

## Phytoplankton Population Changes in Lake Urmia During Dry and Wet Periods

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### Abstract

Hypersaline environments are important natural assets that have significant economic, ecological, scientific and natural value. Management and protection of these variable ecosystems depend on understanding the influence of salinity on biological productivity and community structure. The object of the present study is to investigate the relationships between two basic components in Urmia Lake i.e. microalgae and physico-chemical parameters, particularly salinity level in order to provide a better understanding dynamics of this unique ecosystem. 3 sampling sites were selected in north and south of Urmia Lake. Samplings (2 samples in each site) were carried out monthly from April 2018 to October 2019. Water level, salinity, Electrical Conductivity (EC), Total Dissolved Solids (TDS), pH, transparency, Phosphate and Nitrate ( $\text{PO}_4^{-3}$  and  $\text{NO}_3^-$ ) were analyzed. phytoplankton species composition and density were also determined. Statistical analysis were performed by PAleontological STatistics (PAST) version 3.04. Totally, seven algal species were identified in Urmia Lake in this study. Bacillariophy-

ta with 5 species was the most abundant algal group in the lake. Chlorophyta and Cyanobacteria both had 1 species, however, *Dunaliella salina* as the only representative of green alga alone composed about 99.5 percent of total algal density of Urmia Lake. This study indicated that salinity, TDS and EC have the highest effects on phytoplankton population structure and *Dunaliella* spp. dominance in Urmia Lake. However, other factors such as  $\text{PO}_4^{-3}$  and  $\text{NO}_3^-$  might have been masked by three main factors.

**Keywords:** Phytoplankton population, *Dunaliella salina*, Urmia Lake, Hypersaline.

### Introduction

Hypersaline environments are integral and dynamic part of the biosphere while the biogeochemical processes occurring in their unique ecosystems have considerable environmental, ecological, natural, social and economic values (Mohebbi, 2010; Shadrin, 2009). Lake Urmia is globally critical as second largest hypersaline lake in the world. It was designated as a wetland of international

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importance by Ramsar Convention and a Biosphere Reserve by UNESCO in 1971 and 1976 respectively (Chander, 2012; Ghaheri et al., 1999; Nouri et al., 2017; Eimanifar and Mohebbi, 2007). There are 5 Ramsar registered sites in the lake's basin, including Lake Urmia and some of its periphery wetlands (Karimi, 2013). Lake Urmia and its periphery wetlands compose 9 globally important bird areas in Lake Urmia basin (Karimi 2013). Urmia Lake once had a surface area of about 5000 km<sup>2</sup> (Asem et al., 2012). The lake is the largest habitat of a particular brine shrimp, *Artemia urmiana*, that is major food source of some migratory birds (Ahmadi et al., 2011; AghaKouchak et al., 2015). The lake's water level has declined significantly, endangering this remarkable ecosystem (Abbaspour and Nazaridoust 2007; Hassanzadeh et al., 2011; Sima and Tajrishi, 2013; Tourian et al., 2015). By 2012, Lake Urmia's water level had dropped about 5 m from its ordinary level, which is the lowest level from 1966 (Karimi, 2013). Vast areas of surrounding lands have been converted to salt marshes and, in southern and southeastern regions, the coastline has retreated several kilometers. Salt crystals can be seen on the Lake surface year round which has disrupted the water birds feeding and migration (Asem et al., 2012). Despite the reduction in precipitation and increased temperature, it appears that water problems in the Lake Urmia basin are mostly man-made and because of the lack of management, low irrigation efficiency, extensive agriculture and cultivation of water-intensive crops (Hassanzadeh et al.,

2011) which has resulted in decreased basin water level. A dike-type causeway (15.4 Km) was constructed to cross the lake at its narrowest part and equipped with a bridge (1.25 Km) to provide limited water exchange between northern and southern parts of the lake in the early 2000s. Water level changing often leads to fluctuation of water salinity, which may cause a changeover in the hydrobiological regime of the lake. In hypersaline lakes, the food chain shortage takes place, and the cascade effect is observed in their dynamics (Golubkuv et al., 2018). Phytoplankton species substitution with the salinity gradient is obviously observed in solar saltern ponds and a range of salt-tolerance in different phytoplankton taxa is seen in these systems (Madkour and Gaballah, 2007). By salinity increasing in the ponds the number of species decreased rapidly and significantly, ending with only one species (*Dunaliella salina*) in the crystallizer pond.

This research was carried out to investigate phytoplankton composition, abundance and fluctuation of some physical and chemical factors in Urmia Lake during dry and wet periods.

### Materials and Methods

Three sampling sites were selected in north and south parts of the lake. In a few months samples were taken from 4 stations. Sampling was carried out in 18 month period from April 2018 to October 2019. In each site, 2 samples were taken for chemical, phytoplankton population analysis, respectively.

Salinity was measured by a refract meter model ATAGO (Japan). In dry period, when salinity was higher than the apparatus measurement range, the water was diluted with distilled water, then the salinity was measured. In this condition, the real salinity was calculated by multiplying in correct dilution coefficient. Temperature was measured in situ by alcoholic thermometer. EC, TDS and pH were measured by WTW LF 320 EC meter and a Testo 320 PH meter, respectively. Dissolved nutrients ( $\text{PO}_4^{-3}$  and  $\text{NO}_3^-$ ) were determined using a spectrophotometer model T80+ UV/VIS (PG Instruments Ltd, UK). The method for  $\text{PO}_4^{-3}$  determination is based on the formation of phosphomolybdate with added ammonium molybdate followed by reduction with hydrazine in acidic medium. Orthophosphate and molybdate ions condense in acidic solution to give molybdophosphoric (phosphomolybdic) acid, which upon selective reduction (perhaps with hydrazinium sulphate) produces a blue colour, due to molybdenum blue of uncertain composition. The intensity of blue colour is proportional to the amount of phosphate. Transparency of water was measured with a 30 cm Secchi disc. Water level fluctuations of Urmia Lake was extracted from West Azarbaijan Water management Company's web site (<http://www.agrw.ir>). Phytoplankton samples were preserved in logul solution, in cold, dark condition. Phytoplankton counting and identification were done using 5 mL settling chambers with a Nikon TS100 inverted microscope at 400× magnification by Utermöhl's method (1958).

At least 50 fields or 100 individuals of abundant species were counted in each sample (Venrick, 1978). Phytoplankton community in each site was analyzed in terms of species composition, species diversity and density. Statistical analysis were performed by PAleontological STatistics (PAST) version 3.04 (Hammer et al., 2001). Detrended Correspondent Analysis (DCA) was performed to observe the distribution of the sampled waters on the basis of their environmental parameters. The data were standardized (mean = 0, variance = 1) before running the analysis. The Euclidean distance was determined among the studied samples from standardized data. Correlation and regression coefficient ( $R^2$ ) of total phytoplankton density and some physical and chemical factors were determined with Excel 2013.

## Results

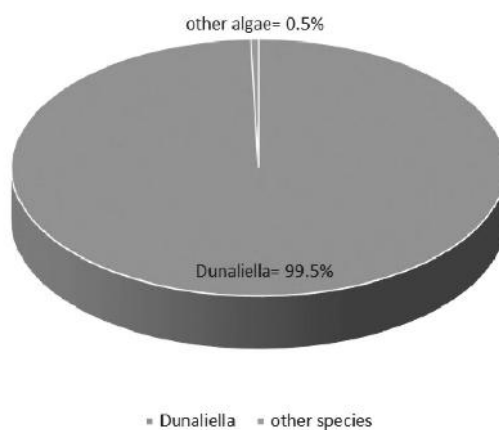
Totally, seven algal species were identified in Urmia Lake in this study. Bacillariophyta with 5 species was the most abundant algal group in the lake. Chlorophyta and Cyanobacteria both had 1 species, however, *Dunaliella salina* as the only representative of the green alga alone composed of about 99.5 percent of the total algal density of Urmia Lake (Table 1 and Figure 1).

Water level fluctuation of Lake Urmia shows an annual cyclic pattern (Figure 2), which dry and wet periods coincide with water level fall and rise, respectively. The highest and the lowest water level was observed in May 2019 and September 2018 respectively.

As shown in Figure 3, Salinity, TDS and EC

**Table 1.** Algal species density in Urmia Lake during April 2018- September 2019.

Density (%)	Mean Density (Ind/L)	Algal species	Spring 2018	Summer 2018	Autumn 2018	Winter 2018	Spring 2019	Summer 2019
99.5	2150652	<i>Dunaliella salina</i> (Dunal) Teodoresco	+	+	+	+	+	+
	3582	<i>Oscillatoria</i> sp.	+	+	-	-	+	-
	2403	<i>Navicula</i> sp.	+	+	+	+	+	-
0.5	420	<i>Cocconies pediculus</i> Her.	-	+	-	+	-	-
	2403	<i>Nitzschia</i> sp.	-	-	+	+	+	-
	420	<i>Synedra ulna</i> (Nitzsch) Ehrenberg	-	+	+	-	-	-
	85	<i>Symbella prostrata</i> (Berkeley) Cleve	-	-	+	-	-	-

**Fig. 1.** Algal composition of Urmia Lake during April 2018 to September 2019.

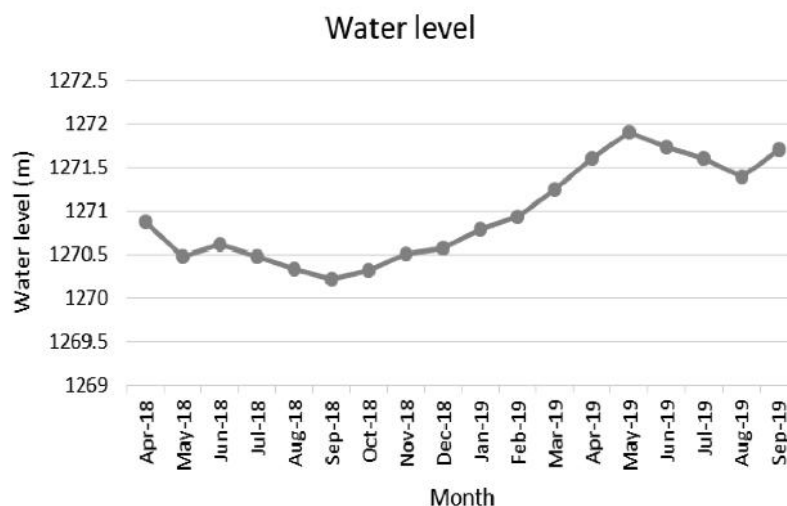


Fig. 2. Water level fluctuations of Urmia lake during sampling.

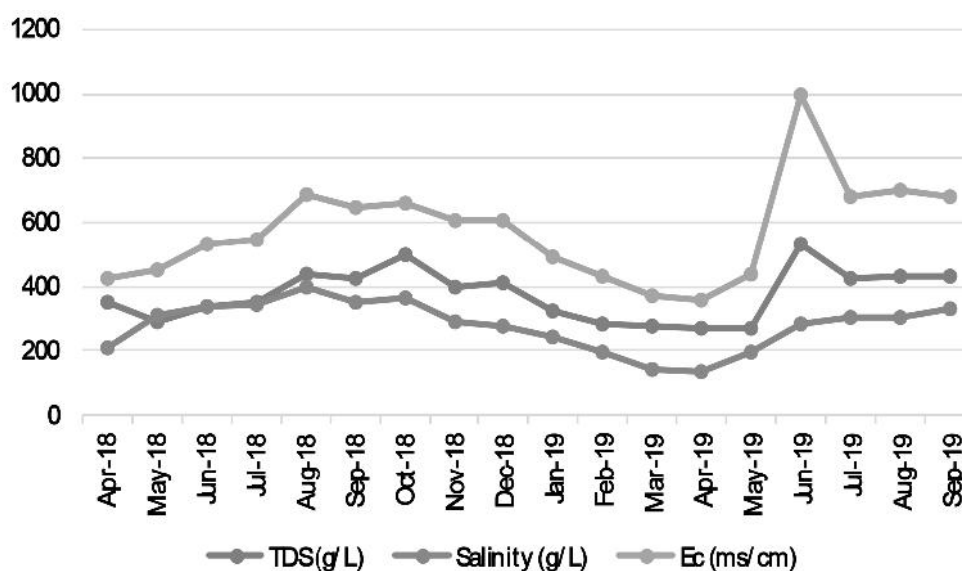


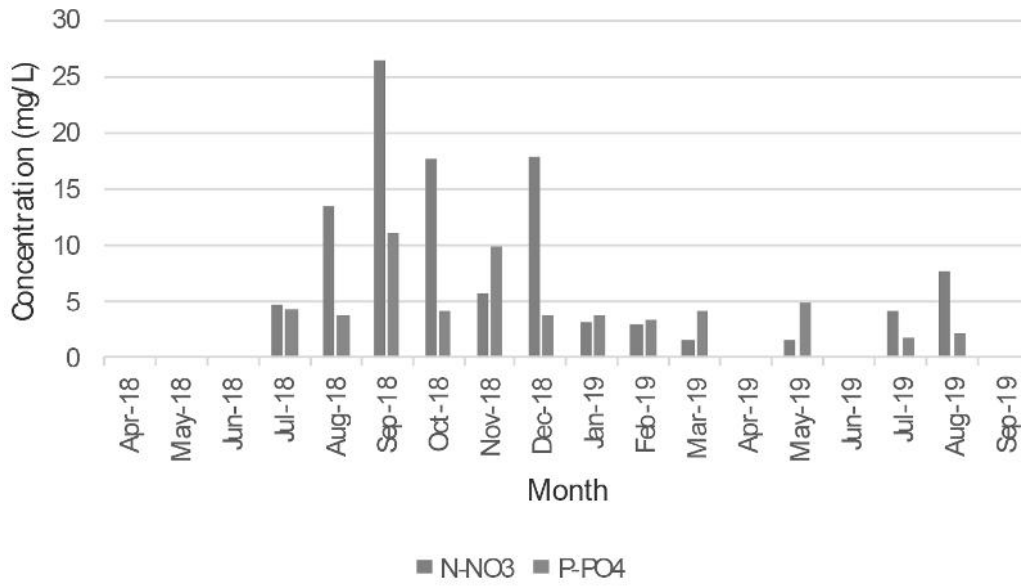
Fig. 3. Salinity, TDS and EC of Urmia lake.

of the lake indicate a similar annual cyclic pattern, so that the highest (396 gr/l) and the lowest salinity (135 gr/l) were in August 2018 and April 2019, respectively. This infers the identical origin of the three parameters because all of them reflect the minerals in water.

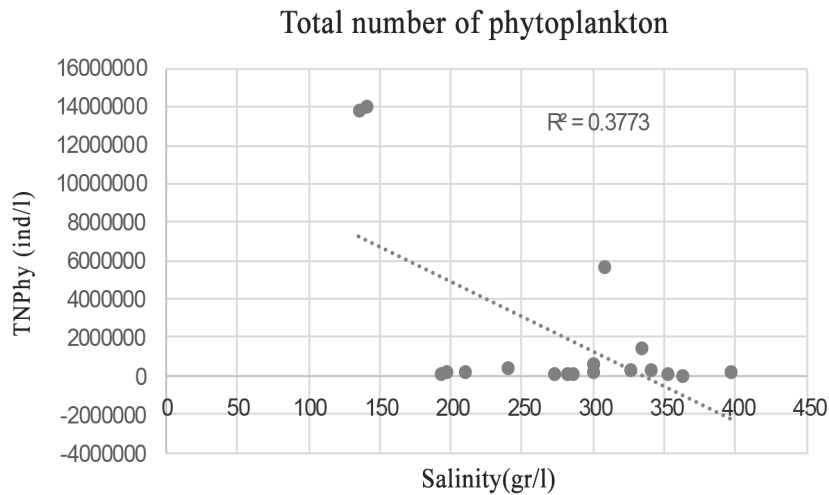
As shown in Figure 4 the highest and low-

est Nitrate and Phosphate concentrations in Urmia Lake were 26.6, 11.25 mg/l and 1.58, 1.7 mg/l, respectively. Both are the main nutrients in the water ecosystems that may affect the biological section.

In this study, there was a slight negative correlation between phytoplankton density and salinity in the lake. Correlation between phy-



**Fig. 4.** Nitrate and Phosphate concentrations in Urmia lake during sampling.



**Fig. 5.** Correlation between total density of phytoplankton and salinity in Urmia lake during the study period.

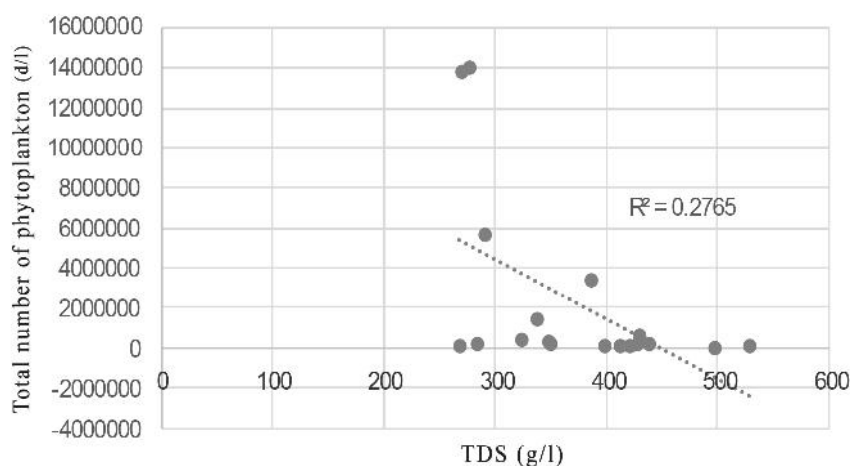
toplankton density and salinity in Urmia Lake is presented in Figure 5.

Correlation between phytoplankton density and (TDS) in Urmia Lake is indicated in Figure 6. As shown there is a slight negative correlation between phytoplankton density and TDS of Lake Urmia in the present study.

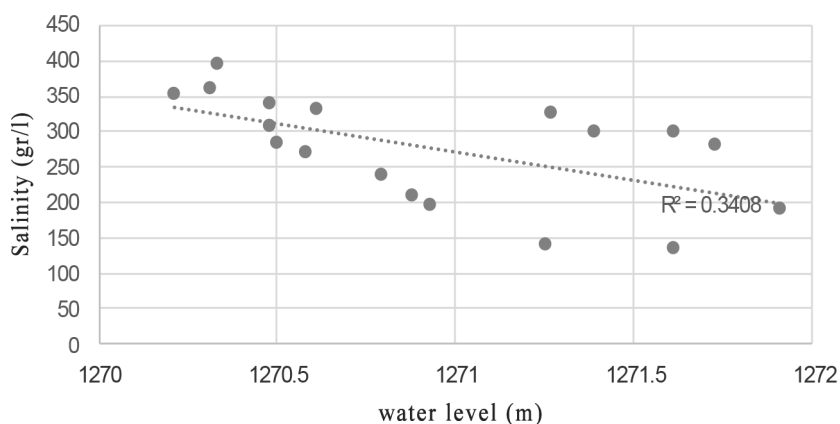
Salinity and water level are correlated negatively (Figure 7). As water level rises the salinity of the lake water drops slowly and contrariwise.

EC and phytoplankton density in the lake are negatively correlated in this study (Fig. 8).

*Dunaliella* density in Urmia Lake as the dom-



**Fig. 6.** Correlation between total density of phytoplankton and TDS in Urmia lake.



**Fig. 7.** Correlation between salinity and water level in Urmia lake.

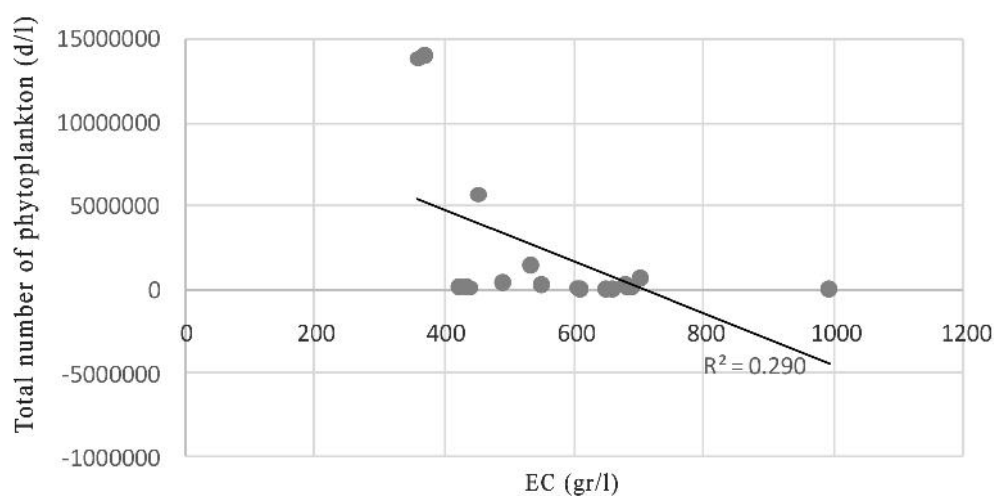
inant alga has negative correlation with water salinity, by increasing the salinity, *Dunaliella* density is dropped (Figure 9).

Detrended Correspondence Analysis (DCA) of algal species and physico-chemical factors in Urmia Lake during April 2018 to September 2019 is indicated in Figure 10. *D. salina* and total phytoplankton density are the main factors that influence the sampling sites.

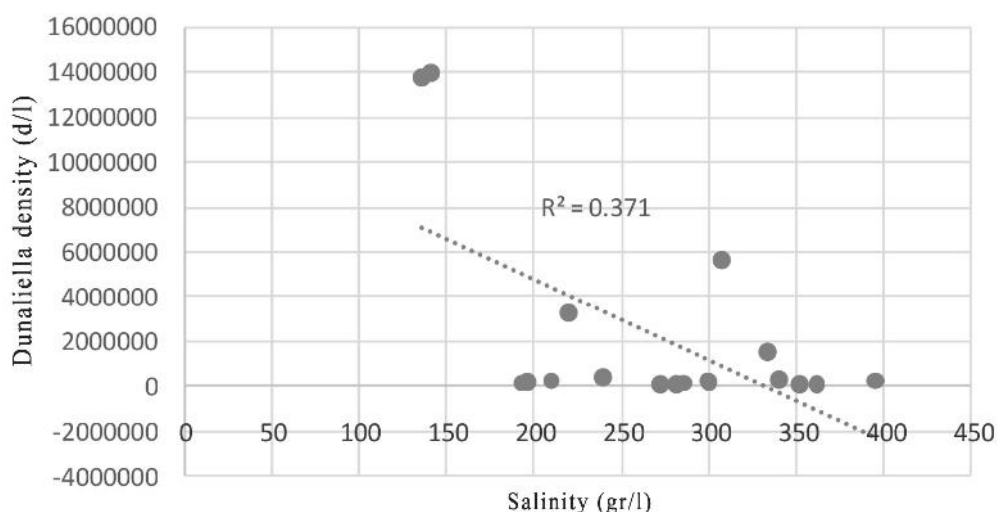
### Discussion

The Urmia Lake ecosystem is not complex, but it is not as simple as often portrayed. In temperate hypersaline lakes, salinity is determining factor in phytoplankton species composition and diversity. In most of these lakes, *Dunaliella* spp. mostly dominate microalgae, due to their higher salinity tolerance. Microalgal composition of Urmia





**Fig. 8.** Correlation between total density of phytoplankton and (EC) in Urmia lake.

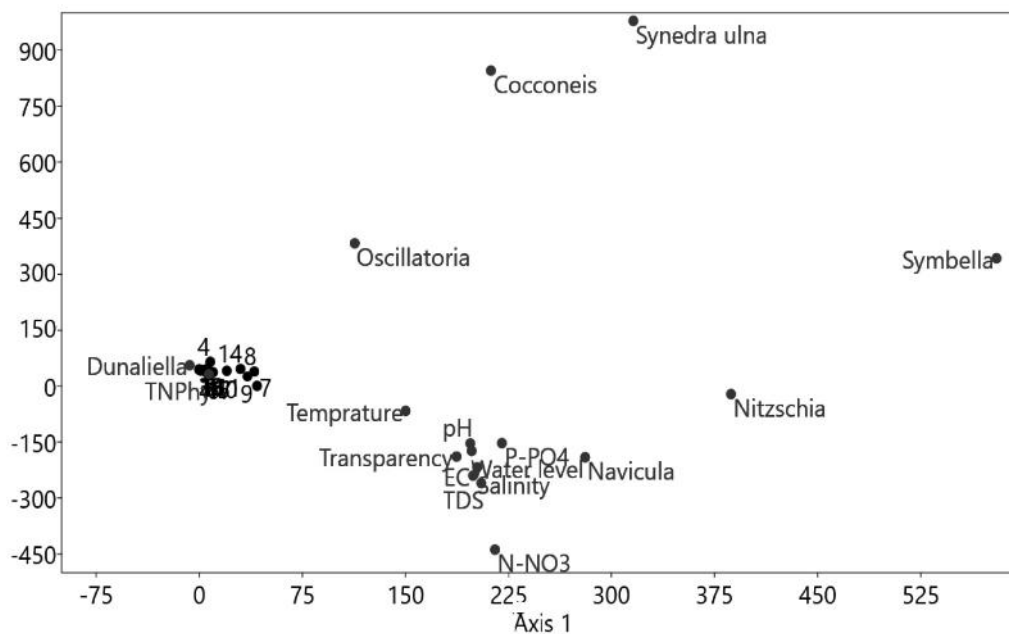


**Fig. 9.** Correlation between *D. salina* density and salinity in Urmia lake.

Lake is roughly similar to the phytoplankton composition in the Great Salt Lake (USA), which consists predominantly of *Dunaliella*, and diatoms like *Nitzschia* and *Navicula* (Sorgeloos, 1997). Currently, most hypersaline lakes, show an increase of salinity due to human impact and climate change (increasing temperature and reduced precipitation). Before Lake Urmia's shrinkage, Ryahi et al.

(1994) observed 6 cyanophyta, 4 Chlorophyta, 2 Bacillariophyta, while Mohebbi et al. (2006) reported 3 Cyanophyta, 2 Chlorophyta, 11 Bacillariophyta. *Enteromorpha intestinalis* (Linnaeus) Nees, a green macroalga has once been reported by Günther (1899) and Saberi (1978) in this lake, but this alga has not been observed or reported since then. This could be attributed to low-





**Fig. 10.** Detrended Correspondent Analysis (DCA) of algal species and physico-chemical factors in Urmia lake.

er salinities observed at that times. Asadi et al (2011) reported 4 Cyanobacteria species from Urmia Lake and 14 species from some flowing rivers to this lake. After shrinking, algal species diversity and density have dramatically been decreased, but high contribution of *Dunaliella* in the lake's algae composition (up to 95%) and in high water level period, is still noticeable (Mohebbi et al., 2017). Also, dramatic reduction in algal density was observed during drought season (i.e. summer and autumn).

Quantitative analysis of chlorophyll a and algal density indicated that primary production in Urmia Lake is lower than its sister the Great Salt Lake and that *Dunaliella* is the dominant species (more than 95% of total phytoplankton in number) of both lakes (Van Stappen et al., 2001; Gliwicz et al., 1995; Mohebbi et al., 2006). *Dunaliella sa-*

*lina* is a unicellular green alga found in high salt concentration water bodies. It produces a distinct pink and red colour characteristic of saltern ponds. *Dunaliella* species are able to tolerate variable NaCl concentrations, ranging from 0.2% to approximately 35% (Hamed et al., 2017). This microalga is a natural source of carotenoids used as human food as well as animal feed (shrimps). The high content of  $\beta$ -carotene makes *Dunaliella* attractive to biotechnologists for large-scale production in high-salinity, shallow, open ponds under high solar radiation (>30 C). Desiccation of Lake Urmia threatens the valuable genetic resources and biodiversity of algae. Therefore, isolation and identification of *Dunaliella* endogenous strains by molecular methods is important (Hejazi et al., 2016). In Lake Urmia, like other temperate hypersaline lakes, *Dunaliella* spp.

mostly dominate due to their higher salinity tolerance (Mohebbi, 2010; Mohebbi et al., 2006; Dolapsakis et al., 2005; Giliwicz et al., 1995). Recently, four species of *Dunaliella* have been identified using 18S rDNA gene sequencing from this lake (Ghorbani et al., 2013), including *D. bardawil*, *D. parva*, *D. salina* and *D. tertiolecta*. *Dunaliella* is a preferred food source for *Artemia* due to its more positive effects on growth, survival rate and reproductive characteristics of *Artemia* (Mohebbi et al., 2016). By salinity increasing in Lake Urmia, *Dunaliella* spp. accumulated large amounts of beta-carotene in their chloroplasts, the mechanism by which this alga could tolerate high irradiance and salinity (Garcia et al., 2007).

In summer 2012, an algal bloom occurred at the northwest of Lake Urmia in a relatively small scale (Manaffar and Ghorbani, 2015). Molecular analysis of samples (18sr DNA and ITS region sequencing), indicated dominance of *Dunaliella tertiolecta* ( $1.2 \times 10^6$  cells/ml) density. This indicated eutrophication of the lake which was induced by high levels of P and N and may partly be related to low water level. Alvarez et al. (2006) found that the main factors controlling phytoplankton dynamics in shallow hypersaline lakes of Spain are water regime and consequent changes in TDS concentration. This is agreed with our study in which TDS is positively ( $R^2 = 0.276$ , Figure 6) related to phytoplankton density. They also noted that TDS concentration masks other factors such as nutrient concentration. In this study, we did not observe any correlations between

$\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$  and phytoplankton population changes. This infers that salinity masks these nutrients effects. When salinity in the Great Salt Lake is high, phytoplankton diversity is low and composition is dominated by two species of halotolerant green algae *Dunaliella* (Wurtsbaugh, 1995; Stephens, 1998); this result is according with our findings. When salinity in Urmia Lake is high, the phytoplankton diversity is reduced to only *Dunaliella* genus. The ecosystem's inter-annual variation is most strongly exhibited when brine shrimp are absent (e.g., annual peak phytoplankton abundance), which suggests that the strong effect of brine shrimp can mask or alter annual differences in abiotic influences (Belovsky et al., 2011). In the absence of brine shrimp, maximum annual phytoplankton abundance is primarily determined by nutrient availability. With brine shrimp, phytoplankton abundance should depend on its annual maximum in the absence of brine shrimp, intensity of brine shrimp grazing, and its ability to recover after grazing. Therefore, brine shrimp abundance is critical and we hypothesized that brine shrimp populations were ultimately limited by phytoplankton availability.

The upper limit for diatom growth is about 180 ppt NaCl. However, some species of *Navicula* and *Nitzschia* might have been adapted to higher salinities in Urmia Lake.

Shallow hypersaline lakes are controlled by several factors such as climate, water depth, salinity, zooplankton, turbidity that give rise to very complex patterns. Combination of factors direct the system in a particular di-

rection in each cycle.

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