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Seasonal Dynamics and Biomass of Mixotrophic Flagellate *Dinobryon sertularia* Ehrenberg (Chrysophyceae) in Derbent Reservoir (Samsun, Turkey)

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Abstract

Mixotrophic protists, combining both heterotrophy and phototrophy, are found abundantly in eutrophic waters. *Dinobryon sertularia* Ehr. from Chrysophyceae (golden algae) are mixotrophic organisms often make up blooms and colony in pools, lakes and dam reservoirs. This study was carried out in Derbent Dam Lake in the Middle Black Sea Region. Seasonal dynamics and biomass of *D. sertularia* were investigated at four stations between February 2001 and July 2002. *D. sertularia* consisted of 47-60% in spring, 61-79% in summer, and 82-88% in autumn of all phytoplankton population. In August 2001, *D. sertularia* was calculated in the highest amounts numbers (18,740-13,140 cells ml^{-1}). This species provided important contributions to phytoplankton biomass. In the present study, it was found that there is an inverse correlation between water depth and phytoplankton biomass production. According to Bray-Curtis Similarity Indice, 88% similarity was determined among Station 3, Station 4 and 4-6 m of Station 1. *D. sertularia* was dominant and common organism in all seasons. The reservoir water was defined as oligomesotrophic and alkaline characters (pH 7.98), and average temperature was 15.56°C.

Keywords: *Dinobryon sertularia*, mixotrophy, phytoplankton, biomass, Derbent Reservoir.

Derbent Rezervuarı'nda (Samsun, Türkiye) Miksotrofik Flagellat *Dinobryon sertularia* Ehrenberg (Chrysophyceae)'nın Biyoması ve Mevsimsel Dinamiği

Özet

Fototrofi ile heterotrofiyi birleştiren miksotrofik protistler ötrofik sularda bol bulunurlar. Tatlısu fitoplanktonik flagellatlılarından olan *Dinobryon sertularia* Ehr. Chrysophyceae'den (altın sarısı algler), koloni oluşturan, planktonik, göl, gölet ve baraj gölü fitoplanktonunda zaman zaman aşırı çoğalmalar yapan miksotrofik bir organizmadır. Bu çalışma Orta Karadeniz Bölgesi'nde yer alan Derbent Baraj Gölü'nde yapılmıştır. *D. sertularia*'nın mevsimsel dinamiği ve biyoması dört istasyonda Şubat 2001-Temmuz 2002 arasında incelenmiştir. *D. sertularia*, ilkbaharda fitoplanktonun %47-60'ını, yazın %61-79'unu, sonbaharda ise %82-88'ini oluşturmuştur. Ağustos (2001) ayında en yüksek sayılarda kaydedilen *D. sertularia* (18.740-13.140 cells ml^{-1}), bu ayda fitoplankton biyomasına önemli katkı sağlamıştır. Mevcut çalışmada biyomas üretimi ve derinlik arasında zıt bir ilişkinin olduğu tespit edilmiştir. Bray-Curtis benzerlik indeksine göre 3. ve 4. istasyonlar ile 1. istasyonun 4-6 m derinlikleri arasında %88 oranında benzerlik kaydedilmiştir. Oligo-mezotrofik karakterli, bazik özellik gösteren (7,98) ve ortalama sıcaklığı 15,56 °C olan Derbent Rezervuarı'nda *D. sertularia* her mevsim dominant ve çok yaygın organizmalar içinde yer almıştır.

Anahtar Kelimeler: *Dinobryon sertularia*, Miksotrofi, Fitoplankton, Biyomas, Derbent Baraj Gölü.

Introduction

Mixotrophy, known as a nutritional strategy, is a feeding capacity of certain organisms in autotrophic and heterotrophic environments. It is mostly used for protists being phototrophic and phagotrophic. These organisms can combine heterotrophy and phototrophy (Stockner, 1988). Protists are commonly found in coastal and open oceans, including bays, euphotic zones, mesotrophic and eutrophic waters from equator to polar areas.

The phenomenon of bacterial ingestion or uptake unicellular algae by phytoflagellates has been observed not only recently but also in the past (Pascher, 1911; Aaronson and Baker, 1959; Pringsheim, 1963). Mixotrophic species are known in many groups such as Chrysophyceae, Prymnesiophyceae, Xanthophyceae, Dinophyceae and Cryptophyceae (Porter, 1988). They may reach similar ingestion rates as purely heterotrophic flagellates in oceans (Estep *et al*., 1986; Arenovski *et al*., 1995) and lakes (Bird and Kalff, 1986; Sanders *et al*., 1989). Besides, they also contribute to the nutrient supply via ingestion of bacteria, as a carbon source (Doddema and Van Der Veer, 1983; Nygaard and Tobiesen, 1993; Li *et al*., 2000).

Mixotrophy is quite prevalent in most of phytoplanktonic organisms including ciliates, sarcodines and flagellates as Chrysophyta, Cryptophyta and Dinophyta. Some genus included in the phytoplankton communities as *Chrysochromulina, Ochromonas, Dinobryon, Gymnodinium, Peridinium, Cryptomonas* and *Rhodomonas* were defined as mixotrophic algae in literature (Sanders and Porter, 1988; Tranvik *et al*., 1989; Boraas *et al*., 1992; Jones, 1994). Microalgae are mostly thought as facultative photosynthetic organisms. However, an important part of microalgae species have heterotrophic or mixotrophic growth ability. These organisms can also display heterotrophic development in most of the taxonomic classes (Droop, 1974; Hellebust and Lewin, 1977; Neilsen and Lewin, 1974; Kaplan *et al*., 1986). In general, photosynthetic cells can obtain nutrients from an organic carbon source in insufficient light conditions. In other words, they have growth ability in lightness environments. Algae that have growth ability as heterotrophic and mesotrophic can increase their biomass at high level by taking advantage of this feeding strategies.

Chrysophyta members found in fresh waters and pools are mostly present in winter. They reach maximum amount in spring and autumn months. *Dinobryon* is generally known as a bacteria nourished as microtrophic.

Dinobryon can growth in the phytoplankton of both acidic and basic waters. Some species can be found in the mucilage colonies of Cyanobacteria or on the frustule of diatom *Tabellaria* (Prescott, 1951). *Dinobryon* can prevalently find in the phytoplankton of boreal and temperate areas where the water has poor nutrient and alkalinity. Also, it is very widespread in the lakes of the areas where the soils are mountainous and acidic (Canter-Lund and Lund, 1995). The potassium concentration of environment can be toxic to the most of the freshwater *Dinobryon* species. *D. sertularia* develops in water at 20ºC or less (Lehman, 1980). *Dinobryon* including oligotrophic lake phytoplankton has a high tolerance to all conditions except low phosphate (Tanyolaç, 1993). Among the *Dinobryon* genus, there are many species having a phagocytic strategy in addition to photosynthesis. *D. sertularia* feed on bacteria and use the carbon. These species die in lightness and can not use the organic carbon. Phagotrophy does not seem to be dependent on metabolic energy supply in the species, it is rather dependent on the physiological state of the cell (Jones and Rees, 1994).

So far, some studies have been made in Derbent Reservoir (Taş, 2006; Taş and Gönülol, 2007). *D. sertularia,* represented with only species in Chrysophyta division, was quite abundant found in all months and seem to be a very effective organism in the phytoplankton biomass. In this study, the seasonal dynamics and biomass of *D. sertularia* was investigated.

Material and Methods

Study Site

Derbent Reservoir (DR) is located on Samsun province in the Middle Black Sea Region in Turkey (41°25'06″N-35°49'52″E). The reservoir was made of rock filling style and constructed on the Kızılırmak River which is the longest river (1,355 km) in Turkey (Figure 1). The reservoir was built with aim to control

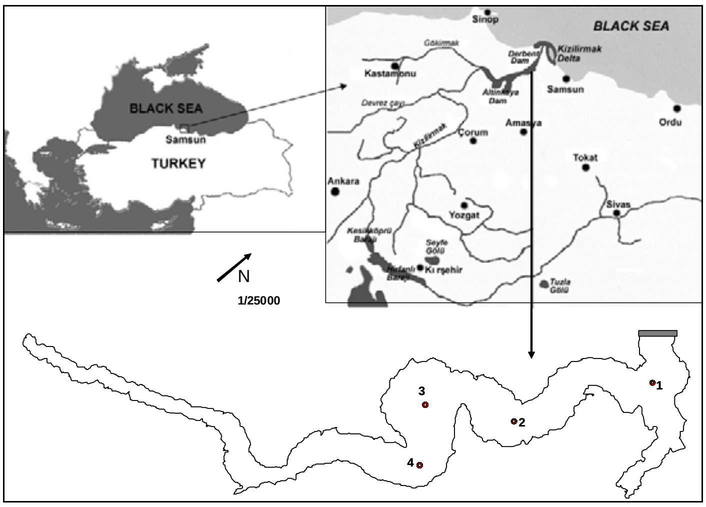

Figure 1. The map of Derbent Reservoir and the sampling stations for the present study.

flood and product energy and irrigation of the Delta where Ramsar Site (wetland of international importance). The surrounding of the reservoir is used as recreational activities. In DR, Rainbow trout (*Oncorhynchus mykiss*) aquaculture and natural fishing have been operated for 20 years. Some of fish species in the lake are: *Cyprinus carpio*, *Capoeta* sp., *Leuciscus cephalus*, and *Perca fluviatilis* living in the lake. The hydrological characteristics of DR were given in Table 1.

The area is characterized by the coastal Black Sea climate regime. The weather is hot in summer and mild and rainy in winter. The study area exhibits West Mediterranean type of rain regime. The amount of rainfall increases in February (102.4 mm) and December (68.0 mm) (Anonymous, 2002).

Sampling and Measurements

Samples were collected monthly from four different stations in DR between February 2001 and June 2002 (Figure 1). With aim to determine physical and chemical characteristics, the station 1 (st 1) was selected from the deepest and the largest zone. Temperature and oxygen values were measured in all stations by Oksiguard Handy Mk III digital apparatus. Other analyses were made by using standard techniques in water analyses laboratory at Samsun DSI (APHA, 1992).

Phytoplankton samples were taken from the surface (0-20 cm) in all stations. Just for st 1, samples were collected from 2, 4, 6, 8, and 10 m depth, with 2.0 liter capacity standard water sampler acc. to Ruttner (Hydro-Bios). Organisms in the water were collected by applying the sedimentation method of Utermohl (Utermohl, 1958). Counting was made with Olympus inverted plankton microscope (Lund *et al*., 1958). Biomass measure was made by taking into account of the volume and the weight of the organisms that its numbers is known. *D. sertularia* was identified according to Graham and Wilcox (2000) and John *et al.* (2003). BioDiversity Pro 2.0 and SPSS 13.0 software were used in the analyses of datas.

Table 1. The hydrological characteristics of the Derbent Reservoir

Altitude (m)	60
Height (m)	29
Min. operating code (m)	54.50
Max. operating code (m)	57.50
Max. water level (m)	60.0
Lake area according to min. operating code (ha)	525
Lake area according to min. operating code (ha)	1650
Lake volume according to min. operating code (m^3)	$167x10^6$
Lake volume according to max. operating code (m^3)	$213x10^6$
Annual average stream (m^3)	$5.4x10^9$

Results

Frequency coefficient of *D. sertularia* was calculated by presence-absence table for quantitative samples. According to this, the most prevalent species were recorded as 61-100% at all stations and all depths of st 1 (Figure 2). *D. sertularia* was found significantly dense in the phytoplankton (Figure 3).

Once the seasonal dynamics of *D. sertularia* had been investigated, algal blooms were observed in spring months and the end of summer months (Figure 4). *D. sertularia* caused little blooms in winter months but blooms increased in spring months. In all stations, this amount was changed between 2-88% in the phytoplankton density. *D. sertularia* was dominant by constituting 47-60% of total organisms in spring months. But, this species was not observed in any

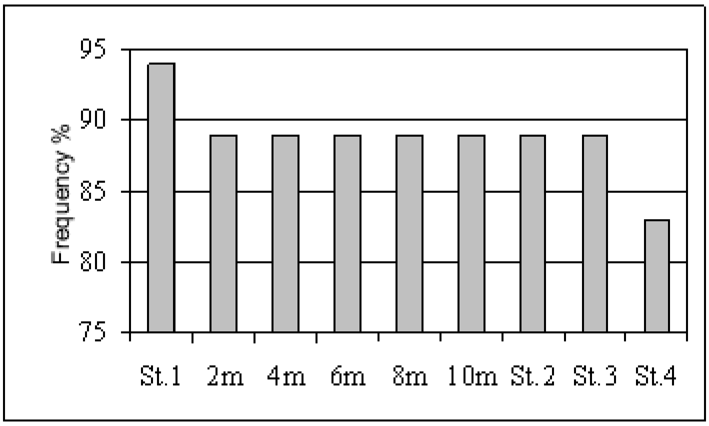

Figure 2. The abundance distribution of *Dinobryon sertularia* in the depths of the st. 1 and st. 2, 3, 4.
$f=(N_a/N)X100$ (N_a: sampling number containing a species, N: All sampling number)
rare present species 1-15%, frequent present species 16-40%, prevalent present species 41-60%, very prevalent species 61-100%.

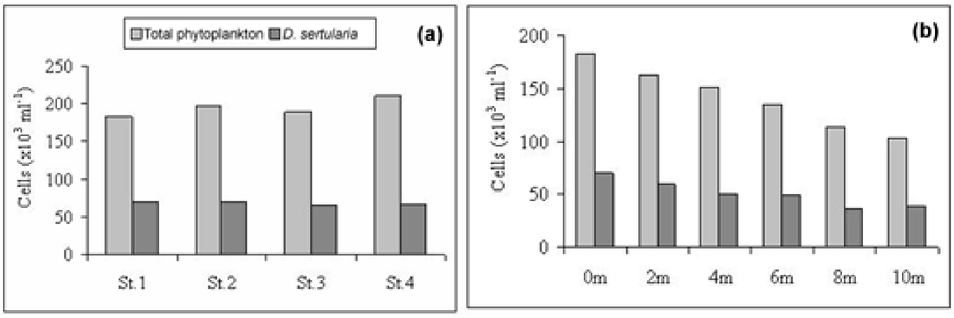

Figure 3. The cell density of *D. sertularia* in the surface waters of the all stations (a) and in the depths of the st.1 (b) within the total phytoplankton.

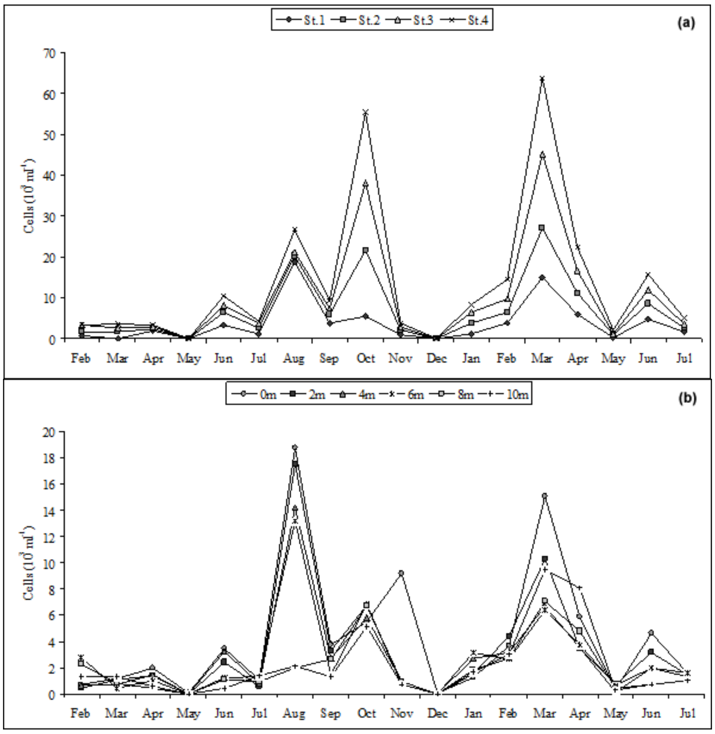

Figure 4. The monthly variation at cell density of *D. sertularia* in all stations and the depths.

stations in May 2001. However, it was recorded as dominant organism in May 2002. The ratio of *D. sertularia* was between 3-18% of the phytoplankton at the beginning of summer and green algae were dominant organism in this period (*Chlorella vulgaris, C. ellipsoidea, Tetraedron minimum, T. muticum*). At the end of summer, *D. sertularia* was found as dominant organism by making blooms at 79%, 78%, 67% and 61% ratio the stations of 1, 2, 3, and 4, respectively. Also, blooms occurred in autumn months. This species was recorded at 82%, 83%, 88% and 86% ratio in DR phytoplankton in the stations from 1 to 4, respectively (Figure 5). In August 2001, it was recorded at the highest amount as 8,740-13,140 cells ml^{-1} in the surface, 2 m, 4 m, and 6 m depth of st 1. And also, it was recorded as 16,100 cells ml^{-1}, 18,075 cells ml^{-1} and 18,525 cells ml^{-1}in st 2, 3, 4 in October 2001, respectively. In March 2002, this species was determined as 7,050 cells ml^{-1} at 8 m depth and 9450 cells ml^{-1} at 10 m depth in st 1 (Figure 4-5).

D. sertularia have provided very important contributions to the phytoplankton biomass of DR apart from the st 1. The highest phytoplankton biomass in the surface stations was enrolled in spring months and the end of summer months (Figure 6). In August, the highest biomass recorded as 6.56 µg ml^{-1} in st 1 and 5.48 µg ml^{-1} in st 2. The biomass was 2.72 µg ml^{-1} in st 3, and 1.95 µg ml^{-1} in st 4. In the middle of autumn, the highest biomass was found as 6.13 µg ml^{-1} in st 4.

The seasonal variation of the biomass in the surface waters was determined to be similar to st 1, according to vertical analysis (Figure 6). But, it was

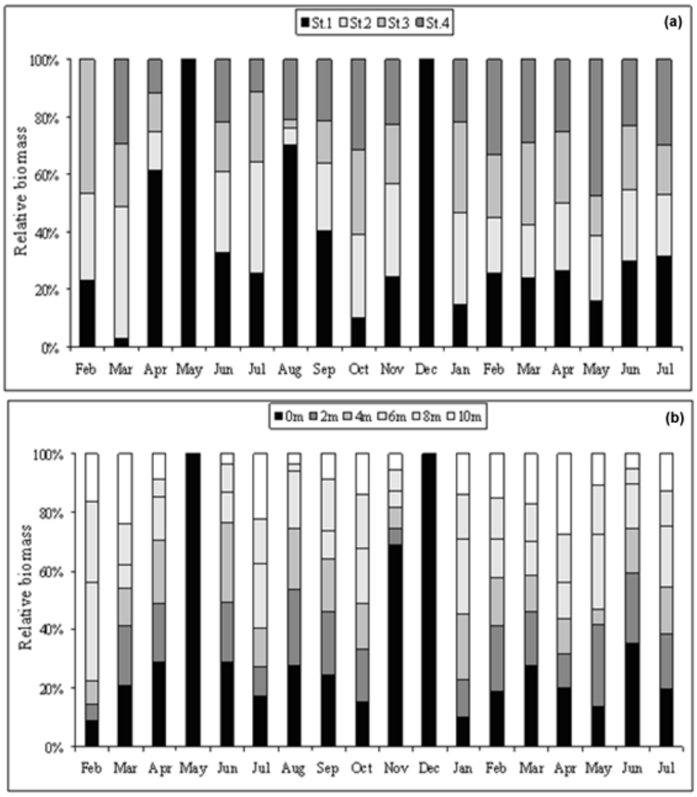

Figure 5. Monthly variations in relative biomass of *D. sertularia* in the surface waters of the all stations (a) and in the depths of the st. 1 (b).

observed to be decrease in biomass amount towards depths. The highest biomass was determined as 6.11 µg ml^{-1} (st 2), 4.97 µg ml^{-1} (st 3) and 4.60 µg ml^{-1} (st 4) in August. In October, the biomass was not high at the surface water of the stations. This amount was found as 2.36 µg ml^{-1} and 1.79 µg ml^{-1} in October. The highest biomass in March was found as 3.60 µg ml^{-1} in 2 m and 3.30 µg ml^{-1} in 10 m at spring bloom in 2002. It was calculated as 2.84 µg ml^{-1} in 10 m in April.

A Bray-Curtis similarity index was undertaken by using cluster analysis for the density of *D. sertularia* at the surface and from the depths of the stations. In the end of analysis, the highest similarity was seen (88.8%) at the surface of the st 3-st 4 and also 4 m-6 m (88.8%) in the depths of st 1 (Figure 7).

Physical and chemical analysis results of st 1 were given in Table 2. Cation arrangement of the reservoir water with base character were found as $Na^+ > Ca^{++} > Mg^{++} > K^+ > Fe^{++} > NH_4^+$-N and also, anion arrangement was found as $SO_4^= > Cl^- > NO_3^-$-N > NO_2^--N. Having the highest biomass, water temperature was at 25.5°C water temperature, the highest biomass was recorded in August month. In autumn bloom, the water temperature was measured as 17.2°C. In spring bloom, it was measured as 9.4°C in March and 11.5°C in April (Taş, 2006).

In order to determine its relationship with environmental conditions of *Dinobryon sertularia,* correlation analysis and anova test were performed by using SPSS 13.0 package program. According to the results obtained from two-way-analysis of variance (ANOVA) test, it was determined to be a positive relation among dissolved oxygen ($P<0.001$), pH ($P<0.001$) and total alkalinity ($P<0.009$) with the numbers of the *D. sertularia.*

In respect of correlation analysis, there was a relatively negative relationship between the numbers of the organism and total alkalinity in the lake water ($r= -0.497$, $P<0.05$). It was seen to be a quite positive relations among Electrical Conductivity (EC)-$SO_4^=$ ($r=0.900$), Cl^--EC ($r=0.808$), $SO_4^=$- Cl^- ($r=0.643$) Na^+-EC ($r=0.757$), Na^+-Cl^- ($r=0.678$), K^+-Cl^- ($r=0.632$), $SO_4^=$-Na^+ ($r=0.820$), Ca^{++}-Na^+ ($r=0.700$), Ca^{++}-$SO_4^=$ ($r=0.669$), $SO_4^=$-Fe^{++} ($r=0.616$) ($P<0.01$) in the physico-chemical parameters of the reservoir water, respectively. Despite this, it was found to be relatively negative relations among TAL- NO_2^- ($r=-0.528$), EC-TAL ($r=-0.528$), $SO_4^=$-TAL ($r=-0.491$), K^+-TAL ($r=-0.509$) ($P<0.05$) in the lake water, respectively.

Discussion

Confusion on feeding terminology of algae results from the similarity of nourishment way or transition phase in nourishment mode. Therefore, algae usually can be classified according to nourishing style. But, they can not classify as certainly by this way. Because, they have changing capacity of nourishment way in terms of

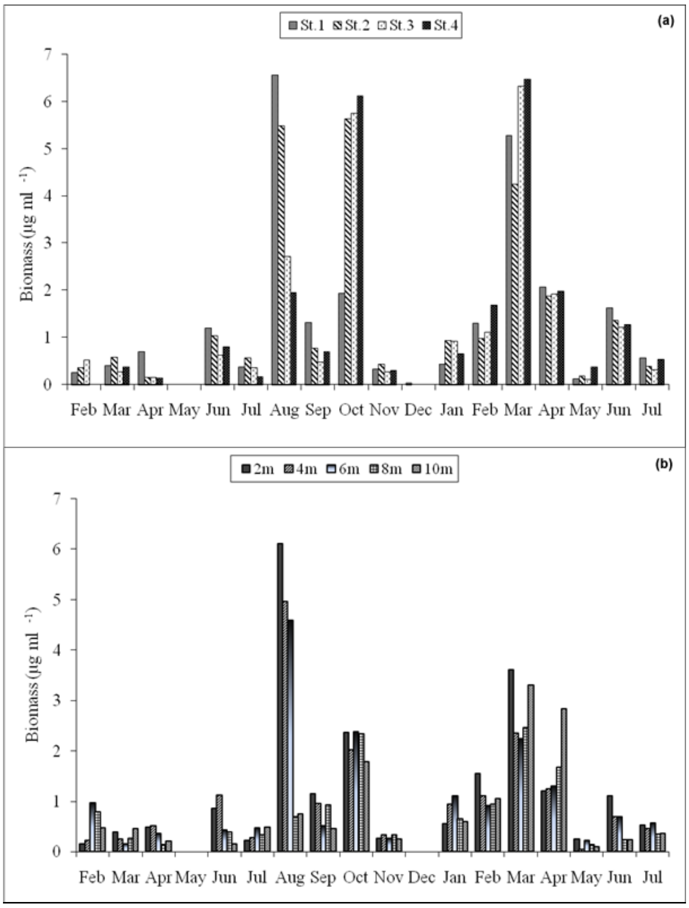

Figure 6. The biomass variation of *D. sertularia* in the surface waters of the all stations (a) and in the depths of the st.1 (b).

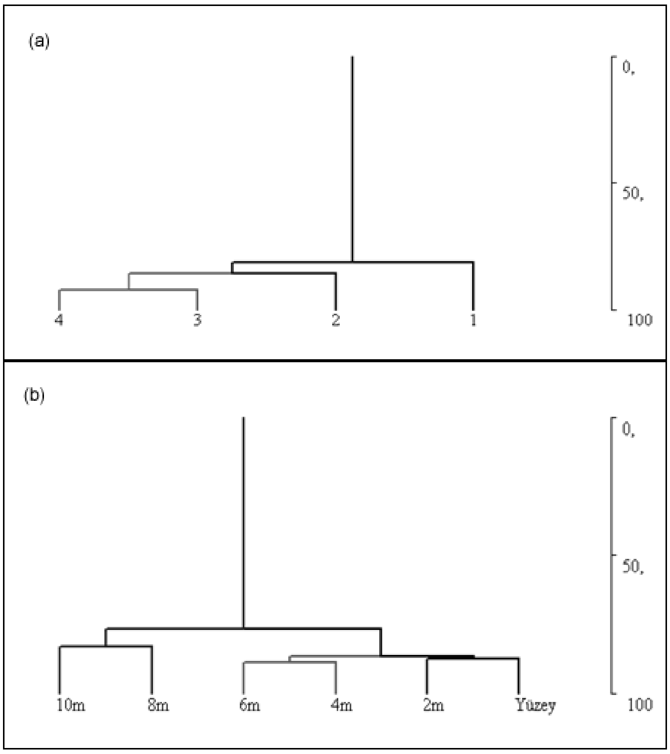

Figure 7. The hierarchical clustering among the surface stations (a) and the depths of the st.1 (b) according to the Bray-Curtis similarity index.

Table 2. Variation of the most important environmental characteristics in the Derbent Reservoir

	Mean Variable	Minimum	Maximum
Temperature (°C)	15.56	4.0	26.1
Dissolved O_2 (mg L^{-1})	10.39	8.1	12.0
pH	7.98	7.1	8.6
Conductivity (µS cm^{-1})	1525	1040	1992
Total alkalinity (mg L^{-1} $CaCO_3$)	163.7	130.0	190.0
Total hardness (mg L^{-1} $CaCO_3$)	439.2	355.0	587.5
Na^+ (mg L^{-1})	171.3	92.0	200.1
K^+ (mg L^{-1})	4.47	2.53	5.46
Ca^{2+} (mg L^{-1})	102.73	71.0	115.06
Mg^{2+} (mg L^{-1})	42.39	3.25	72.96
Fe^{2+} (mg L^{-1})	0.21	*nd*	1.51
Cl^- (mg L^{-1})	246.54	159.75	280.45
o-PO_4^{3-} (mg L^{-1})	0.053	nd	0.16
SO_4^{2-} (mg L^{-1})	295.16	164.16	448.32
NH_4^+-N (mg L^{-1})	0.18	nd	0.5
NO_2^--N (mg L^{-1})	0.05	nd	0.009
NO_3^--N (mg L^{-1})	0.96	0.33	2.33

nd: not determined

environmental conditions.

Mixotrophic organisms play an important role in the flow of energy and substance through food web in aquatic ecosystems. A current concept concerning organic matter dynamics within pelagic food webs consists of two processes: the traditional food web (nutrients–phytoplankton–zooplankton–fish), which considers that the phytoplankton is the dominant ‘producer’, and the microbial loop (Azam *et al*., 1983), which implies that a portion of matter and energy flows through unicellular organisms, such as bacteria, to mixotrophic and heterotrophic protists, bearing in mind that several phytoplanktonic organisms can also be consumers. Based on these two concepts, the small-sized fractions of phytoplankton communities play fundamental roles in the pelagic trophic interactions. In particular, in oligotrophic waters the autotrophic picoplankton can dominate the phytoplankton biomass and production (Stockner and Antia, 1986; Stoecker, 1998), and besides, together with bacteria, constitute the prey for the protistan assemblage. The nanoplanktonic size fraction, to which most of the mixotrophic protists belong, also represents the food for herbivorous zooplanktonic organisms.

Dinobryon benefits from organic elements as vitamins and minerals. When mineral elements are insufficient, it demonstrates mixotrophic or heterotrophic activity. It is known that *D. sertularia* displays facultative bacterial nourishing from literature and has mixotrophic strategy (Porter, 1988; Jones, 1994; Isaksson, 1998). This characteristic of *Dinobryon* is significantly effective adaptation for this type of aquatic environment (Isaksson, 1998). Mixotrophic property of *Dinobryon* provides important advantages when nutrients are limited (Gaedke, 1998; Kümmerlin, 1998). Similar findings are reported some investigations (Pugnetti and Bettinetti, 1999).

According to the results obtained from the study, total 180 taxa belonging to 8 divisions were identified in the phytoplankton of the DR. *Dinobryon sertularia* from Chrysophyta was found as only species. This species has often made blooms in the phytoplankton (Taş and Gönülol, 2007).

D. sertularia was fairly abundant in the DR phytoplankton having oligo-mesotroph characteristics (Taş and Gönülol, 2007). This species was determined in some locations of Suat Uğurlu Dam Lake (Yazıcı and Gönülol, 1994) and Akgöl Lake (Ersanlı *et al*., 2006). The crysophyte *Dinobryon* spp. are the most dominant mixotroph in Lake Constance (Gaedke, 1998). In a study made in the North Patagonian Lakes, it is reported that *D. divergens* and *D. sertularia* are mostly dominant in microphytoplankton (Queimalinos, 2002). This genus is commonly found in water where is insufficient phosphorus (Lee, 1980; Sandgren, 1988), and it is an important grazer of bacteria (Bird and Kalff, 1986). Orthophosphate in the surface water changed in the range 0-0.16 mg L^{-1}. Average value was determined as 0.053 mg L^{-1} (Taş 2006). Having the highest biomass, orthophosphate was found as 0.001 mg L^{-1} in the end of summer. In autumn bloom, orthophosphate was not determined in October month. But, it was found as 0.09 mg L^{-1} in March and 0.05 mg L^{-1} in April in spring months.

Water temperature is very important regarding the seasonal variation of phytoplankton (Richardson *et al*., 2000). When *Dinobryon* spp. compared with other species, it has quite high tolerance to low temperatures. These tolerance levels provide the dominancy of various species in different seasons (Fogg, 1975). By means of this potential, *D. sertularia* made blooms in different seasons and temperatures in DR. Blooms provided significant contributions on phytoplankton biomass. The biomass of *D. sertularia* was found at changing ratios as 0-6.56 µg ml^{-1} in the surface water of all stations and 0-6.11 µg ml^{-1} in the depths of st 1. Chrysophytes found in cold freshwater lakes and ponds are reported to make frequently water blooms in spring months (Graham and Wilcox, 2000). While only *D. sertularia* from Chrysophytes was found in DR phytoplankton, it was determined to be a very common species by making blooms in autumn, spring months and the end of summer. According to physical and chemical analyses of the water, DR has the characteristics of oligo-mesotrophic lakes.

When physical and chemical parameters of the lake water were examined, there are some relations between both these parameters and organism number. According to the results obtained from ANOVA analysis, it was determined to be a positive relation among dissolved oxygen, pH and total alkalinity (TAL) with the numbers of the *D. sertularia*. Also, In respect of correlation analysis, there was a relatively negative relationship between the numbers of the organism and total alkalinity in the lake water. Once these results compare with previous studies, important similarities are seen among physical and chemical features of the lake water (Jeong, 2001; Kim *et al.*, 2007; Tay and Kortatsi, 2008; Shakeri *et al.*, 2009)

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