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Research Article

The study on dinocysts in the southern coastal waters of Caspian Sea

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Abstract

Algal cysts data has the potential to link with environmental parameters. In the pelagic zone, various factors such as sediment turbulence, the unusual rising of temperature, and nutrient can cause the entrance of surface sediment cysts into the water column. This paper aims to study dinocysts fluctuation in the southern part of the Caspian Sea. 563 water samples were collected during a period from 2009 to 2013 as well as a year from 2018 to 2019. The dinocysts were identified by reliable references. Analysis of variance was used to determine significant differences in dinocysts abundance between the seasons, depths, layers, and regions. The microscopic results showed that the cysts of *Dissodinium pseudolunula*, *Pyrocystis* sp., and *Gonyaulax polyedra* were identified. The percentage of presence frequency of cysts in years of 2009-2010, 2011-2012, 2012-2013, and 2018-2019 were obtained 46, 4, 9, and 34%, respectively. The different stages of the *Dissodinium pseudolunula* and *Pyrocystis* sp. cysts formed the highest percent frequency of presence. The cyst of *Gonyaulax polyedra* (as an indicator of warm water) was increased in the water column compared to previous years, which probably is a reflection of increases in sea surface temperature in this region.

Keywords: Dinocyst, Environmental indicators, Southern Caspian Sea, Iran

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Introduction

Microalgae are the basis of life in aquatic ecosystems, they are also considered in terms of causing environmental problems (Bravo and Figueroa, 2014). The adverse consequences of harmful and toxic algal blooms have led to "cyst" researches, because in many cases the sudden increase in algal population cannot be explained only by studying the vegetative phase divisions. Cyst germination and successful growth of newly germinated cysts play an important role in phytoplankton dynamics and blooms (McQuoid, 2005). The cyst formation is occurring in both diatoms and dinoflagellates. Diatoms cysts are subject to dissolution and loss of opal relative to calcium carbonate. So, diatoms cyst unlike dinocysts, are frequently sparse, poorly preserved, and have low diversity in the Caspian Sea (as part of Black Sea Corridor or BSC) (Mudie *et al*., 2017). Based on several studies of the southern Caspian Sea basin, dinoflagellates form the second dominant division in the microalgae population. Therefore, their role in the population dynamics of Caspian Sea microalgae is noteworthy. In addition, dinoflagellate blooms have occurred in two recent decades in this region (Nasrollahzadeh Saravi *et al*., 2015b; 2016). More than 10% of about 2000 marine species of dinoflagellates produce cysts as part of their life cycle. Cysts are divided into two groups: temporary (thin wall) and dormant (thick wall) cysts. Each of these two groups has its own morphological, biochemical, physiological characteristics (Perez *et al.*, 1998). The dormant cysts usually remain in the deeper layer of sediment. Cyst formation is in reaction to both biotic and abiotic parameters such as temperature, food, grazing pressure, or as a part of the life cycle. Cysts enter to vegetative phase and form bloom when favorable conditions are brought backed (Anderson *et al.*, 1987; Rengefors and Anderson, 1998). Ishikawa and Taniguchi (1997) studied in situ patterns of cysts, and bloom formation of dinoflagellates in the Onagawa Bay (Japan). They concluded that cysts play an important role as seeds of the vegetative forms in all the species, but the bloom is performed in a species-specific behavior under fluctuation of the season and environment conditions. In general, the cysts are considered as harmful algal blooms potential and toxin production (Dale, 2001; Garces *et al.*, 2002). Therefore, encystment is an important factor in bloom termination (Wang *et al.*, 2007).

There are many published data on biodiversity and density of vegetative phase of microalgae in the southern basin of the Caspian Sea. Examples are the researches of Roohi *et al*. (2010), Fazli *et al*. (2010), Bagheri *et al*. (2012), and Nasrollahzadeh Saravi *et al*. (2014, 2015b), However available information on cysts are limited in this region. Mudie *et al*. (2017) identified 79 dinocyst species in surface sediment samples of the BSC (Marmara, Black, Azov, and Caspian Seas). Lewis *et al.* (2018) reported the dinocysts of

Kolkwitziella acuta, Lingulodinium machaerophorum, Pentapharsodinium dalei, Scrippsiella plana Luo, *Gonyaulax baltica, Gonyaulax sp., Gymnodinium aureolum, Scrippsiella acuminate, Woloszynskia* sp. Which were recorded in the sediment of the Gorgan transect of the Caspian Sea.

The satellite temperature records of the southern Caspian Sea have been indicated a warming trend in all seasons, especially in summer from 1982 to 2012. The same trend was reported in the Black, Aral, and Ghare Baghaz-Gol Seas. Therefore, this warming process might be an index of global warming (Leroy *et al.*, 2013). The various studies indicated that the Caspian Sea ecosystem is influenced by climate change, overfishing, anthropogenic activities, and invasive species (Roohi *et al.*, 2010; Nasrollahzadeh Saravi *et al.*, 2014, 2015a; Makhlough *et al.*, 2017a,b; Makhlough *et al.*, 2018; Makhlough *et al.*, 2019). Water temperature and nutrients (DIN and DIP) changes in southern of Caspian Sea are shown in Table 1.

Table 1: Mean of the physicochemical variables at different seasons and years in the southern Caspian Sea (surface layer).

	Years of study	Water temperature (°C)				DIP-P ($\mu g.L^{-1}$)				DIN ($\mu g.L^{-1}$)			
		Spring	Summer	Fall	Winter	Spring	Summer	Fall	Winter	Spring	Summer	Fall	Winter
1	1996-1997	20.77	27.16	18.31	11.30	11.28	10.45	6.62	18.88	35.1	15.0	17.4	33.6
2	2009-2010	20.76	26.48	17.88	9.59	5.77	9.62	8.64	6.50	60.5	71.4	93.5	64.8
3	2011-2012	17.45	27.57	16.75	7.25	9.47	8.77	11.99	8.88	89.8	70.6	75.8	74.9
4	2012-2013	23.21	27.15	18.19	10.52	12.39	12.99	19.00	25.52	96.0	91.4	90.6	78.3
5	2018-2019	19.43	27.78	18.95	11.00	10.19	7.71	5.00	4.50	103.8	83.1	82.6	72.0

1-Hosseini, 1999; 2-Nasrollahzadeh Saravi *et al.*, 2010; 3-Nasrollahzadeh Saravi 2011b; 4-Nasrollahzadeh Saravi *et al.*, 2016; 5-Nasrollahzadeh Saravi *et al.*, 2019.

Cysts data from the surface layer of sediments have the potential to link with environmental parameters (Dale, 2001). The identified cysts in sediments in the northern part of the Caspian Sea are characteristics of freshwater and oligo-haline, while in the southern part, they indicate warmer waters and higher salinity (Leroy *et al.*, 2013). In the pelagic zone, various factors such as sediment turbulence (floods and waves), unusual nutrient intake, and rising of temperature, can drive surface sediment cysts moving into the water column (Bravo and Figueroa, 2014) and stimulate cyst germination.

Although the study of algal cysts in the water column is not a selective method, it can nevertheless be helpful in preparing a list of algal cysts, especially in an ecosystem where there is a lack of cysts information.

Identification of cysts in the water column can also explain the sudden increase in plankton forms resulting from the germination of released cysts in the water column. Therefore, considering the history of dinoflagellate bloom in the Caspian Sea and its importance in the planktonic structure of this water body, the present study aims to survey the fluctuations of dinocysts in water column of the southern Caspian Sea.

Materials and methods

The study was conducted seasonally in the years of 2009-2010, 2011-2012, 2012-2013, and 2018-2019 (Table 2 and Fig. 1) in Astara (1), Anzali (2), Sefidrud (3) transects represented as west area, Tonekabon (4), Nowshahr (5), Babolsar (6) transects as center area and Amirabad (7), Turkman (8) transects as east area (Table 2).

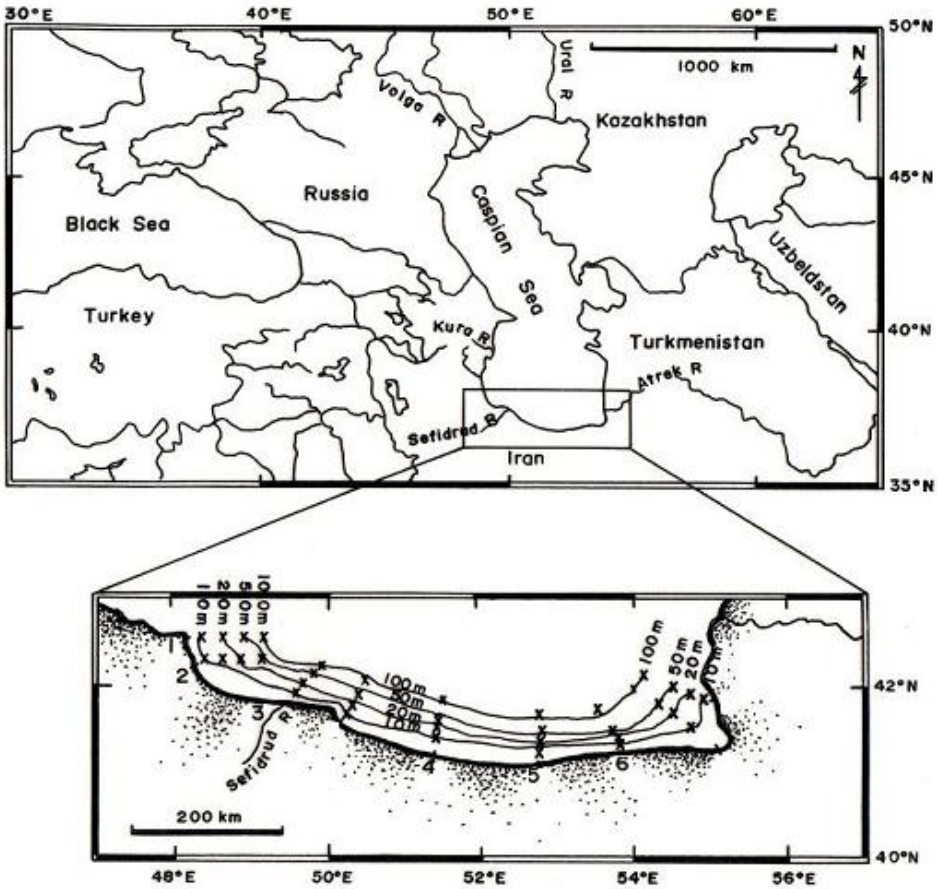

Figure 1: Map of the southern part of the Caspian Sea showing sites of sampling along eight (1-8) transects (labeled ×) and different depths.

The Niskin sampler collected water samples for the study of dinocyst and its vegetative forms. The samples were kept in 0.5 L bottles and preserved using buffered formaldehyde to yield a final concentration of 2%. The water samples were prepared using the sedimentation and centrifuge method

(APHA, 2005). A subsample of 0.1 ml was analyzed under a light microscope (Nikon, LABOPHOT-2, Japan) (coverslip 22×22mm and with magnifications of 100, 200, 400×). The dinocysts and their vegetative forms were identified and enumerated with the help of Elbrachter and Drebes (1978), Nehring (1993), Dale (2001), Wehr and Sheath (2003), Bravo and Figueroa (2014), and Mudie *et al*. (2017). The percent frequency of presence of dinocysts was determined by calculating the number of water samples with cysts divided to total number of samples in seasons, depths, and layers multiplied by 100 to express the temporal and spatial distribution of the cyst. Analysis of variance (ANOVA) was used to determine the statistically significant differences in dinocysts abundance between the seasons, depths, layers, and regions.

Table 2: Characteristics of sampling stations in the southern Caspian Sea.

Transects	Depth(m) of stations	Sampling layers (m)	Number of samples	Years of study
Astara (1), Anzali (2), Sefidrud (3), Tonekabon (4), Nowshahr (5), Babolsar (6), Amirabad (7), Turkman (8)	5,10, 20, 50, 100	Surface, 5, 10, 20, 50, 100	268	2009-2010
Anzali (2),Nowshahr (5),Amirabad (7)	5,10, 20	Surface, 5, 10, 20	84	2011-2012
Anzali (2), Tonekabon (4), Nowshahr (5), Amirabad (7)	5,10, 20	Surface, 5, 10, 20	99	2012-2013
Astara (1), Anzali (2), Sefidrud (3), Tonekabon (4), Nowshahr (5), Babolsar (6), Amirabad (7), Turkman (8)	5,10, 20	Surface, 5, 10, 20	112	2018-2019

Results

The identified dinocysts were included *Gonyaulax polyedra*, *Dissodinium pseudolunula*, *Pyrocystis* sp., *Kryptoperidinium foliaceu.* Meanwhile, some unknown cysts were recorded in microscopic observations. The percent frequency of cysts was 46, 4, 9, and 34%, in the years 2009-2010, 2011-2012, 2012-2013, and 2018-2019, respectively. The different stages of the *Dissodinium pseudolunula* and *Pyrocystis* sp. cysts formed the highest percent frequency during the study periods. The maximum (207143±80778 N/m^3) and minimum (7143±2000 N/m^3) abundance of *Dissodinium pseudolunula* and *Pyrocystis* sp. cysts were recorded in years of (2018-2019) and (2011-2012), respectively. The highest abundance of the cyst (709822±354800 N/m^3) was recorded in 2018-2019, which followed the high abundance of *Kryptoperidinium foliaceum* cyst (Table 3).

The percent frequency of cysts in fall and winter (15, 16) was lower than in spring and summer (40 and 41). In

other words, in cold seasons the cysts were accumulated in some stations. The total cyst abundance decreased from spring (445181±240306 N/m^3) to fall (34286±3526 N/m^3), then it increased in winter.

Table 3: The abundance (N/m^3±SE) of dinocysts at different years and seasons in the southern Caspian Sea.

Years	2009-2010	2011-2012	2012-2013	2018-2019
Gonyaulax polyedra	4851±3121	-	-	-
Dissodinium pseudolunula+Pyrocystis	190672±35998	7143±2000	65657±16651	207143±80778
Kryptoperidinium foliaceum	-	-	-	491965±353875
Total cysts	197388±35917	7143±2000	65657±16651	709822±354800
Seasons	**Spring**	**Summer**	**Fall**	**Winter**
Gonyaulax polyedra	6024±4887	1220±1212	952±947	-
Dissodinium pseudolunula +Pyrocystis	123494±30158	139634±28109	33333±3449	269531±79071
Kryptoperidinium foliaceum	310843±240997	23171±8242	-	-
Total cysts	445181±240306	169512±27712	34286±3526	269531±79071

Gonyaulax polyedra and *Kryptoperidinium foliaceum* cysts showed the maximum abundance in spring, however, the maximum abundance of *Dissodinium pseudolunula* and *Pyrocystis* cysts were recorded in winter (Table 3). The abundance *of Dissodinium pseudolunula* and *Pyrocystis* cysts infall were significantly lower than in other seasons (ANOVA, $p<0.05$). Tables 4 and 5 show the abundance of dinocysts at different depths, layers, and regions. The percent frequency of cyst at depths of 5, 10, 20, 50, and 100 meter were obtained 21, 24, 32, 49, and 46, respectively (Table 4).

Table 4: The abundance (N/m^3±SE) of dinocysts at different depths and layers of the southern Caspian Sea.

Depths	5m	10m	20m	50m	100m
Gonyaulax polyedra	1234±1225	1460±1443	599±590	10959±10947	1389±1377
Dissodinium pseudolunula+Pyrocystis	71605±24170	118248±35052	198204±65797	146575±19474	216667±67440
Kryptoperidinium foliaceum	512346±487928	51095±21026	41317±15026	-	-
Total cysts	590123±483433	175912±35511	243712±65120	157534±20193	218056±67316
Layers	**Surface**	**10m**	**20m**	**50m**	**100m**
Gonyaulax polyedra	4015±2945	1613±1603	-	-	-
Dissodinium pseudolunula+Pyrocystis	198540±43816	109677±21064	96939±43348	82609±18360	69231±37879
Kryptoperidinium foliaceum	168248±144311	53226±23514	27551±13765	-	-
Total cysts	372993±147843	168548±27280	130612±43657	82609±18360	69231±37879

The cysts of *Gonyaulax polyedra*, *Dissodinium pseudolunula*, and *Pyrocystis* observed at all sampling depths. The maximum abundance of cysts (590123±483433 N/m^3) and minimum (157534±20193 N/m^3) were recorded at 5 and 50 m depths, respectively. The mean abundance of cysts showed a significant difference among 5m with 50 m and 100 m of sampling depths (ANOVA, $p<0.05$). The percent frequency of cysts in water layers varied from 28 to 39, with no significant difference between layers (ANOVA, $p>0.05$) (Table 4). The cyst abundance at 5-20 m depths was approximately two folds more than depths of 50-100 m. Similarly, the abundance of cysts in the surface to 20 m of layers was approximately three folds more than layers of 50-100 m (Table 4). Statistical analysis showed a significant decrease in cyst abundance from the upper layer to the 50-100 m of layers ($p<0.05$). According to Table 5, the maximum abundance of *Gonyaulax polyedra*, *Dissodinium pseudolunula+Pyrocystis*, and *Kryptoperidinium foliaceum* cysts were recorded in the west, center and east parts of the study area, respectively. However, there was no significant different of total cyst between three regions ($p>0.05$).

Table 5: The abundance (N/m^3±SE) of dinocysts at different regions of the southern Caspian Sea.

	West	Center	East
Gonyaulax polyedra	7383±5483	1290±1270	-
Dissodinium pseudolunula + Pyrocystis	189262±54541	266452±65656	74375±14364
Kryptoperidinium foliaceum	18792±8509	50968±20211	279375±249020
Total cysts	218121±53681	324516±65833	356250±247236

The regional/seasonal data of total cysts showed that the maximum abundance at west, center, and east regions in winter, fall, and spring seasons, respectively. The seasonal abundance of *Gonyaulax polyedra* cyst of water was compared with its vegetative form in Figure 2. The patterns of seasonal changes were the same in two forms (cyst and vegetative forms) of the species and the abundance was decreased from spring to next seasons.

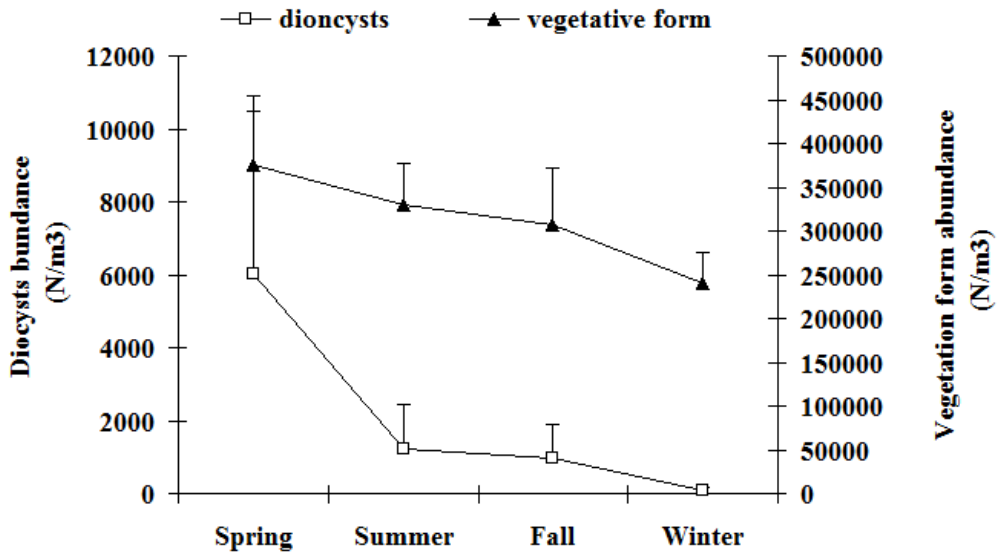

Figure 2: The seasonal abundance of *Gonyaulax polyedra* cyst (N/m^3±SE) and vegetative (100 N/m^3±SE) forms in the southern Caspian Sea.

Discussions

According to satellite data, the sea surface temperature (SST) in the southern Caspian Sea has been an alarming upward trend, especially in the summer (Leroy *et al*., 2013). The same trend was obtained in the field study of the temperature of the area (Table 1). It seems that along with the climate parameter changes and ongoing eutrophication in the Caspian Sea (Nasrollahzadeh *et al.*, 2008, 2018), dinocysts of the water column have been intensified based on the present study as well. So far, about 40 vegetative forms of dinoflagellates species have been identified in the Iranian basin of the Caspian Sea (Nasrollahzadeh Saravi *et al*., 2015b). In the present study, 4 dinocysts species (*Gonyaulax polyedra*, *Dissodinium pseudolunula*, *Pyrocystis* sp., *Kryptoperidinium foliaceum*) were identified. It approximately was 10% of the number of dinoflagellates species in the water columns. Mudie *et al.* (2017) reported 9 species of dinocysts in sediments of different regions of the Caspian Sea, which constituted about 20% of the species identified in the vegetative phase.

In general, the construction of thermocline starts in the late spring and is completed in the summer in the Caspian Sea. Then, it begins to distribute in the fall and eventually destructed in the winter. Water stratification and subsequent mixing of water layers are important events that effect on many biotic and abiotic processes in the study area (Sapozhnikov *et al.*, 2006; Nasrollahzadeh Saravi, 2011b). Full mixing of water in the winter is accompanied by increased turbidity and nutrients in the water column (Niyazi *et al.*, 2016). It seems that the entry of nutrients into the water column

provides a good chance for the cysts movement (from sediment to water) to form a vegetative stage. The sharp increase of cyst abundance was recorded from fall into the winter and the maximum abundance of cyst record in spring (Table 3). It might be due to the high nutrients from the previous season (Nasrollahzadeh *et al.*, 2010) or the flood event in the spring of 2019 (Table 1) that supports the cyst shift to the vegetative stage. During the periods of the study, vegetative forms of *Dissodinium pseudolunula* and *Pyrocystis* were not noticeable (Makhlough *et al.*, 2012; Nasrollahzadeh Saravi *et al*., 2014, 2015b) and there is no published data on the vegetative form of *Kryptoperidinium foliaceum* in this area. Lewis *et al*. (2018) isolated the motile stage of *Kryptoperidinium foliaceum* from the Caspian Sea sediment slurry cultures. The present study showed a simultaneous increase in the cyst and vegetative forms of *Gonyaulax polyedra* in water with increases in temperature and nutrients (Tables 1 and 3). The study by Leroy *et al*. (2013) also showed that global warming has led to an increase in the abundance and frequency of *Gonyaulax polyedra* cysts on sediment and its vegetative form in the water of the Caspian Sea after the year 1967. Unlike heterotrophic species which are increasing in wintertime (Ribeiro *et al.*, 2016), the autotrophic dinoflagellates (such as *Gonyaulax polyedra*) are turbid-sensitive and typically limited to warm and stratified layers. The present study showed that the abundance of vegetative and cysts of *Gonyaulax polyedra* in spring and summer were higher than in winter along with the increase of temperature. In addition, the microscopic observation of Caspian Sea water during biological pollution in 2009 showed the contribution of *Gonyaulax polyedra* as species that accompanied the bloom of *Nodularia spumigena* (Nasrollahzadeh Saravi *et al.*, 2011a). Therefore, the species are considered as an indicator of climate change and pollution (Leroy *et al*., 2013).

The study of Bringue *et al.* (2013) showed that the minimum temperature for the growth of *Gonyaulax polyedra* was 15°C, and the increase of SST was the most important environmental factor in *Gonyaulax polyedra* germination. In the present study, the water temperature was obtained 13-22°C and 23-29°C during the spring and summer seasons (Table 1), respectively, which confirmed the aforementioned finding.

The water temperature in summer was higher than spring. However, the seasonal changes (Fig. 2) showed that the abundance of *Gonyaulax polyedra* (cyst and vegetative forms) in summer was lower than in spring. In other words, the high abundance has not occurred at the point of maximum temperature. Kremp and Anderson (2000) explained two reasons (reduced potential for germination towards the end of an annual cycle and genetic characters) for decreasing the trend of *Gonyaulax polyedra* abundance from

spring toward winter .They concluded that the observed results probably indicating to lower role of external parameters (nutrient, temperature, and light intensity) compare to internal factors (such as maturation and an annual cycle) in controlling germination of *Gonyaulax polyedra* after a sudden increase of germination in the spring. The seasonal changes of dinoflagellate *Scrippsiella hangoei* in northern Baltic Sea (Kremp and Anderson, 2000) were similar to *Gonyaulax polyedra* temporal fluctuations in the present study. The sudden increase in the cyst and vegetative forms of *Gonyaulax polyedra* in spring (after the flood) in the Caspian Sea indicated the large seed banking, which can cause large reproduction of the species.

The increase of dinocysts has an impact on the food chains of the ecosystem (Ribeiro *et al*., 2016). The dinocysts were observed in all layers and depths (Table 4), which has the potential to increase dinoflagellates in the water column. The maximum value of cysts in the upper layer probably indicating more opportunity for cysts to enter the growth stage.

As the scientific forecast about climate parameters change effects (CEP, 1998), the severe floods happened in the spring of 2019 in the southern Caspian Sea, especially in the east region. The flood causes ground and soil flooding and increases the flow of nutrients (N, P, and Si) rich water into the sea. In the spring of 2019, the phosphorous concentration (21.4 µg/L) increased two to three folds compared to the previous season (3.5-12.3 µg/L) on the east coast (Turkman transect) (Nasrollahzadeh Saravi *et al.*, 2019). The study of Mudie *et al*. (2017) showed different ecological assemblages of cysts in BSC were correlated with seasonal and annual SST, salinity, phosphate, nitrate, and bottom water oxygen. In the present study, the Microscopic observation showed that the high abundance of heterotrophic cysts of dinoflagellate *Kryptoperidinium foliaceum* (40 million N/m^3) at the surface layer (5m depth) of Turkman coast (close to the estuarine of Gorgan River). The cyst of *Kryptoperidinium foliaceum* was reported in surface sediments of Gorgan Gulf (Caspian Sea) in Lewis *et al.* (2018).

In the center region of the Caspian Sea, maximum cyst abundance was in fall, which is probably related to high levels of nutrients. The increase of nutrients (especially in sediment) accompanied by algal blooms in the central region of the Caspian Sea (during late summer to fall) was reported in a study by Nasrollahzadeh Saravi *et al*. (2011a). Noteworthy is that the effective factors on increasing cyst abundance have regional and seasonal dynamics. Based on the result, the maximum abundance of phytoplankton in winter was recorded in the west region, which is naturally rich in nutrients due to the flood of rivers and freshwater resources (Nasrollahzadeh Saravi, 2008). In such areas,

determining increases in nutrients loading due to human activities or climate change is difficult (Ribeiro *et al.*, 2016). There was no significant difference of abundance in individual cysts species between regions, however, *Gonyaulax polyedra*, (*Dissodinium pseudolunula*+*Pyrocystis*) and *Kryptoperidinium foliaceum* cysts showed a tendency to increase in a specific region, which was in the west, center, and east region, respectively. The FAO (2008) emphasized that nutrients from anthropogenic sources in coastal areas would increase HAB species with poses a threat to food safety.

The climate parameters (an increase of temperature and flood events) and anthropogenic sources of nutrients have an important role on dinocysts dynamic throughout the Caspian Sea, especially in coastal areas. The dinoflagellate species could be indicators of harmful potential and food safety impact. Overall, the result of the present study is helpful in fish culture activities and environment management.

Acknowledgments

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