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Spatial Distribution of *Asterionella formosa* Hassall, *Cyclotella ocellata* Pantocsek and *Fragilaria crotonensis* Kitton in the Zayandehrud Reservoir Dam, Iran

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ABSTRACT The main diatomic structure-forming species of the Zayandehrud Reservoir was investigated by collecting samples from the surface, five, seven and 10 m depths from January 2011 to October 2012 along four linear transects. *Asterionella formosa* Hassall, *Cyclotella ocellata* Pantocsek and *Fragilaria crotonensis* Kitton were revealed as the three structure-forming species among diatomic algae in the reservoir. The number of these diatomic cells increased from the reservoir banks to its central areas, but their abundance was non-uniformly distributed at the 10-meter water column at different sites, except for *C. ocellata* that showed a practically uniform distribution at the peak of its maximum growth. Overall, the frequency and cover of these structure-forming diatoms changed under the influence of different abiotic variables such as electrical conductivity (EC) and nitrate (NO_3-N).

Key words: *Dam lake, Diatoms, Phytoplankton, Structure-forming species*

1 INTRODUCTION

The phytoplankton and its structure better said structure-forming species, which have a high frequency of occurrence, high number of cells or biomass at optimum conditions, are the most important component of every aquatic ecosystem (Pashkevich and Shuyskiy, 2002), because the dynamics of zooplankton and fish are connected directly with them in a water body (Olrik *et al.*, 2007; Gołdyn and Kowalczewska-Madura, 2008; Banu, 2012). However, unnatural or excessive growth of structure-forming species of algae may impact negatively on other organisms, ecosystems or fisheries and may even be harmful (Smayda, 1997). Biological monitoring of the dominant species in a water body is a priority task during the observation of its ecological state since it heightens appreciably the reliability of an ecological forecast (Trifonova *et al.*, 2008).

On the other hand, key algal species can be different indicators. The assessment of the trophic status of a water body is one of practical and theoretical importance. It has been well-known for a long time that planktonic dominant algae would be good indicators to determine trophic water types (Rawson, 1956; Brook, 1965). Bioindicators are a useful instrument for estimating the anthropogenic influence on aquatic ecosystems (Lata Dora *et al.*, 2010; Li *et al.*,

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2010; Voudouris and Voutsa, 2012). Now, algae organisms living in the world's oceans are used as an important indicator of climate change (Smol and Cumming, 2000; Kadir *et al*., 2013).

Located at a height of about 1950 m above sea level in the Zagros Mountains in the Esfahan-Sirjan Basin, the Zayandehrud Reservoir is very important source of freshwater in Central Iran as it supplies water to several provinces. The average depth of this reservoir is 40 m and the maximum depth can reach down to 100 m near the dam; its water-surface area is 48 km^2 on average and the volume is 1090 million m^3, but these values vary constantly.

In algological aspect, the Zayandehrud Reservoir has been investigated in a great deal but for short periods (Zarei Darki, 2001, 2004; Shams and Afsharzadeh, 2007). According to all these studies, *Asterionella formosa* Hassall, *Cyclotella ocellata* Pantocsek and *Fragilaria crotonensis* Kitton were the dominating structure-forming diatomic species in the Zayandehrud Reservoir. The aim of this study was to investigate the horizontal and vertical distribution of structure-forming species of diatoms in this water body and its relationship to environmental variables.

2 MATERIALS AND METHODS

2.1 Study sites

Zayandehrud Reservoir is located at a height of about 1950 m above sea level in the Zagros Mountains in the Esfahan-Sirjan Basin (50° 15'-50° 45' N; 32° 30'-33° 00' E) (Moenian, 2008). Four transects were designated, each bearing three sites (left and right banks, and a middle site) for sampling (Figure 1). The first transects was selected to reflect river algal flora, while the second and third transects took into consideration human influence as they were located close to a residential area, and the fourth one for being in the vicinity of the dam that characterized lacustrine algal flora of the reservoir. The average depth of the reservoir during sampling ranged from 20-30 m with a maximum depth of 51 m. The geographical coordinates and depths of the middle sites during sampling are presented in Table 1.

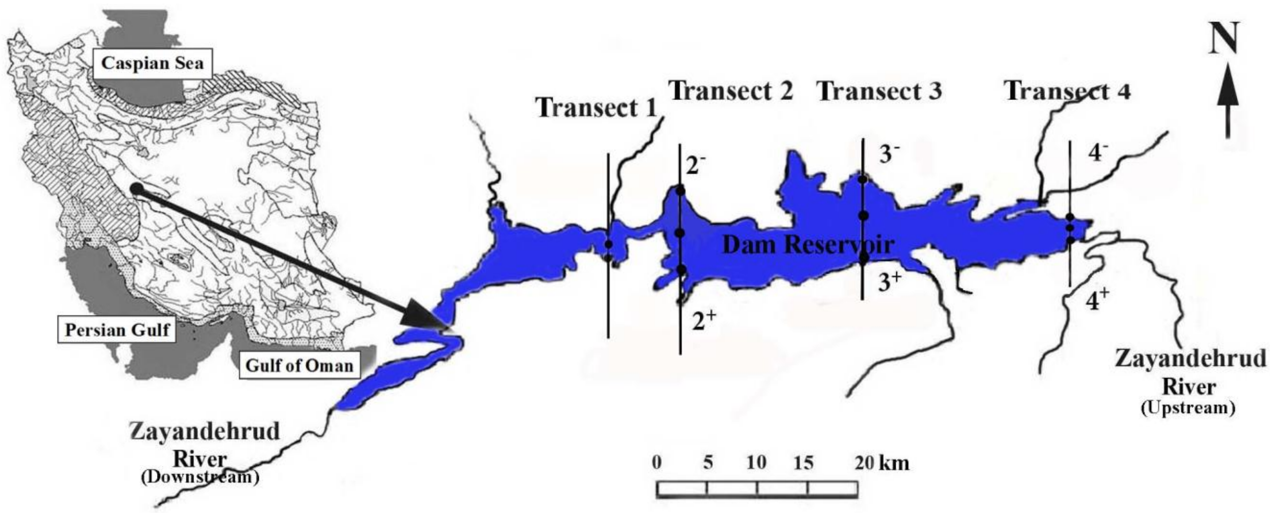

Figure 1 Schematic map of Zayandehrud Reservoir showing the sampling sites

The transects corresponding sampling sites on the left bank are denoted by 2^-, 3^-, 4^- while on the right bank by 2^+, 3^+, 4^+, and the middle sites by 1, 2, 3, 4

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Table 1 Geographic coordinates and depths of the middle site of each transects on the Zayandehrud Reservoir

Transects	**Geographic coordinates**	**Depth (m)**			
		January	**April**	**July**	**October**
1	50°31'45.74"E-32°42'56.38"N	1.2	1.4	1.5	1.0
2	50°36'31.58"E-32°43'09.32"N	10.5	7	8.3	5.5
3	50°39'56.68"E-32°44'08.97"N	32	27	28.4	23.5
4	50°44'02.37"E-32°44'01.72"N	51	48	49.3	43

2.2 Sampling

Samples were collected from January 2011 to October 2012 in the middle of each season. In the mid-points of transects, samples were taken from the surface, 5, 7, and 10 m depths. A total of 152 phytoplankton samples were collected. Qualitative samples were taken by a planktonic net filtering 100 liters of reservoir water; quantitative samples were carried out by the Ruttner bathometer. Samples were preserved with Lugol's solution immediately in the field and fixed with 37% formaldehyde stock solution after a few weeks (Wasser *et al.*, 1989).

The water temperature, transparency, conductivity, dissolved oxygen, pH were measured at all sites. Transparence of the water was estimated by a Secchi disk. Profiles of temperature, dissolved oxygen, pH and conductivity were measured with a Hach's versatile HQ40d dual-input multi-parameter. Samples also were taken to determine the chemical and biological variables (chemical oxygen demand; COD; biochemical oxygen demand: BOD; total nitrogen: $N_{total;}$ orthophosphate: PO_4-N; organic carbon: C_{org}) in accordance with the environmental regulations of surface water quality (Oleksiv, 1992).

2.3 Sample analysis

Phytoplankton samples were condensed by a settling method (Wasser *et al.*, 1989) and quantitatively analyzed with a Nikon-Eclipse E200 microscope in the laboratory of the Marine Biology Department, Tarbiat Modares University (Iran). The diatoms were processed for the purpose of deleting organic contents of the cell by warm methods before microscopic analysis (Proshkina-Lavrenko, 1974; Lange-Bertalot, 2001). Cells were counted in the Gorjaev's chamber. Frustules and valve structure of diatoms were investigated using scanning electronic microscopes (Jeol JSM-840) in the Islamic Azad University, Majlesi Branch. Identification of the diatomic species was carried out using the basic systematic reports (Zabelina *et al.*, 1951; Krammer and Lange-Bertalot, 1991a, b).

Nonparametric correlation (Spearman) analyses were used to determine relationships among the biomasses of investigated species and the above-mentioned environmental factors.

3 RESULTS

Throughout the investigation, diatoms, dinoflagellates, and golden algae dominated quantitatively in the phytoplankton of the Zayandehrud Reservoir. *Asterionella formosa* Hassall, *Cyclotella ocellata* Pantocsek and *Fragilaria crotonensis* Kitton – were the structure-forming species among diatomic algae of the reservoir.

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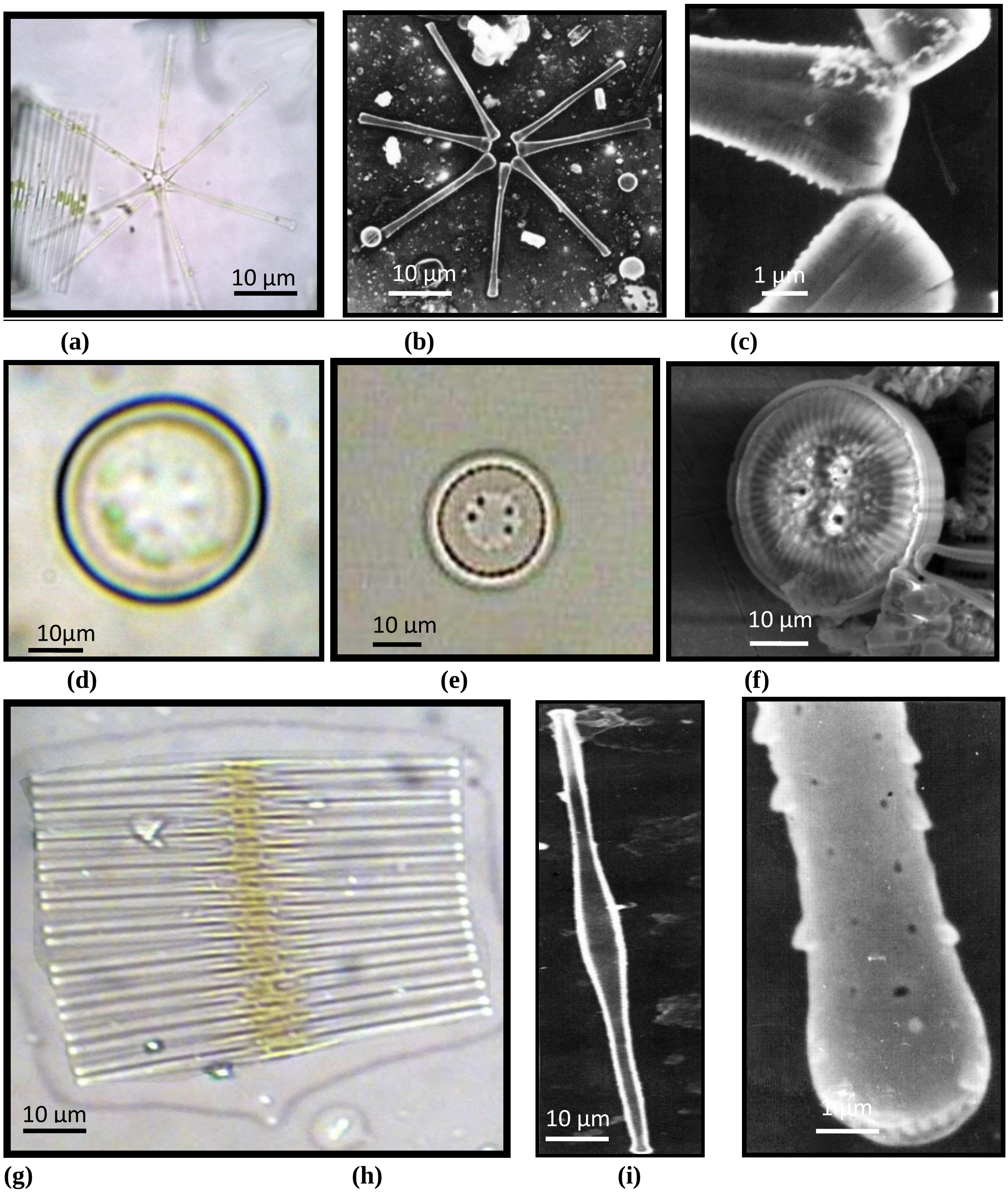

Figure 2 Structure-forming species of diatomic algae in the reservoir:
(a-c) *A. formosa;* (d-f) *C. ocellata;* (g-i) *F. crotonensis*

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A. formosa Hassall 1850 (Figure 2 (a-c)) (= *Asterionella gracillima* var. *formosa* (Hassall) Wislouch 1921, *Asterionella gracillima* (Hantzsch) Heiberg 1863).

It had 8-celled stellate colonies. Frustule with girdle of each cell was a narrow-linear with expanded ends; among them, the basal pole of the cell was typically wider than the apical pole. Valves were linear with the length from 40 to 130 μ. The mid-valve width ranged 1-2 μ. The stria were very tender, 25-28 in 10 μ.

It is a widespread oligo-beta-mesosaprobic species which presented in the plankton of the reservoir from winter to spring with its peak in winter. Ranges of measured abiotic variables are presented in Table 2. A maximum cell number was revealed in the vicinity of the dam (Table 3). On the whole, *A. formosa* was not presented at all sites of the reservoir in the spring. It was not at all detected in the summer and autumn samples.

Absolute predomination of this species as compared with the other examined species was observed at the first transect in the spring plankton (Figure 3). Also an important contribution of *A. formosa* to algal flora at the surface was revealed at the middle site of the second transects both in winter and in spring.

Vertical distribution of this species showed a quite evenly distribution from the surface to 7-meter depth (Figure 4), but start declining to 10 meter depth. At the middle site of the fourth transect, repeated outbreak was at a depth of 7 m.

C. ocellata Pantocsek 1901 (Figure 2 (d-f)). Cells were single; frustule was disk-shaped with almost flat valves 6-20 μ in diameter. The marginal zone was about one half of the length of the radius with a prominent radial striae of 15 in10 μ. The central portion was bluntly limited by different-sized and spaced colliculate protuberances.

Table 2 Ranges of some abiotic variables of plankton in the Zayandehrud Reservoir in 2011-2012

Ranges of variables	Species		
	A. formosa	***C. ocellata***	***F. crotonensis***
Temperature (T, °C)	7-23	6-25	7-24
Transparency (cm)	40-550	20-620	40-550
pH	7.73-8.62	7.58-8.81	7.61-8.70
EC (μS cm^{-1})	304-413	231-480	311-410
NO_3-N (mg l^{-1})	4.14-9.17	3.79-17.80	4.1-12.61
PO_4-N (mg l^{-1})	0.03-0.09	0.02-0.19	0.03-0.12

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Table 3 The number of cells of the investigated species at different sites and seasons

Species	Cell number ($\times10^3$ cells l^{-1}) Sites: 1*	2^-	2	2^+	3^-	3	3^+	4^-	4	4^+
Winter (January 2011)										
A. formosa	10	10	45	10	35	50	80	20	110	45
C. ocellata	10	540	100	890	440	910	220	330	460	90
F. crotonensis	-**	-	20	-	-	20	80	35	245	55
Spring (April 2011)										
A. formosa	20	-	80	-	-	45	-	-	10	-
C. ocellata	-	-	10	-	-	10	-	-	20	-
F. crotonensis	-	10	35	10	80	460	-	-	90	-
Summer (July 2011)										
C. ocellata	10	-	10	-	-	10	-	-	10	-
Autumn (October 2011)										
C. ocellata	10	-	10	10	-	-	10	10	-	-

* 2^-, 3^-, 4^- are sites on the left bank, 2^+, 3^+, 4^+ are sites on the right bank and 1, 2, 3, 4 are the middle sites;
** Species weren't revealed at the present sites

Under the prevailing environmental parameters of the Zayandehrud Reservoir (Table 2), *C. ocellata* was found to be main oligosaprobic structure-forming and diatomic dominant species in that reservoir. To a greater or lesser extent, species was present in the plankton throughout the whole year (Table 3). In the winter, *C. ocellata* was shown with the largest cell number; in the spring, it was revealed a smaller number of cells and only at some sites. Along transects, cell numbers increased from the banks to their central sites. It is interesting to note that contribution of *C. ocellata* reduced near dam in the winter while contribution of *A. formosa* and *F. crotonensis* increased here (Figure 3).

Active growth of species began at a depth of 10 m in the late autumn and reached its peak into the water column in the winter (Figure 4). Species was almost evenly distributed in the ten-meter water layer.

F. crotonensis Kitton (Figure 2 (g-i)) (= *Fragilaria smithiana* Grunow in van Heurck 1881, *Synedra crotonensis* (Kitton) Cleve and Möller 1878, *Nematoplata crotonensis* (Kitton) Kuntze 1898).

Valves were lanceolate with capitate ends, 40-170 µ long. The central area was wider than the rest of the valve and 1-3 µ wide. Cells formed ribbon-like colonies joined by linking spines. The axial area was lanceolate, with a clear fascia at the central area. Striae were distinct, 15-18 in 10 µ composed of apically elongated areolae (lineolae).

F. crotonensis, that is an oligo-beta-mesosaprobic species, was vernal diatomic dominant in the Zayandehrud Reservoir. Species was met in the plankton of the reservoir from January to May in range of measured abiotic variables presented in Table 2.

In winter, *F. crotonensis* was more concentrated in the vicinity of the dam. In the

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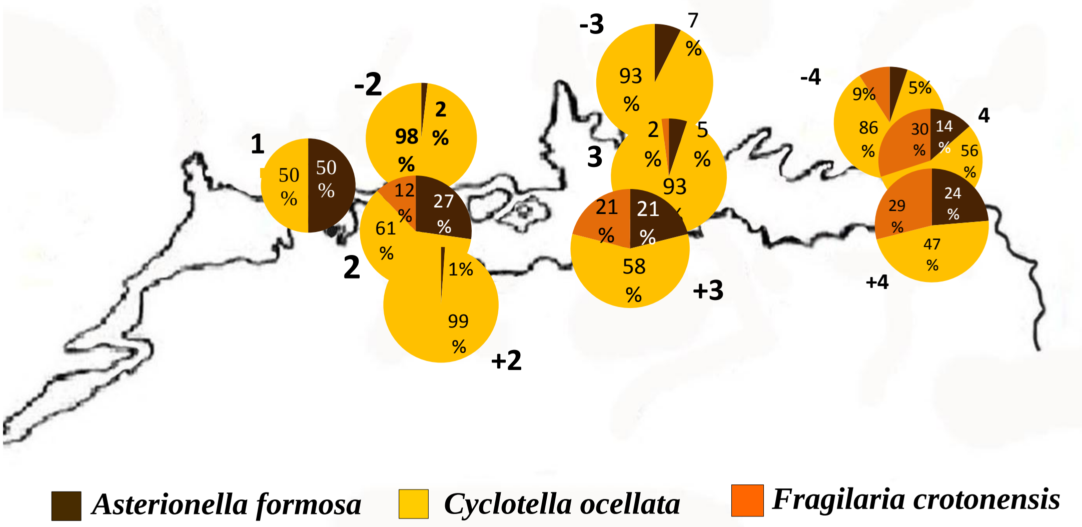
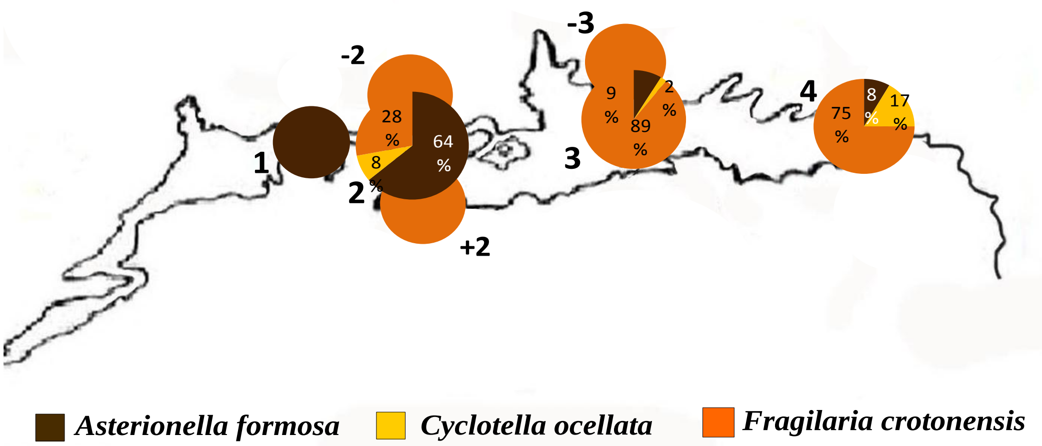
spring, it formed long colonies distributed more or less evenly across the horizontal surface of the reservoir from the second to fourth transect. The maximum number of cells were found at the third transect (Table 3). Definitely, this species was dominant and made the highest contribution to diatomic algal flora in this season but it was not absolutely found in summer and autumn (Figure 3).



(a)



(b)

Figure 3 Relative **c**ontribution (%) of each diatomic structure-forming species to algal flora at the surface: (a) in January 2011, (b) in April 2011

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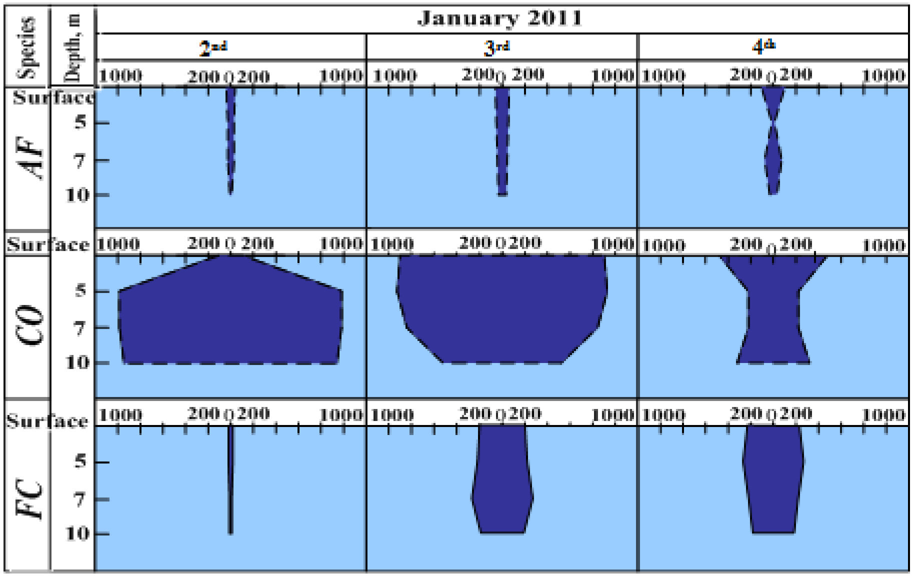

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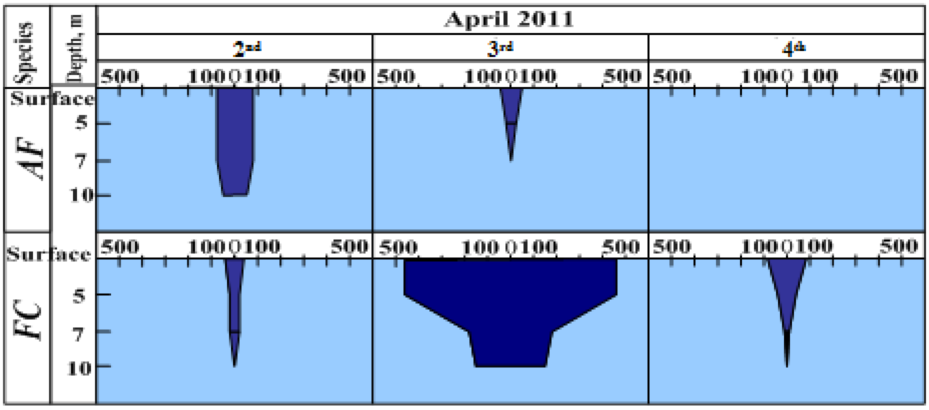

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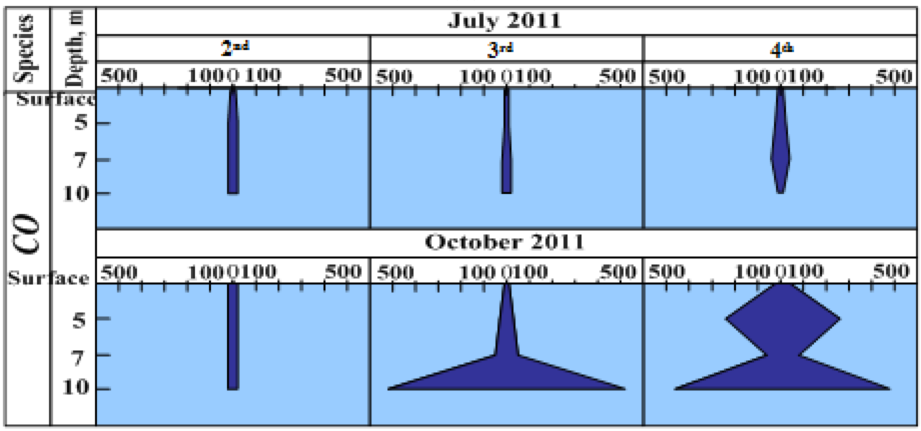

(c)

Figure 4 Vertical distributions of *A. formosa*, *C. ocellata* and *F. crotonensis (AF*, *CO,* and *FC)* at the second, third, and fourth transects (2, 3, 4) in different months ($\times 10^3$ cells l^{-1}): (a) in January, (b) in April, (c) in July and October

In the 10 m water column, *F. crotonensis* was distributed uniformly enough in winter; some increase occurred at 5 and 7 m (Figure 4). In spring, when the depth increased, its number of cells decreased so species preferred surface water.

The identified species were correlated with most of the expected environmental factors (Table 4). *A. formosa* biomass was positively correlated with EC, NO_3-N, DO (all $P < 0.05$), and negatively correlated with temperature and sulfate (SO_4-N). In addition, positive correlations were observed between *F. crotonensis* biomass and EC and NO_3-N; conversely negative relationships were found with TDS. *C. ocellata* biomass was significantly positively correlated with Fe, Mn, EC, TDS, pH, DO and negatively correlated with temperature.

Table 4 Results from Spearman's correlation between dominant species biomass and environmental variables

Abiotic variables	**Abundance**		
	AF	**FC**	**CO**
T	-0.41*	-0.05	-0.51*
pH	0.27	0.11	0.44*
TDS	-0.20	-0.44*	0.40*
EC	0.66*	0.48*	0.56*
NO_2-N	-0.03	-0.03	0.35
NO_3-N	0.56*	0.40*	0.19
PO_4-N	0.45*	0.34	0.07
SO_4-N	-0.44*	-0.37	-0.29
NH_4-N	-0.21	-0.31	-0.02
DO	0.59*	0.24	0.55*
Fe	0.45*	0.03	0.68*
Mn	-0.03	-0.32	0.67*

* Negative and positive correlating values, p<0.05

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4 DISCUSSION

These three structure-forming species (viz. *A. formosa*, *C. ocellata* and *F. crotonensis*) in the Zayandehrud Reservoir are frequently encountered in many reservoirs and lakes around the world (Kadri and Sen, 1998; Negro *et al.,* 2000; Jasmine *et al.,* 2005; Trifonova *et al.*, 2008; Znachor *et al.,* 2013). In our case, they comprised from 25 to 95% of the total phytoplankton abundance. The maximum density of the examined species was recorded in winter. The number of *C. ocellata* cells prevailed largely over those of *A. formosa* and *F. crotonensis* that is evidence of low eutrophication of the water body (Bellinger and Sigee, 2010).

Species *A. formosa* and *F. crotonensis* are studied enough from the morphological point of view (Zabelina *et al.,* 1951; Krammer and Lange-Bertalot, 1991a,b). In the reservoir, the investigated specimens did not differ in size from any described species in other literature. *A. formosa* formed clear 8-cell colonies even in winter, indicating that light was not a limiting factor (Wagner, 2008). Perhaps, this is due to the fact that the Zayandehrud Reservoir never freezes completely (except along the banks depending on the cold severity) and the water temperature does not go below 5°C.

These three species are considered as indicators of soft water (Negro *et al*., 2000). In the Zayandehrud Reservoir, conductivity averaged 420 $\mu S\ cm^{-1}$ and pH was in the range of 7.8 to 8.6 during the winter period throughout the water body when they were in bloom.

The dominance of these species was significantly correlated with the decrease of dissolved nutrients. In general, diatoms which are good competitors for nutrients, especially phosphorus (Becker *et al.,* 2008). In the present investigation, the active cell fission of these three species occurred at a nitrogen value of 4 mg l^{-1} and 0.03 mg l^{-1} of orthophosphates in the water surface (nutrients increased with increase in the depth). In other words, these taxa are abundant when P availability is very low and the supply of N is moderate to high (Jasmine *et al.,* 2005). However, the available autecological data on *C. ocellata* are based on studies in many different geographical regions (e.g. Canada, several sites in Europe, North America), which could indicate that the calculated total phosphorus optima for this species depends on the region where it grows (Cremer and Wagner, 2003).

The shortage of dissolved silicon (Si) in the water as a result of winter and spring exhaustion due to mass blooming of diatoms also can give a reduction of their share in the summer and autumn phytoplankton and the restructuring of its taxonomic structure (Znachor and Nedoma, 2008). Unfortunately, this microelement was not measured in the present paper.

Certainly, it is also necessary to take into account the temperature values. It is well known that the majority of diatoms vegetate at low temperatures (Ruth, 1971). With increase in temperature, other of algal species in different divisions that have a regulating impact on the abundance, biomass, dimension and species structure of diatoms begin to grow; it is so-called allelopathic relationships (Smayda, 1997). Although *C. ocellata* is considered as a summer form in temperate zone (Trifonova *et al.*, 2008), it reached its maximum number of cells in the Zayandehrud Reservoir in winter. In summer, maybe its growth is inhibited by active vegetation of dinoflagellates such as *Ceratium hirundinella* (O.F.Müller) Dujardin, *Peridiniopsis quadridens* (Stein) Bourrelly, *Peridinium cinctum* (O.F.Müller) Ehrenberg that were encountered in the Zayandehrud Reservoir. On the other hand, there are the presuppositions that several species or ecological races exist within this taxon, all showing different ecological requirements (Cremer and Wagner, 2003). Species *F. crotonensis* are noted as a typical summer form for temperate zone in the reviewed literature (Trifonova *et al.,* 2008). In the Zayandehrud Reservoir, it grows quite well at low temperature, although it preferred to actively divide in spring at temperature of 15 °C. The active growth period of this species also has been noted in the Keban Reservoir in spring (Turkey) (Kadri and Sen, 1998).

The studied species are actively consumed by zooplankton (Monakov, 1998), which could be considered as another reason for their reduction after the spring warming that was associated with the reproduction of such encountered zooplankton species as *Notholca squamula*, *Cyclocyprys* sp., *Keratella cochlearis*, *Leydigia acanthocercoids*, and *Chydorous sphaericus*.

5 CONCLUSION

Our observations showed the domination of structure-forming diatoms among other divisions of algae in winter-spring and late autumn seasons. Along transects, the number of cells of the analyzed species increased from the banks to its central sites. In ten-meter water column, their distribution was not uniform, so it was difficult to predict some pattern. Only *C. ocellata* showed a practically uniform distribution in the investigated water column in the peak of its maximum growth. Overall, the frequency and cover of these structure-forming diatoms changed under the influence of different abiotic variables more such as EC and NO3-N.

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پراکنش مکانی ***Asterionella formosa*** **Hassall,** ***Cyclotella ocellata*** **Pantocsek** و ***Fragilaria crotonensis*** **Kitton در سد مخزنی زاینده‌رود، ایران**

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چکیده گونه‌های اصلی تشکیل‌دهنده ساختار دیاتوم‌های سد زاینده‌رود و پراکنش افقی و عمودی آن‌ها در فصل‌های مختلف با نمونه‌برداری از سطح و عمق‌های پنج، هفت و ۱۰ متر در بهمن ماه ۱۳۹۰ تا آبان ۱۳۹۱ از ایستگاه‌های مستقر روی چهار ترانسکت بررسی شد. گونه‌های *Asterionella formosa* Hassall, *Cyclotella ocellata* Pantocsek و *Fragilaria crotonensis* Kitton به‌عنوان گونه‌های تشکیل‌دهنده ساختار جلبک‌های دیاتوم در مخزن سد بودند. در طول ترانسکت‌ها تعداد سلول‌های آنالیز شده از سواحل به طرف مرکز افزایش یافته، اما وفور پراکنش آن‌ها در ستون ده-متری آب به‌طور یکسان نبوده است. تنها *Cyclotella ocellata* در ستون آب مورد مطالعه با حداکثر رشد خود تقریباً به‌طور یکسان انتشار یافت. به‌طور کلی فراوانی و پوشش نمایندگان ساختار تشکیل‌دهنده دیاتوم‌ها، تحت تاثیر متغیرهای غیر زنده مختلف نظیر EC و NO_3-N تغییر یافته است. نتایج تحقیق حاضر به درک بهتر اکولوژی و وابستگی محل زندگی گونه‌های مطالعه شده در پژوهش کمک خواهد نمود.

کلمات کلیدی: دریاچه سد، دیاتوم‌ها، فیتوپلانکتون، گونه‌های تشکیل‌دهنده ساختار

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