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Hydrobiological variables as a regulatory factor on the abundance of heterotrophic flagellates in an urban pond

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Abstract The seasonal abundance of flagellates has been monitored over a period of 1 year from December 2013 to November 2014 (divided into 4 conjugative seasons namely winter, spring, summer, and autumn) in an experimental pond located in Rajshahi City Corporation area, Bangladesh. To our knowledge, this study is the first to shed light on the occurrence and possible interrelationships among heterotrophic flagellates (HF), bacteria and zooplankton in Bangladesh and the result obtained by this study will be beneficial for similar water ecosystem all over the world. Standard methods were used to determine the prescribed hydrological parameters and zooplankton cell density. Maximum HF abundance (14 346.00 cells/mL) was found in the spring and the minimum (5 215.00 cells/mL) occurred in the summer. Inverse to HF, significantly (P<0.05) higher zooplankton abundance was found during the winter (782.00±47.62 cells/mL) and the lowest value was found in the autumn (448.00±39.15 cells/mL). Whereas similar to the HF, total bacterial abundance was significantly higher during the spring ((2.25±1.05)×10^5 cells/mL) and lower in the summer ((0.79±0.06)×10^5 cells/mL). Multivariate analyses (ANOSIM and MDS) have shown significant seasonal differences for cell numbers where MDS ordination plot and cluster analysis based on similarity in the genera abundance of HF revealed overlapping condition between winter and spring. Canonical correspondence analysis (CCA) also showed a distinct separation among the genera based on the prevailing hydrological situation and indicated that temperature, pH, BOD_5, and NO_3^- were the most important environmental variables in determining the observed variation in HF community structure. Among the biological factors, zooplankton showed negative but total bacteria were positively correlated with HF abundance.

Keyword: heterotrophic flagellates; abundance; hydrobiological factors; urban pond

1 INTRODUCTION

Heterotrophic flagellates form an important and diverse component of the communities living in aquatic ecosystems as they are the predators on bacteria and small phytoplankton, prey for larger zooplankton, and facilitate re-mineralization and recycling of elements essential for phytoplankton and microbial growth (Aydin and Demirsoy, 2012). Free-living heterotrophic flagellates are distinguished from the other protists by their smallest size and high reproduction rate. These smallest organisms represent an essential link in microbial food loops, ensuring efficient methods of the transformation of substances and energy in aquatic ecosystems (Tikhonenkov et al., 2015). In the microbial loop, heterotrophic bacteria convert dissolved organic matter of autochthonous or allochthonous origin into particulate organic carbon. Therefore, the heterotrophic bacteria are then grazed by heterotrophic flagellates and small ciliates. Additionally, some HF is found to be capable to prey on both bacteria and phytoplankton and regenerating nitrogen from grazed microbes (Goldman and Caron, 1985). The hydrological regime is considered the relevant factors driving the structure and dynamic of the planktonic flagellate protozoa community in the aquatic ecosystem (Jiang and Shen, 2005, Camargo and Velho, 2010). In general, this community can also be controlled by biotic factors, such as food resource availability, predation pressure and trophic state (Samuelsson et al., 2006; Araújo and Godinho, 2008; Camargo and

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Velho, 2011). Seasonal variation in rainfall regime can control flagellates by modifying community structure and their metabolism in the environment (Cushing and Allan, 2001; Araújo and Godinho, 2008). Therefore, major factors controlling the structure of the flagellate community is essential to monitor, management, conservation, and restoration of aquatic ecosystems (Camargo et al., 2012). Several studies on heterotrophic flagellate's diversity, composition and species richness, seasonal dynamics (Kiss et al., 2009; Camargo and Velho, 2011), distribution (Camargo et al., 2012; Tikhonenkov et al., 2015) and grazing experiments (Guillou et al., 2001; Thurman et al., 2012) have been conducted in abroad but information related to heterotrophic flagellates species composition, seasonal occurrence, and abundance, and their prey organisms are evidently unknown in Bangladeshi waters. In the present study, we focus on the seasonal changes of heterotrophic flagellate cell density with the changes in hydrobiological factors of pond water.

2 MATERIAL AND METHOD

2.1 Study area

The study was conducted in an urban fishpond located in Rajshahi City Corporation for a period of 12 months from December 2013 to November 2014. The respective study period is categorized into 4 seasons according to Wang et al. (2016), where winter season was consisted of December 2013 to February 2014, spring as March to May 2014, summer as June to August 2014 and autumn as September to November 2014. The pond was rainfed and devoid of inlet and outlet facilities and got over flooded during the rainy season and it was greatly modified by human activities such as clandestine disposal of domestic effluents, solid waste, debris of construction materials, cans, plastic bottles, tires, old furniture etc.

2.2 Analysis of water quality

Surface water samples were collected once a month from 10 to 11 am. For accuracy of sampling, samples were taken from three distinct places of the pond. For the estimation of different chemical parameters, water samples were collected in plastic bottles. Celsius thermometer was used to measure surface water temperature. The dissolved oxygen (DO) concentration and Biological Oxygen Demand (BOD) of the collected water samples were determined in laboratory condition by the Winkler's titration method. NO_3^- and pH were measured using a Hach Kit (Model FF-2, USA). Oxidation Reduction Potential (E_h) and Oxidation Reduction Index (rH_2) were calculated by using the formula given by Mukherjee (1996).

2.3 Determination of Zooplankton

For zooplankton study, 10-L of water sample was collected from pre-selected places in plastic containers. The mesh size of the plankton net was 25 μm. Zooplankton samples were preserved in 5% formalin for further studies in the laboratory. In the laboratory, 2–3 drops of the settled zooplankton sample were placed on a glass slide. The quantitative estimation of zooplankton was done by Sedgewick-Rafter counting chamber (S-R cell) following the method described by Rahman (1992)

$$N=(A\times 1000\times C)/V\times F\times L,$$

where, N is the number of plankton cells, A is the total number of zooplankton counted, C is the volume of the final concentrate of the sample in mL, V is the volume of a field, F is the number of the field counted, and L is the volume of original water in liter.

2.4 Heterotrophic flagellates and bacteria count method

HF and bacterial counts were conducted with epifluorescence microscopy following Hobbie et al., (1977). For counting, 1 mL of filtered acridine orange solution was used to obtain a final solution of 0.01%. The samples were then filtered through 0.2-mm Nuclepore filters pre-stained with Irgalan black. Counting was carried out with a 100× immersion objective on an epifluorescence microscope fitted with a mercury lamp and interference filters chosen to give blue excitation light. Direct counts were made with the aid of an ocular grid (Fenchel, 1982).

2.5 Statistical Analysis

One-way analysis of variance (ANOVA) at 5% level of significance was used to test for significance in the seasonal variation in hydrological parameters as well as total zooplankton and bacterial cell abundance and relationship between HF and other two biological variables (zooplankton and bacteria) was determined by correlation coefficient analysis using Statistical Package for Social Sciences (SPSS) version 20.0. Duncan's post hoc tests were employed to check for the difference in water quality and

Table 1 Hydrobiological variables (mean±SD) of the experimental pond during the study period

	Winter	Spring	Summer	Autumn	*F*-value	*P*-value
Environmental variable						
Temperature (°C)	18.11±1.55^{c}	31.09±2.66^{ab}	32.17±1.34^{a}	28.51±4.45^{b}	47.900	0.000
pH	7.81±0.40^{a}	7.56±0.07^{ab}	6.79±0.20^{c}	7.37±0.27^{b}	23.941	0.000
DO (mg/L)	6.25±1.28^{a}	4.96±1.36^{b}	3.79±0.26^{c}	5.17±0.71^{b}	8.981	0.000
BOD_5 (mg/L)	1.10±0.45^{b}	1.32±0.40^{b}	3.23±0.47^{a}	1.45±0.80^{b}	28.567	0.000
NO_3^- (mg/L)	0.20±0.10^{b}	0.35±0.11^{a}	0.30±0.12^{ab}	0.29±0.13^{ab}	2.627	0.067
E_h (mv)	0.44±0.05^{a}	0.41±0.06^{ab}	0.37±0.05^{b}	0.38±0.02^{b}	3.973	0.016
rH_2	30.38±1.38^{a}	29.28±1.74^{ab}	28.40±1.46^{b}	27.98±2.05^{b}	3.601	0.024
Biological variable						
TZ (cells/mL)	782.00±47.62	427.33±15.63	583.00±84.48	448.00±39.15	6.812	0.001
TB ($\times10^5$ cells/mL)	1.17±0.23	2.25±1.05	0.79±0.06	0.98±0.13	18.665	0.000

Key: TZ: total zooplankton; TB: total bacteria.

abundance of the HF and other biological variables between every pair of seasons. Aiming to summarize graphically the patterns of seasonal distribution of genera abundance, multidimensional scaling analysis (nMDS) was used. To construct the similarity matrices, Bray-Curtis coefficient was carried and to test if the abundance data of HF shows significant seasonal distribution, it was employed one-way ANOSIM analysis using PAST (version 3.0) software. Canonical correspondence analysis (CCA) was also carried out using PAST (version 3.0) software to elucidate the possible relationships between genera of HF and the hydrological variables.

3 RESULT

3.1 Hydrobiological variables

Over the study period significant seasonal differences were reported for all of the hydrological variables such as temperature (F=47.900; P=0.000), pH (F=23.941; P=0.000), DO (F=8.981; P=0.000) E_h (F=5.95; P=0.004 5) and rH_2 (F=3.37, P=0.03) (Table 1). As expected, water temperature was higher during summer (32.17±1.34°C) and pH was acidic in nature (6.79±0.20). Inverse to temperature, the concentration of dissolved oxygen was higher during winter (6.25±1.28 mg/L) than in other seasons. BOD_5 value showed its higher value during summer, while NO_3^- seasonality indicated a significant increase in spring and lower value in the winter. Significantly higher value of both E_h and rH_2 was found during the winter, whereas, lower value of E_h and rH_2 was recorded in summer and autumn respectively. There was a significant difference in the mean values of biological variables during the study period (Table 1). The highest value of total zooplankton abundance (TZ) was found during the winter (782.00±47.62 cells/mL) and the lowest value was found in the spring (427.33±15.63 cells/mL). Similar to the TZ, total bacterial abundance (TB) was higher during the spring ((2.25±1.05)×10^5 cells/mL) and lowest in summer ((0.79±0.06)×10^5 cells/mL).

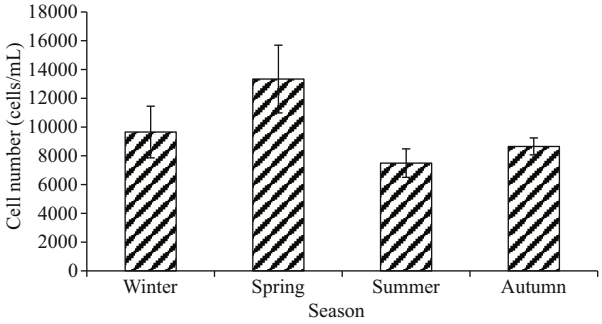

Fig.1 Seasonal variation of heterotrophic flagellates cell number during the study period

3.2 Abundance and distribution pattern of heterotrophic flagellates

A total of 12 genera were identified from the experimental pond during the study period. The total abundance of HF varied appreciably from 5 215.00 to 14 346 cells/mL with a mean of 9 743.58 cells/mL. Total numbers of HF varied significantly with season (ANOSIM, P<0.002, R=0.84) and they were much greater in spring (13 337.67 cells/mL) and winter (9 653.33 cells/mL) than in autumn (8 651.67 cells/mL) and summer (7 491.67 cells/mL) (Fig.1). The main genera shaping the overall HF community during the study period were *Euglena* (41.62%), *Trachelomonas* (13.54%), *Ceratium* (9.13%), *Peridinium* (8.39%)

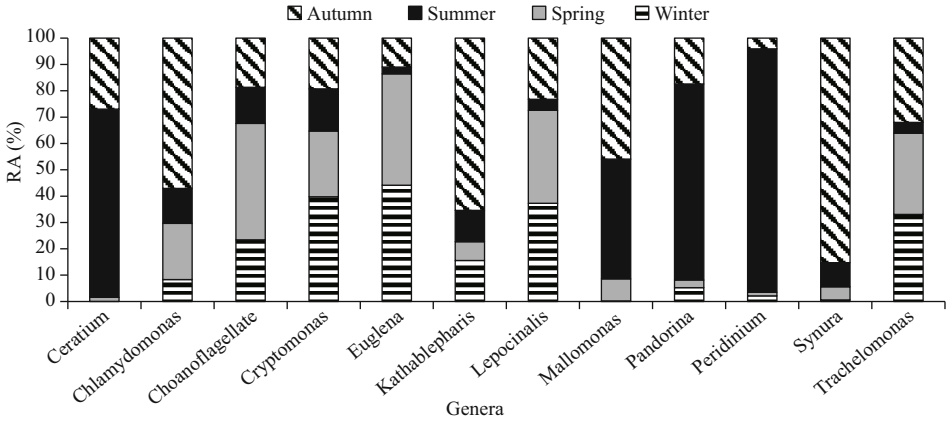

Fig.2 Relative abundance of HF genera during the study period

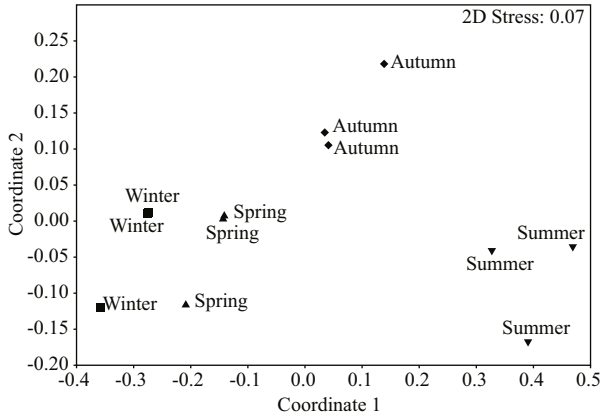

Fig.3 Nonmetric multidimensional scaling plot comparing the HF abundance samples collected in winter, spring, summer and autumn seasons during the study period

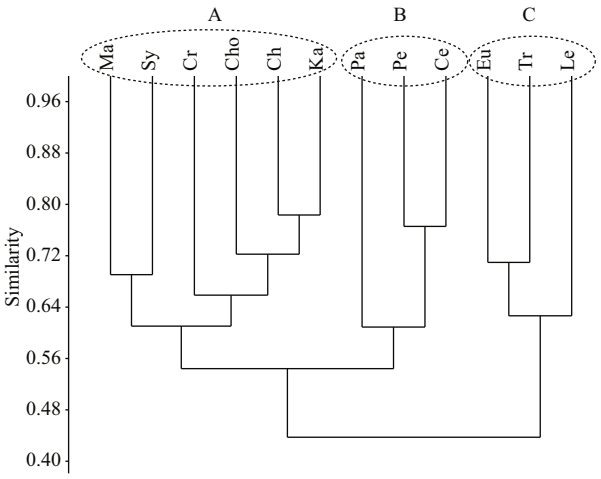

Fig.4 Classification of samples by their genera abundance in winter, spring, summer and autumn seasons

A–C different groups of communities. Ce: *Ceratium*; Ch: *Chalamydomonas*; Cho: *Choanoflagellate*; Cr: *Cryptomonas*; Eu: *Euglena*; Ka: *Kathablepharis*; Le: *Lepocinalis*; Ma: *Mallomonas*; Pa: *Pandorina*; Pe: *Peridinium*; Sy: *Synura*; Tr: *Trachelomonas*.

and *Lepocinclis* (7.96%), which contributed to 80.64% of the overall genera. During spring and winter, relative abundance (RA) of *Euglena* was higher than of the other genera (Fig.2), but during summer and autumn, *Ceratium* and *Synura* showed higher RA, respectively.

The difference in abundance of HF was also clearly reflected on the MDS plots of genera assemblage centroids, which exhibited no overlap in the ordination among the summer and autumn. However, the overlap between winter and spring season indicated some similarities between these seasons (Fig.3). Hierarchical cluster analysis (Fig.4) was performed to classify the samples by genera abundance among the seasons to reveal genera that tends to be associated with seasonal variation. Classification of samples indicates three groups of communities (Fig.3). Ordination of samples shows the results similar to MDS plot, where, autumn and summer constitute cluster "A" and "C", whereas winter and spring combined to form the cluster "B" (Fig.4).

3.3 Influence of environmental and biological variables on HF abundance

Regarding the ordination of CCA, the first two axes were considered, which expressed the highest variability in abundance data (Fig.5). They explained 90.61% of the cumulative constrained variance in the genera environment biplot (axis 1, 63.03%, eigenvalue 0.609; axis 2, 23.58%, eigenvalue 0.214). The main factors determining the differences in communities along axis 1 are temperature and BOD_5. Along axis 2, the most influencing parameter is NO_3^-. These influencing factors cause the genera to separate in distinguished groups based on seasonal basis: (A) genera (*Ceratium*, *Pandorina*, and *Peridinium*) that were more abundant in summer season and mostly influenced by higher value of temperature and BOD_5; (B) genera (*Chlamydomonas*, *Kathablepharis*,

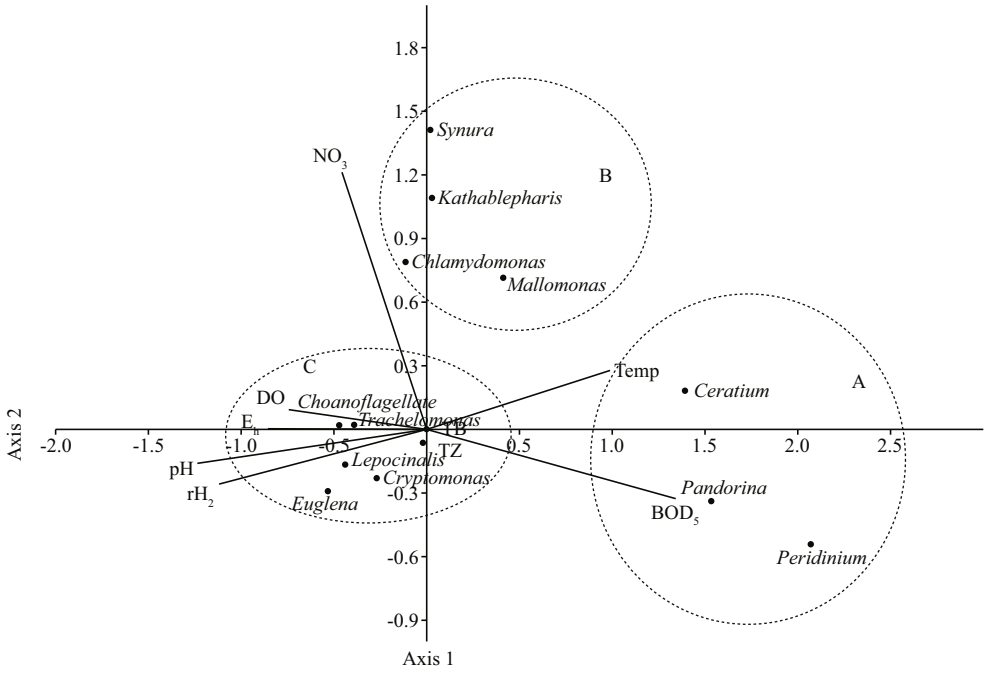

Fig.5 Canonical correspondence analysis (CCA) biplot

Analysis, with the impact of the abiotic variables: Temp: temperature; DO: dissolved oxygen; BOD_5: biological oxygen demand. A: summer; B: monsoon; C: winter.

Table 2 Correlation among heterotrophic flagellates, total zooplankton, and total bacteria

	Heterotrophic flagellates	Total zooplankton	Total bacteria
Heterotrophic flagellates	1		
Total zooplankton	-0.218	1	
Total bacteria	0.433	-0.301	1

Mallomonas and *Synura*) more abundant in autumn; and (C) indicates genera (*Choanoflagellate*, *Cryptomonas*, *Euglena*, *Lepocinalis*, and *Trachelomonas*) overlaps in the winter and spring and prefer higher value of DO, pH, E_h, rH_2. Correlation analysis among the heterotrophic flagellates, total zooplankton, and total bacteria is shown in Table 2. The total cell density of heterotrophic flagellates was negatively correlated with total zooplankton and positively with total bacterial abundance. Total zooplankton abundance was also negatively related to total bacterial cell density during the study period (Table 2).

4 DISCUSSION

During the present study, a total of 12 genera of heterotrophic flagellates were identified and the highest abundance was found for genus *Euglena* which was responsible for 41.62% of total genera identified. *Trachilomonas*, the second highest contributor, represents 13.54% of the total generic abundance of heterotrophic flagellates. Camargo and Velho (2010) also reported a higher abundance of *Euglena* and *Trachilomonas* in their study in floodplain ecosystem compared to the present study. Mean heterotrophic flagellates abundance in the studied pond ranged from 7 491.67 cells/mL in summer to 13 337.67 cells/mL in spring. These results are consistent with other reports that show significantly higher numbers of heterotrophic nanoflagellates in spring compared to other seasons (Monger and Landry, 1991; Hall et al., 1993; Solic and Krstulovic, 1994; Zhao et al., 2003). The results of the One-Way ANOSIM revealed that the genera composition had a significant seasonal pattern, which was also reported by Kiss et al. (2009), Camargo et al. (2012), and Amorim and Araújo (2012). Based on MDS and cluster analysis, we may infer that the temporal pattern observed for the abundance was influenced by environmental parameters. As shown in CCA analysis, the main factors determining the differences of generic status of heterotrophic flagellate communities are temperature, pH, BOD_5, and NO_3^-. Moreover, as reported by Camargo et al. (2012) and Arndt et al. (1996), acidic pH, high water temperature and the oxygen availability are considered the main limiting factor for heterotrophic flagellate growth in a water body. Therefore, lower abundance of heterotrophic

flagellates during summer may be explained by acidic pH, low availability of oxygen, and higher temperatures that may have unsettled the flagellate community structure. On the other hand, during the summer, the pond becomes enriched through drainage of decomposed materials, surface runoff and seepage from surroundings, which increases the biological activity (Naz, 1999). Increased biological activity resulted in the highest value of BOD_5 in summer due to an elevated temperature, acidic pH and reduced available DO in water that makes the water body unfavorable for the growth of flagellates and other organisms (Kosolapova and Kosolapov, 2011). Although, winter holds favorable DO and pH for heterotrophic flagellates growth, lower NO_3^- did not benefit the flagellates as a whole. Therefore, urban influence is considered the most important factor for determining the structure of flagellate community composition. A similar observation was also made by Camargo et al. (2012). The urbanization effects on aquatic environments are referred to as urban stream syndrome (Meyer et al., 2005), where changes in water quality parameters occurred. In the present study, urban stream syndrome was found to occur mainly due to the practice of anthropogenic such as municipal and industrial activities. However, rainfall works as an agent for the structural distribution, dynamics, and metabolism of the environment. Biota in the present study was influenced mostly by the concentration of NO_3^- that was carried to the water body through runoff and trigger changes in the inorganic nutrient concentration of the habitat during the rainy season (Cushing and Allan, 2001). Thus in this period, only species more adapted to these adverse conditions can stay in the environment, which possibly explains the enrichment of three flagellates genera namely *Ceratium*, *Pandorina*, and *Peridinium*. However, in the present study, the oxidation-reduction condition may affect the distribution of heterotrophic flagellates to a certain extent. As reported earlier, anaerobic forms are found in the bottom with a low oxidation-reduction index, while aerobic forms occur in areas that have a higher index (Mukherjee, 1996). Therefore, in the present study, we found that the oxidation-reduction index has decreased during the summer and it negatively influences the HF abundance. The relationship of heterotrophic flagellate with other biological groups through grazing interactions has also been recognized as having a major effect on their abundance. During the study period, heterotrophic flagellates showed a positive correlation with bacterial abundance and negative with total zooplankton cell abundance, which was in agreement with the findings of Nakano and Kawabata (2000) and Kobari et al. (2010) who also observed a positive relationship between heterotrophic flagellates and total bacteria. However, a higher value of bacterial cell density during the spring in the present study may be explained by the increase in decomposition rate of the organic matter, which was facilitated by raising temperature that provided a higher amount of prey to the heterotrophic flagellates. Urban system syndromes also produced more dissolved organic carbon (DOC) in the pond water. Increased level of DOC in water might positively influence the bacterial abundance and ultimately increased the abundance of heterotrophic flagellate. A similar observation was also made by Kosolapova and Kosolapov (2011), who reported that municipal sewage of the city increases the DOC in water, in which the abundance, species diversity, and activity of heterotrophic flagellates and bacteria increased. During the study period, zooplankton grazing was observed to have negative effects on bacterial abundances as well as on heterotrophic flagellate abundance. A similar result was also reported by Gasol and Vaque (1993) in other types of habitat. In a study conducted by Khalifa and Sabae (2012) also stated that total bacterial count was negatively correlated with rotifers abundance based on a prey-predator relationship in a freshwater ecosystem.

5 CONCLUSION

In summary, the results obtained showed that the community succession of flagellates genera in the studied pond depends on both hydrological and biological variables, which can be bothered by the seasonal changes. Hydrobiological changes caused by human activities may also imply a significant effect in flagellate community structure. As the genera of *Euglena*, *Trachilomonas* and *Lepocinalis* are good indicators of pollution and the great success of these groups in the studied pond during spring and winter attributed to some extent of polluted nature of the experimental pond. However, lower abundance of flagellates genera during summer was better explained by rainfall regime.

6 DATA AVAILABILITY STATEMENT

Data are available on request to the authors.

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