Fluctuation in density of ciliates in Bukan dam reservoir, 
Zarrinehrud, Iran

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Abstract
The abundance and species composition of ciliates were analyzed in the Bukan Dam Reservoir (West Azerbaijan, Iran) from January to December 2008. Surface water samples were collected at monthly intervals from the middle (Stations 1 and 3) and shallow (Stations 2 and 4) areas of the lake. Three samples were taken in the water column using a 5-litre sampler. Totally 30 liters water was collected, 200 ml of which was fixed immediately with 8.6 ml of a saturated HgCl₂ solution and stained with 0.04% bromophenol blue. Three subsamples were counted in a 5-ml chamber and examined with a microscope (100 x) as well as Chlorophyll a and a few ecological parameters such as dissolved oxygen, pH, transparency and water temperature were measured. Totally 50 ciliates species were found in Bukan Dam Reservoir. Regarding the density, a high-density period was detected from January to April with a mean density of 2.86 x 10³ cells.l⁻¹ Ciliata at stations 1 and 3 and 4.16 x 10³ cells.l⁻¹ at stations 2 and 4. The high density of ciliate in summer may be due to the increase in the density of bacteria and moderate metazooplankton as a result of the appearance of non-edible algae. The ciliata occurring at the highest densities were Coleps tessellates, Didinium nasutum, Paradileptus elephantinus, Stentor polymorphus, Zosterograptus labiatus, Paramecium bursaria, Cyclidium citrullus, Vorticella campanula, Halteria grandinella and Aspidisca costata. The maximum and minimum chlorophyll a values observed at the reservoir water were 10.39 µg.l⁻¹ and 1.0 µg.l⁻¹ being obtained in May and October respectively.

Keywords: Ciliata, Density, Bukan dam reservoir, Iran

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Introduction
Bukan Dam Reservoir is located in southern part of West Azerbaijan Province, with a surface area of 45x10^6 m^2. It has been formed after constructing of a dam (48m high) in 1969. The total volume of the reservoir is 650x10^6 m^3, useful volume is 486x10^6 m^3 (Annon, 2008). The ecological role of planktonic ciliates as trophic links between bacteria and pelagic zooplankton has been increasingly appreciated during recent years (Foissner et al., 1999). Ciliata are known to play an important role by consuming bacteria and thus reducing their numbers in environments rich in organic matter (Javornicry and Perokesova, 1963). They also consume phytoplankton (Brook, 1952) and are consumed by Cladocera, Copepoda and Rotifera (Rerk et al., 1977) in addition to being highly efficient in releasing phosphorus (Porter et al., 1979).

Study of the zooplankton cycle in a marine environment, showed that protozoa act as a link in the food chain of the sea between small planktonic organisms to the large metazooplanktonic herbivores (Smetacek, 1981). Graze on autotrophic and heterotrophic pico and nanoplankton and functioning as prey for larger zooplankton, contribute to the remineralization and cycling of nutrients (Blomqvist et al., 2001; Ventela et al., 2002). In total, ciliata populations play a pivotal role in the indication of pollution degree in lakes (Sonntag et al., 2002). Alekperov (1977) suggested that the mean number of species in Mangirchober, Varvara and Jeiranbatal reservoirs (located in Azarbaijan) were 50, 91 and 81, respectively. Moreover, the average density of ciliates in Mangirchober, Varvara and Jeiranbatal reservoirs were 5138, 38901 and 13413 (Ind.l^-1), respectively. On the other hand, they concluded that Mangirchober Reservoir was mesotroph and two other reservoirs were eutroph. The studies about the ciliata composition in freshwater are scarce in Iran. Thus, the present investigation was undertaken to study the abundance and composition of planktonic ciliates and some ecological parameters in the middle and shallow area in Bukan Dam Reservoir.

Materials and methods
Sampling was performed in four sampling sites from January to December 2008. Surface water samples were collected in monthly intervals at two different points, (stations 1 and 3) and in the shallowest area of the Reservoir (stations 2 and 4) and examined for ciliates composition and density. Thriplate samples were taken in the middle water column using a 5-litre sampler (Bernatowicz, 1953). Totally 30 liters was collected. 200 ml of each sample was fixed immediately with 8.6 ml of a saturated HgCl_2 solution and stained with 0.04% bromophenol blue (Pace and Orcutt, 1981). Three sub samples were counted in a 5-ml chamber and examined with a microscope (100 x). Ciliata were identified according to Jahn et al. (1949), Pennak (1953), Corliss (1979) and Foissner et al. (1999). Phytoplankton chlorophyll a concentrations were estimated according to the method of Parsons and Strickland (1965) after 24 h extraction in 90% acetone. Additionally, the following physical and chemical factors were examined: transparency, pH, O_2, conductivity and biogens (TN, PO_4, N-
NH$_4$, N-NO$_3$). Transparency, pH, O$_2$ and conductivity was determined *in situ* using the Secchi disc and electrode Jenway 3405 and remaining factors were measured in the laboratory, according to Hermannowicz et al., (1976). The data were processed in Excel and analysed by SPSS software.

**Figure 1:** Location of Bukan Dam Reservoir and the sampling sites (36° 23’ 42˝ N and 46° 33’ 17˝ N)

**Results**
The seasonal variations of transparency, dissolved oxygen and chlorophyll *a*, water temperature and pH were shown in Table 1.

**Figure 2:** Monthly variations of chlorophyll *a* (µg/L) in the present study
Table 1: Physical properties of water in the present study

<table>
<thead>
<tr>
<th></th>
<th>Transparency (m)</th>
<th>Dissolved oxygen (mg.l(^{-1}))</th>
<th>Chlorophyll a (µg.l(^{-1}))</th>
<th>Water Temperature (°C)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan.</td>
<td>1.4</td>
<td>12.00</td>
<td>6.2</td>
<td>6.0</td>
<td>8.4</td>
</tr>
<tr>
<td>Feb.</td>
<td>1.5</td>
<td>14.40</td>
<td>3.8</td>
<td>4.8</td>
<td>7.6</td>
</tr>
<tr>
<td>Mar.</td>
<td>1.8</td>
<td>11.30</td>
<td>5.3</td>
<td>7.8</td>
<td>7.7</td>
</tr>
<tr>
<td>Apr.</td>
<td>1.7</td>
<td>11.81</td>
<td>2.3</td>
<td>14.5</td>
<td>8.1</td>
</tr>
<tr>
<td>May</td>
<td>1.6</td>
<td>9.61</td>
<td>10.4</td>
<td>17.1</td>
<td>8.3</td>
</tr>
<tr>
<td>Jun.</td>
<td>1.7</td>
<td>12.80</td>
<td>1.8</td>
<td>20.0</td>
<td>8.4</td>
</tr>
<tr>
<td>Jul.</td>
<td>1.0</td>
<td>8.00</td>
<td>1.6</td>
<td>24.2</td>
<td>8.0</td>
</tr>
<tr>
<td>Aug.</td>
<td>1.5</td>
<td>10.12</td>
<td>1.7</td>
<td>27.5</td>
<td>7.7</td>
</tr>
<tr>
<td>Sep.</td>
<td>1.5</td>
<td>7.80</td>
<td>2.6</td>
<td>25.2</td>
<td>7.3</td>
</tr>
<tr>
<td>Oct.</td>
<td>1.4</td>
<td>8.00</td>
<td>1.0</td>
<td>18.0</td>
<td>8.1</td>
</tr>
<tr>
<td>Nov.</td>
<td>1.5</td>
<td>5.51</td>
<td>3.8</td>
<td>14.1</td>
<td>8.6</td>
</tr>
<tr>
<td>Dec.</td>
<td>1.4</td>
<td>13.92</td>
<td>2.9</td>
<td>13.3</td>
<td>8.4</td>
</tr>
<tr>
<td>Average</td>
<td>1.5</td>
<td>10.44</td>
<td>3.6</td>
<td>16.0</td>
<td>7.5</td>
</tr>
</tbody>
</table>

Chlorophyll \(a\) fluctuation showed two distinct phases: a period from May to June during which values were higher than those observed at a period from July to October. The maximum and minimum chlorophyll \(a\) values observed at the reservoir water were 10.4 µg.l\(^{-1}\) and 1 µg.l\(^{-1}\), in May and October, respectively (Fig. 2). The maximum transparency value observed at the reservoir water was 1.8 m and the minimum was 1 m, being obtained in March and July, respectively (Fig. 3).

Figure 3: Monthly variations of water transparency (m) in the present study

Figure 4: Monthly variations of dissolved oxygen (mg/l) in the present study
The maximum dissolved oxygen value observed at the reservoir water was 14.40 mg.l\(^{-1}\) and the minimum was 5.51 mg.l\(^{-1}\) being obtained in February and November, respectively (Fig. 4).

Temperature and pH profiles at the reservoir were shown in Figs 5 and 6. High temperatures were recorded from July to September, with a maximum value of 27.5 °C. The minimum value of 4.8 °C was observed in February. pH was between 7.3 and 8.6 during the study period.

![Figure 5: Monthly variations of water temperature (°C) in the present study](image)

![Figure 6: Monthly variations of pH in the present study](image)

### Table 2: Chemical properties of water in the present study

<table>
<thead>
<tr>
<th></th>
<th>Hardness (mg.l(^{-1}))</th>
<th>Ec (µs.cm(^{-1}))</th>
<th>PO4 (mg.l(^{-1}))</th>
<th>TN (mg.l(^{-1}))</th>
<th>BOD (mg.l(^{-1}))</th>
<th>N-NO(_3) (mg.l(^{-1}))</th>
<th>N-NH(_4) (mg.l(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan.</td>
<td>150.40</td>
<td>61.40</td>
<td>0.14</td>
<td>1.09</td>
<td>6.42</td>
<td>1.60</td>
<td>0.20</td>
</tr>
<tr>
<td>Feb.</td>
<td>159.20</td>
<td>229.80</td>
<td>0.16</td>
<td>1.58</td>
<td>2.92</td>
<td>1.13</td>
<td>0.10</td>
</tr>
<tr>
<td>Mar.</td>
<td>145.71</td>
<td>281.57</td>
<td>0.06</td>
<td>1.24</td>
<td>2.65</td>
<td>1.42</td>
<td>0.15</td>
</tr>
<tr>
<td>Apr.</td>
<td>156.80</td>
<td>219.00</td>
<td>0.32</td>
<td>1.62</td>
<td>1.54</td>
<td>1.67</td>
<td>0.06</td>
</tr>
<tr>
<td>May</td>
<td>147.00</td>
<td>212.25</td>
<td>0.22</td>
<td>1.14</td>
<td>2.51</td>
<td>1.38</td>
<td>0.03</td>
</tr>
<tr>
<td>Jun.</td>
<td>158.33</td>
<td>150.10</td>
<td>0.34</td>
<td>1.19</td>
<td>1.41</td>
<td>1.00</td>
<td>0.16</td>
</tr>
<tr>
<td>Jul.</td>
<td>145.67</td>
<td>48.12</td>
<td>0.37</td>
<td>1.70</td>
<td>4.77</td>
<td>1.00</td>
<td>0.23</td>
</tr>
<tr>
<td>Aug.</td>
<td>141.75</td>
<td>46.65</td>
<td>0.04</td>
<td>1.39</td>
<td>2.19</td>
<td>1.00</td>
<td>0.12</td>
</tr>
<tr>
<td>Sep.</td>
<td>161.50</td>
<td>53.79</td>
<td>0.34</td>
<td>1.10</td>
<td>6.59</td>
<td>1.60</td>
<td>0.18</td>
</tr>
<tr>
<td>Oct.</td>
<td>173.00</td>
<td>73.09</td>
<td>0.09</td>
<td>1.22</td>
<td>5.69</td>
<td>1.00</td>
<td>0.15</td>
</tr>
<tr>
<td>Nov.</td>
<td>140.40</td>
<td>230.40</td>
<td>0.11</td>
<td>1.07</td>
<td>7.37</td>
<td>1.40</td>
<td>0.26</td>
</tr>
<tr>
<td>Dec.</td>
<td>172.80</td>
<td>53.64</td>
<td>0.15</td>
<td>1.73</td>
<td>4.87</td>
<td>1.14</td>
<td>0.15</td>
</tr>
<tr>
<td>Average</td>
<td>154.38</td>
<td>138.33</td>
<td>0.19</td>
<td>1.14</td>
<td>4.09</td>
<td>2.33</td>
<td>0.15</td>
</tr>
</tbody>
</table>
The chemical properties of water including hardness, conductivity, PO₄, TN, BOD, N-NO₃ and N-NH₄ varied among months and their particular values were presented in Table 2. The composition and density (mean values) of the major ciliates taxa in the middle and shallow areas of Bukan Dam Reservoir were presented as follows and in Fig. 4, respectively.

Table 3: Mean number of species, density and percent of Ciliates in the present study

<table>
<thead>
<tr>
<th>Classes of ciliata</th>
<th>Number of species (ind.l⁻¹)</th>
<th>Density (ind.l⁻¹)</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinetofragminophora</td>
<td>17</td>
<td>569</td>
<td>32</td>
</tr>
<tr>
<td>Oligohymenophora</td>
<td>15</td>
<td>898</td>
<td>50</td>
</tr>
<tr>
<td>Polyhymenophora</td>
<td>18</td>
<td>315</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>1782</td>
<td>100</td>
</tr>
</tbody>
</table>

Class: Kinetofragminophora

Order: Prostomatida
- Elopes hirtus, Holophrya atra, Holophrya hexatricha, Prorodon brachyodon,
- Prorodon viridis, Prorodon ovum, Prorodon teres and Coleps tesselatus.

Order: Haptorida
- Enchelys pupa, Didinium nasutum, Paradileptus elephantinus, Paradileptus conicus and Lacrymaria olor.

Order: Pleurostomatida
- Litonotus lamella.

Order: Nassulida
- Nassula citrea,
- Zosterograptus labiatus and Trithigmnostoma cucullalus.

Class: Oligohymenophora

Order: Hymnmostomatida
- Tetrahymena pyriformis, Glaucoma chattoni, Paramecium aurelia, Paramecium caudatum, Paramecium bursaria, Frontonia acuminate, Frontonia leucas,
- Frontonia elliptica, Urosentrum turbo and Stokesia vernalis.

Order: Scuticociliatida
- Pleuronema coronatum, Cyclidium glaucoma, Cyclidium citrullus, Vorticella nebulifera and Vorticella campanula.

Class: Polyhymenophora

Order: Heterotrichida
- Metopus sp., Stentor polymorphus, Stentor roeseli and Condylostoma rugosum.

Order: Oligotrichida
- Halteria grandinella, Strombidium viride, Strombidium mirabile, Strombidium fallax, Strombidium gyrans, Strombidium velox, Tintinnidium pusillum and Tintinnopsis cylindrata.

Order: Hypotrichida
- Oxytricha minor, Oxytricha pellionella, Stylonychia mytilus, Euplotes patella, Euplotes eurystomus and Aspidisca costata.
A total number of 50 ciliates species were found in plankton samples. The highest species diversity belonged to Polyhymenophora (18 species) and the lowest species diversity belonged to Oligohymenophora (15 species), which was not statistically significant. The average annual density of ciliata was 1782 Ind.l⁻¹. The highest density belonged to Oligohymenophora (898 ind.l⁻¹) and the lowest belonged to Pleurostomatida (315 ind.l⁻¹) which was statistically significant (P<0.01).

The monthly distribution of Ciliates populations in the Bukan Dam Reservoir was presented graphically in Fig. 7. Three different periods can be observed with respect to the density of protozoa detected, i.e. two high-density periods from January to March and from July to August and a low-density period from September to December.

The mean number of ciliata calculated during the low-density period at autumn was about 1000 ciliata l⁻¹ and 700 ciliata l⁻¹ in the shallow and middle areas, respectively which was not statistically significant. During the high-density period, the mean number at the shallow areas was about 4000 ciliata l⁻¹ while in the middle areas it was 2800 ciliata l⁻¹ which was statistically significant (P<0.05). Thus, during the high density period the mean number of ciliata was approximately four times more than that obtained during the low density period. The densities of ciliates showed clearly marked seasonal changes in Bukan Reservoir. They fluctuated from 1 ind. ml⁻¹ in April to 5 ind. ml⁻¹ in March which was statistically significant (P<0.01). During this period Prostomatida (Eoleps hirtus), Haptorida (Didinium nasutum and Paradileptus elephanteus), Nassulida (Zosterograptus labiatus), Hymnostomatida (Paramecium bursaria), Scuticociliatida (Vorticellacampanula), Heterotrichida (Stentor polymorphus and Metopus.sp), Oligotrichida (Halteria graninella) and Hypotrichida (Aspidisca costata) constituted about 60% of the total number of the ciliate. The present study clearly showed that ciliates density in the middle and shallow area were correlated positively (R² = 0.92) and ciliata density and transparency were negatively correlated (R² = - 0.56).
Discussion
The highest densities of planktonic ciliates in the Bukan Dam Reservoir were observed from January to April. The densities of ciliates showed clearly marked seasonal changes in the reservoir. Also the peaks in the winter and the summer were probably determined by abiotic water factors. They were observed also in other mesotrophic lakes (Takamura et al., 2000; Ventela et al., 2002). Protozoan abundance tended to increase with increase in the lake productivity (Beaver and Crisman, 1990). Similarly the present study showed that ciliates abundance correlated with reservoirs productivity. The concentration of appropriate food (bacteria, nanoflagellates and algae) are probably the major regulator of abundance, biomass and diversity of planktonic ciliata (Wisckowski et al., 2001). Very high densities of ciliata in late winter in Bukan reservoir might have been caused by the phytoplankton density. It seems that with the death of these organisms there is greater decomposition and consequently, a larger number of bacteria and amount of detritus available for the protozoa, which in turn cause an increase in density. Similar situation relating a large abundance of protozoa after the death of the phytoplankton and simultaneous bacterial growth has been previously observed in Lake Dalnee (Sorokin and Paveljeva, 1972). The small bacterivorous ciliata, mainly Scuticociliatida (Cyclidium glaucoma and C. citrullus) are typical of
Mesotrophic lakes (Beaver and Crisman, 1990). Omnivorous *Coleps tesselatus* and *Vorticella* sp. are also dominated in the middle area of lakes in pH>7 and their importance increases with eutrophication (James et al., 1995). The highest densities occurrence of *Coleps tesselates*, *Paradileptus elephantinus*, *Stentor polymorphus*, *Zosterograptus labiatus* and *Cyclidium citrullus* in late winter showed an increase in eutrophication in the reservoir. This happened after entry of water with high loads of nutrients resulted from sewage water of city of Sagez at this season. The densities of ciliata showed significant seasonal changes in Bukan reservoir. The data obtained in the present study particularly chlorophyll *a* concentration and transparency confirmed mesotrophic status of the reservoir and were similar to that observed in other mesotrophic lakes (Carlson, 1977; Wetzel 1983). The results of the present study suggested that Bukan Dam Reservoir had similar species number and relatively identical density with Mangirchober Reservoir which may indicate the same trophy status of these two reservoirs (both reservoirs are mesotrophic).

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