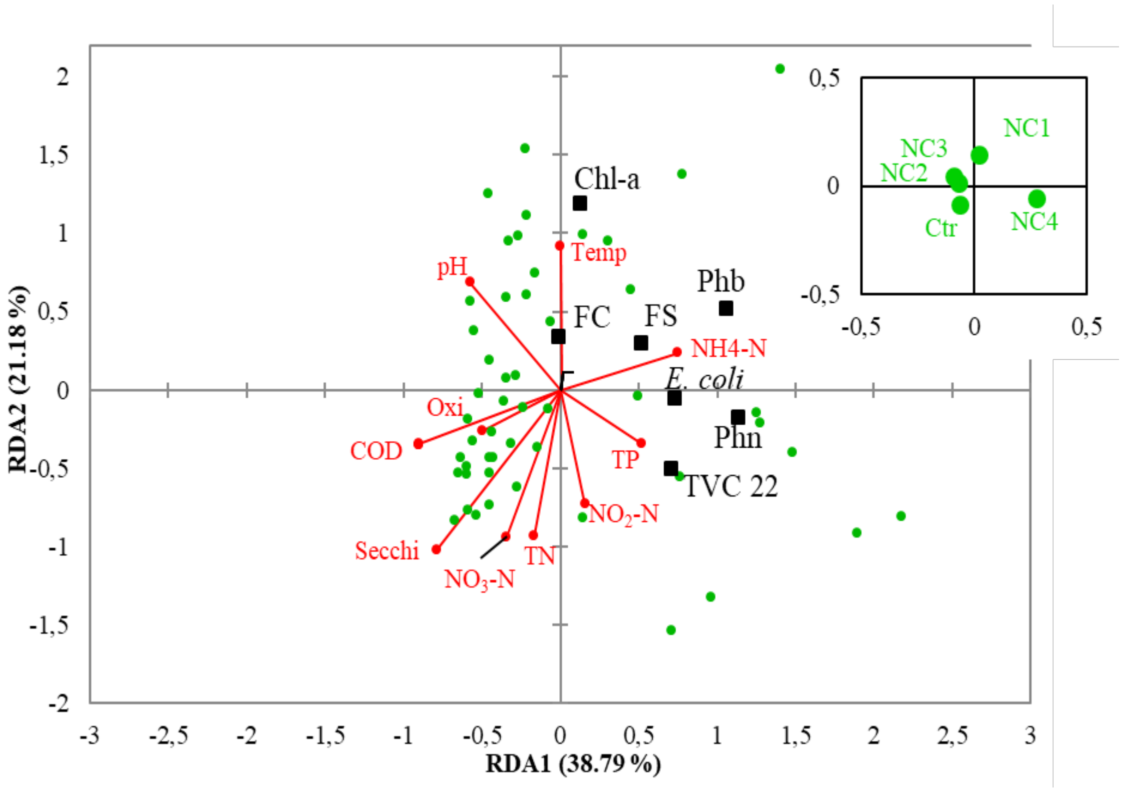

Fig. 3. Biplot in the redundancy analysis (RDA) of microbiological indicators and phytoplankton abundance in relation to environmental parameters.

The microbiological indicators and the phytoplankton seasonal variation limits established in the present study coincide with other observations in Kardzhali Reservoir for the period 2009-2012 (Iliev, 2015; Dochin & Stoyneva, 2014; Dochin, 2015). In the summer, PHn and PHb exceed significantly the values observed in the lacustrine zone of the reservoir in previous periods (Traykov, 2005). The spatial distribution of both biological parameters did not differ significantly between stations, which is untypical for lake-like reservoirs. Under a slow water flow, typical for Kardzhali reservoir, it is expected to observe reduction in the phytoplankton and TVC abundance in the direction form riverine to lacustrine zone, in result of self-purification mechanisms such as sedimentation, dilution, sunlight inactivation, predation and starvation (Medema *et al.*, 2003, Nahurska & Deptula, 2004; Iliev, 2015). Such tendencies are more similar for eutrophic reservoirs (Dochin *et al.*, 2017b), and differ from the earlier established patterns of distribution (Traykov, 2005). Moreover, in the summer period have been established a significant increase in the abundance of potential producers of cyanoprokaryotic toxins, such as representatives of the genera *Anabaena*, *Aphanizomenon*, and *Microcystis*. A bloom of the potential toxin producers of the blue-green *Pseudanabaena c.f. mucicola* and *Gloeotrichia echinulata* have also been reported in the autumn of 2017 at all stations (Dochin & Iliev, 2019).

Multivariate statistical methods used in the present study could help interpret the complex data matrices and to better understand the relationship between water quality and its impact on the biological communities (Shrestha & Kazama, 2007). The results confirmed that the location of the sampling station significantly affects the studied variables and that net-cage aquaculture is a major anthropogenic factor in Kardzhali reservoir. The redundancy analysis corroborated that the environmental factors, such as water temperature and nutrient concentration have a profound impact on the freshwater microbial community and influence their density (LINDSTRÖM *et al.*, 2005; CALIZ & CASAMAYOR 2014; ILIEV, 2015; LEW *et al.*, 2016). Temporal differences in water column bacterial indicators, due to temperature variations, have been previously reported for reservoirs and agricultural watersheds, with greatest abundances in July and August (SIMON & MAKAREWICZ, 2009; NORTH *et al.*, 2014). The increase of microbial population could affect the number of bacteria in the fish during handling and processing (GORLACH-LIRA *et al.*, 2012). Our research confirms that selected environmental factors regulate the microbial community in Kardzhali reservoir ecosystem. However, a significant part of the parameters as pH, well-known to regulate the microbial communities (ZMISLOVSKA *et al.*, 2001; LIN *et al.*, 2012; LEW *et al.*, 2016), had no visible impact in the current conditions of the ecosystem. In this regard, the observation of a secondary increase in the TVC at station NC4 and NC5 is most likely caused by additional input of organic matter in the area. The constant presence of *E.coli* and FC indicates the possible sewage and fecal contamination from the adjacent villages (Mishra, 2010). It showed that cage-farms are not the only source of anthropogenic pressure.

RDA confirmed that the distribution of the phytoplankton was highly related to the parameters Secchi depth and organic content (COD), which is agreement with the result of BRAHIM ERRAHMANI *et al.* (2015) and O'FARELL *et al.* (2002) who pointed out that the phytoplankton growth is strongly influenced by suspended matter content, which reflects on the water transparency. WU *et al.* (2014) also state that the nutrient composition and concentration is the driving force for phytoplankton development. The elevated concertation of phosphates during summer and early autumn and the low during the winter and spring periods are often observed in other lakes and reservoirs (TAMMEORG *et al.*, 2014). As a result, TP does not correlate with the development of phytoplankton communities, and due to its excess, it is no longer a limiting factor. The pH, dissolved oxygen, and temperature described, as basic parameters for phytoplankton development (DOCHIN, 2015; BRAHIM ERRAHMANI *et al.*, 2015) had no effect in the present study, due to the close proximity of the sampling stations to the cage farms.

Conclusions

The present work demonstrates that the environments in reservoirs with intensive aquaculture are characterized by fluctuations of the physicochemical factors, biological and microbiological indicators for water quality, caused by the nutrient loads. The organic inputs affect food-web interactions and appear to be one of the primary mechanisms that influence microbial phytoplankton communities. There was no correlation between TP and the phytoplankton parameters which may indicate that phosphorus was not a limiting factor despite its low natural bioavailability and rapid mineralization rates, suggesting regular phosphorus inputs from the feed and fish feces. The study confirms the reliability of the PCA and other multivariate analysis for the interpretation of the intricate relationships between the parameters in the complex data sets in heavily modified water bodies. RDA clearly revealed that the long-term exploitation is related to changes in physicochemical water quality, which in terms lead to alterations in the phytoplankton and bacterial communities. During the three-year monitoring campaigns, the reservoir was in an excellent sanitary condition. The persistent detection of low number *E. coli* and FC on the other side, in the vicinity of the net-cages and their absence in the control zone, suggests that there are non-point sources of pollution other than aquaculture activities, such as wastewater discharges from the nearby villages. Our findings point out the necessity of constant control and more frequent monitoring, using sensitive indicators such as phytoplankton and microbiological parameters.

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