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SHORT PAPER

An Unusual Red-Tide Event of *Noctiluca Scintillans* (Macartney) in the Southeastern Black Sea

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Abstract

An intense *Noctiluca scintillans* bloom is reported for the first time in the southeastern coast of the Black Sea. During the present study, surface water samples were collected in the vicinity of the port of Rize between 18 and 20 April 2011. The bloom appeared as a sudden increase in cell number (6.81x10^6 cells l^{-1}) and persisted during three days. The bloom led to a red-tide event with typical streaks and patches of reddish viscous surface waters. The abundance of *Noctiluca* was higher than other previous reports in the Black Sea. During the bloom, sea surface temperature ranged between 9.9-11.7°C and salinity ranged between 15.04-17.7 ‰. Nitrite+nitrate, silicate and phosphate concentrations ranged between 0.59 - 7.13 µM l^{-1}, 3.69-10.92 µM l^{-1} and 0.04-0.19 µM l^{-1}, respectively. Meteorological data showed light precipitation and low wind speed before and during the red-tide, indicating a stable stratified surface environment, optimum for the red-tide initiation. Light onshore winds may have caused accumulation of cells near the coast and possibly also contributing for triggering the red-tide. The abundance of *N. scintillans* correlated positively with chl-a concentration (R^2= 0.83) and an intense bloom of diatom *Melosira* spp. (5x10^5 cells l^{-1}) was found concomitantly with *N. scintillans* red-tide. Microscopic examination of live cells from the *N. scintillans* red-tide revealed the presence of high number of *Melosira* spp. within the *N. scintillans* body, confirmating its grazing on these microalgae. The occurrence of this anomalous event may indicate a shift in the planktonic food web as a result of natural variability or anthropogenic influences like eutrophication.

Keywords: Red-tide, *Noctiluca scintillans*, heterotophic dinoflagellate, southeastern Black Sea.

Güneydoğu Karadeniz'de Olağandışı bir *Noctiluca scintillans* Bloomu

Özet

Olağandışı bir *Noctiluca scintillans* bloomu güneydoğu Karadeniz'den ilk kez rapor edilmektedir. Çalışma esnasında yüzey suyu örnekleri 18-20 Nisan 2011 tarihleri arasında Rize Limanı ve çevresinden toplanmıştır. Hücre sayısındaki (6,81 x 106 hücre l^{-1}) ani artış ile başlayan bloom üç gün sürmüştür. Liman içinde ve kıyı boyunca surface suyunda tipik yoğun toplanmalar ve kırmızımsı bantlar gözlenmiştir. Red-tide süresince denizsuyu yüzey sıcaklığı 9,9-11,7°C, tuzluluk ‰ 15,04-17,7 arasında değişmiştir. Nitrit+nitrat, silikat ve fosfat konsantrasyonları ise sırasıyla 0,59- 7,13 µM l^{-1}, 3,69- 10,92 µM l^{-1} ve 0,04- 0,19 µM l^{-1} arasında değişmiştir. Meteorolojik veri, red-tide'dan önce ve sonra hâkim olan hafif yağış ve düşük rüzgâr hızının belirlediği durgun tabakalaşmış yüzeyin red-tide'ın başlaması için uygun çevre olduğunu göstermiştir. Ayrıca red-tide'ın en yoğun olduğu periyotta kıyıdan karaya doğru esen hafif rüzgâr, hücrelerin kıyıda akümülasyonuna ve muhtemelen red-tide'nın başlamasına katkıda bulunmuş olabilir. *Noctiluca* bolluğu ile chl-a konsantrasyonu arasında pozitif yönlü korelasyon bulunmuştur (R^2= 0,83) ve liman içinde *N.scintillans* red-tide'ı ile eş zamanlı olarak diatom *Melosira* spp.'ye (5x10^5 hücre l^{-1}) ait yoğun bir bloom tespit edilmiştir. Mikroskobik incelemeler esnasında canlı *N. scintillans* hücreleri içerisinde *Melosira* spp.'nin varlığı, *N. scintillans*'ın bu türler üzerinden otlandığını göstermiştir. Sıra dışı bu olayın meydana gelmesi, doğal değişimin ya da ötrofikasyon gibi antropojenik etkilerin bir sonucu olarak planktonik besin zincirinde bir değişime işaret ediyor olabilir.

Anahtar Kelimeler: Red-tide, *Noctiluca scintillans*, heterotofik dinoflagellat, Güneydoğu Karadeniz.

Introduction

The large size heterotrophic dinoflagellate *Noctiluca scintillans* is one of the most common red-tide forming organisms. It is found in all temperate and tropical waters of the world as well in the major upwelling regions (Elbrächter and Qi, 1998; Harrison *et al.*, 2011), and blooms have been reported from several coastal areas in the world (Porumb, 1992; Huang and Qi, 1997; Quevedo *et al*.,1999; Rodríguez *et al*., 2005; Mohanty *et al*., 2007; Baek *et al*., 2009; Gopakumar *et al.*, 2009; Padmakumar *et al.*, 2010). The blooms typically occur from spring to summer and in extreme events can cause viscous discolorated surface waters as the cells concentrate near the surface (Elbrächter and Qi, 1998; Miyaguchi *et al*., 2006; Baek *et al.*, 2009; Padmakumar *et al*., 2010). Positive buoyancy of cells provided by the large cell vacuoles filled with ammonium ions, is considered one of the factors leading *N. scintillans* to accumulate in surface waters. It has been reported that when optimum hydrographical and biological conditions (salinity, temperature, chlorophyll-a, water stability) are provided, abundance of *N. scintillans* increase and then accumulate via wind, current and tide (Huang and Qi, 1997; Elbrächter and Qi, 1998; Umani *et al*. 2004; Miyaguchi *et al*., 2006). *N. scintillans* exhibits a phagotrophic feeding habit and primarily diet consist of bacteria, diatom, other dinoflagellates, copepod nauplii and fish larvae (Elbrächter and Qi, 1998). Previous studies have shown that *N. scintillans* competes with other grazers, directly by predation of invertebrate eggs (Elbrächter and Qi, 1998; Quevedo *et al*., 1999) and indirectly by competition for food (Umani *et al*., 2004). Although *N. scintillans* itself is not toxic, it is classified as a HAB species as it can cause fish and marine invertebrate kills through oxygen depletion, gill clogging or generation of high levels of ammonia in the surrounding waters (Okaichi and Nishio, 1976; Elbrächter and Qi, 1998).

In the Black Sea, *N. scintillans* is one of the common and numerous components of the heterotrophic plankton (Erkan *et al*., 2000; Kovalev *et al*., 2001; Feyzioglu and Sivri, 2003; Ozdemir and Ak, 2012; Mikaelyan *et al.*, 2014). The development of populations start in March and by the end of May *N. scintillans* reaches the maximum abundance (Nikishina *et al*., 2011; Mikaelyan *et al.*, 2014). Mean densities were about 1-6 cells l^{-1} in the 1970s and increased by 8-10 times in the 1980s (Harrison *et al*., 2011). *N. scintillans* biomass decreased from the 1980s to the earlier 1990s due to gelatinous organisms such as *Mnemiopsis leidyi* and *Aurelia aurita* which compete with *N. scintillans* for food (Kovalev and Piontkovski, 1998). However, Shiganova *et al*. (2008) also observed that the abundance of *N. scintillans* increased in some parts of the Black Sea in 1990s. Despite being a common species, mass development leading to a red-tide is not common. This paper reports a red-tide event in coastal waters of Rize in April 2011. To our knowledge this is the first report of a red-tide event in this area, although it is possible that other events may have occurred before. The bloom persisted three days and disappeared concomitantly with decreasing chl-*a*. The red-tide was monitored continuously between 18 to 20 April 2011 and its results are reported here. Complementary environmental and meteorological data was used to understand the mechanisms that triggered the bloom.

Materials and Methods

Study Area and Sampling

Sampling was conducted between 18-20 April 2011 in the vicinity of the port of Rize (41º02'12"N, 40º31'06"E) which is located in the southeastern coast of the Black Sea (Figure 1). During the three days, samples were collected at 8 am, 13 pm and 19 pm in 4 stations. The stations were progressively away from the coast. Three sampling stations were located in the area where the red-tide occurred. We noted that the boat typically dispersed the patches during sampling. The last station was one mile from the coast, to be used as a reference point. The water depth at the sampling stations ranged between 3 and 30 m. Samples were taken from surface by 1 l polyethylene bottles for nutrients and plankton determination. Part of the water sample was preserved immediately in 2 % formaldehyde and cells were enumerated within 2

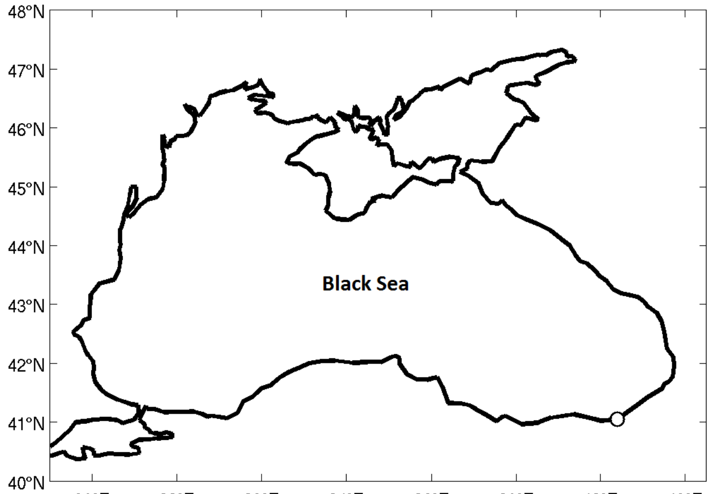

Figure 1. Location of red-tide event, Rize, Turkey.

hours of sampling.

Temperature, salinity, pH and dissolved oxygen were measured in situ with an YSI probe. Nitrite+nitrate, silicate and phosphate analysis were made according to Parson *et al*. (1984). In vivo Chlorophyll-*a* concentration was measured by Turner Handheld Aquafluor Fluorometer. The fluorometer was calibrated with a pure chl-*a* standard solution (Sigma), the concentration of which was determined spectrophotometrically (Parsons *et al.* 1984).

Meteorological data was used to study the environmental factors that may have triggered the bloom. Hourly means of air temperature and wind speed, and daily means of precipitation, at a meteorological station in Rize, were provided by the Turkish State Meteorological Service, Ankara.

N. scintillans and Phytoplankton

N. scintillans cells were counted with a Sedgewick-Rafter counting chamber using Nikon SMZ 745 T dissection microscopy. Phytoplankton species were counted with a Sedgwick-Rafter counting chamber using Nikon T 100 inverted microscope at 200X magnification. Photographs were taken using Nikon DS-Fi1 CCD camera and the cell diameters of 100, randomly chosen, cells of *N. scintillans* were measured from each sample using NIS-Elements D imaging software. Cell volume was calculated on the assumption that *N. scintillans* cells are spherical. Random subsamples of up to 20 cells from each sample were examined for evidence of feeding. Cells were scanned under high power and any prey viewed inside the cell were identified to lowest possible taxa according to Hasle and Syversten (1997) for diatoms and Steidinger and Tangen (1997) for dinoflagellates.

Results

Hydrographic and Meteorological Conditions

Physico-chemical properties of the near-shore waters off the Rize Bay, during the red-tide event are shown in Table 1. The surface sea-water temperature during this period ranged from 9.9°C to 11.7°C. Surface salinity ranged from 15.04 ‰ to 17.9 ‰. Dissolved oxygen value ranged between 5.69-8.61 mg L^{-1} at surface water. The concentrations of Nitrate + Nitrite during the red-tide varied between 0.59 µM l^{-1} to 7.13 µM l^{-1} and a sudden decrease from coastal to offshore was observed. Silicate concentration showed similar changes between stations and ranged between 3.69 µMl^{-1} to 10.92 µM l^{-1}. Phosphate concentration was less than 0.19 µM l^{-1} at all sampling stations.

Meteorological data for the sampling period is shown in Figure 2. During the bloom period both wind and precipitation were weak, and wind direction fluctuated between the W and E quadrants. In the day before the bloom (17 April), relatively higher precipitation occurred (15 mm/day) and the wind was extremely low, with absence (0 km/h) of wind from the afternoon until the morning of the next day (18 April). Wind direction in the day of the bloom was ENE which is directly towards the sampling area. Air temperature showed an increase towards the end of the sampling period, with diurnal maximums around mid-day.

The Bloom and Chlorophyll *a*

The dense bloom of the dinoflagellate *N. scintillans* was observed with a displacement volume of 769 ml l^{-1} on 18 April 2011 in the southeastern coast of Black Sea (Figure 3). This bloom appeared with bright red discoloration and persisted for three days, and then suddenly disappeared. The cell number of *N. scintillans* was 2.83 x 10^6 cells l^{-1} during onset of the bloom at 8:00 am. The largest number of *N. scintillans* cells (6.81 x 10^6 cells l^{-1}) was recorded in 5 hours after first sampling and gradually decreased till last day of red-tide (Figure 4). In the reference point, Station 4 (1 mile off from the red-tide patches), the cell number of Noctiluca ranged between 0.75-1.1 x 10^3 cells l^{-1} during red-tide. Maximum cell diameter was found as 800.9 µm whereas minimum cell diameter was 425 µm. Mean cell diameter were found 602 µm. The mean cell volume of *N. scintillans* was 1.35x10^8 µm^3.

The average chl-*a* concentration at stations showed different pattern (0.23-2.64 µg l^{-1}). A major peak (5.57 µg l^{-1}) recorded at Sta.1 and also a minor peak (1.48 µg l^{-1}) recorded at Sta.2 during first day of bloom. The lowest concentrations (0.17 and 0.19 µg l^{-1}) recorded at Sta.3 and Sta.4, respectively. Diminution of chl-*a* concentration was concomitantly observed with the waning period of bloom. The abundance of *N. scintillans* was well correlated with chl-*a* during bloom period (R^2= 0.83, Figure 5).

Microscopic examination of live *N. scintillans* cells collected during the bloom revealed the presence of microalgae species, including diatoms and autotrophic dinoflagellates. The largest cell density of microalgae within the *N. scintillans* body was represented by the diatom *Melosira* spp. (Figure 6). Cell density of *Melosira* spp. at Sta. 1 in the peak of bloom was 5x10^5 cells l^{-1}, however their density sharply decreased afterwards simultaneously with chl-*a*. *N. scintillans* was found to be a single dominant species constituting 99% of the current population, while *Melosira* spp. dominated the phytoplankton density (80 %).

Discussion

A very intense bloom was observed in the southeastern Black Sea between 18 to 20 April 2011. Table 1 shows a resume of other intense blooms in different geographical regions. The cell numbers reported in this study (6.81 x 10^6 cells l^{-1}) were within

Table 1. Comparison between present study and previous red-tide events from different regions with cell number, cell size and hydrographical parameters

Area	Noctiluca abundance (cells l^{-1})	Time	Size (µm)	Temperature Mean (C°) (min.-max.)	Salinity (‰) (min.-max.)	DO (µg l^{-1}) (min.-max.)	Nitrite (µM l^{-1}) (min.-max.)	Nitrate (µM l^{-1}) (min.-max.)	Posphate (µM l^{-1}) (min.-max.)	Silicate (µM l^{-1}) (min.–max.)	Chl-*a* (µg l^{-1}) (min.–max.)	Reference
Dapeng Bay, South China Sea	2.8×10^5	April	400-1200	20.6-21.3	30.57-30.83	-	-	-	-	-	1.49-1.64	Huang and Qi, 1997
Central Cantabrian Coast, Northern Spain	1.13×10^6	April	-	13.4	30-35	-	-	-	-	-	-	Quevedo *et al*., 1999
Bahía de Mazatlán Bay, México	1.3×10^6	January	220-500	19.3 (13-25)	-	-	-	-	-	-	-	Rodríguez *et al*., 2005
Northwestern bay of Bengal	2.38×10^5	April	-	28.5 (27.8–29.1)	32.65 (30.21–34.33)	4.6 (3.3–5.3)	0.59 (0.42-0.76)	4.29 (1.97-6.21)	2.10 (1.41–2.88)	32.61 (19.19-44.61)	-	Mohanty *et al*. 2007
Coastal waters of Saudi Arabia, Red Sea	$2.5\text{-}3\times10^6$ 2.7×10^6	February March	-	22-24 24-25	37-38 >38	3-3.5 2.5-3	-	>0.32 >0.32	0.05-0.1 >1.05	>2.63 >2.63	-	Mohamed and Mesaad, 2007
Sagami bay, Japan	2.3×10^6	April	-	17-18	>30	-	6-7(NO_{2+3})		~0.5	~30	>2	Baek *et al*. 2009
Gulf of Mannar, Southeast coast of India	1.35×10^6	October	400-1200	29.5	34.2	4.86	-	-	8.28	-	-	Gopakumar *et al*. 2009
Southwest coast of India	8.1×10^8	August	500-1000	26.03-27.24	34.11	6.89	-	0.44	0.19	18.29	12.34	Padmakumar *et al*. 2010
Southern Brazil	1.44×10^5	December	600-1000	-	-	-	-	-	-	-	-	Cardoso, 2012
Dardanelles, Sea of Marmara, Turkey	2.2×10^5	May	-	~12	~23	~10	~0.15 (NO_{2+3})		~0.1	~1	~3.5	Turkoglu, 2013
Coastal waters of the southeastern Black Sea	6.81×10^6	April	425-800.9	10.98 (10.4-11.7)	15.67 (15.04-16.08)	6.64 (5.69-7.32)	6.29(NO_{2+3})	-	0.12	8.51	2.64 (0.26-5.57)	Present study

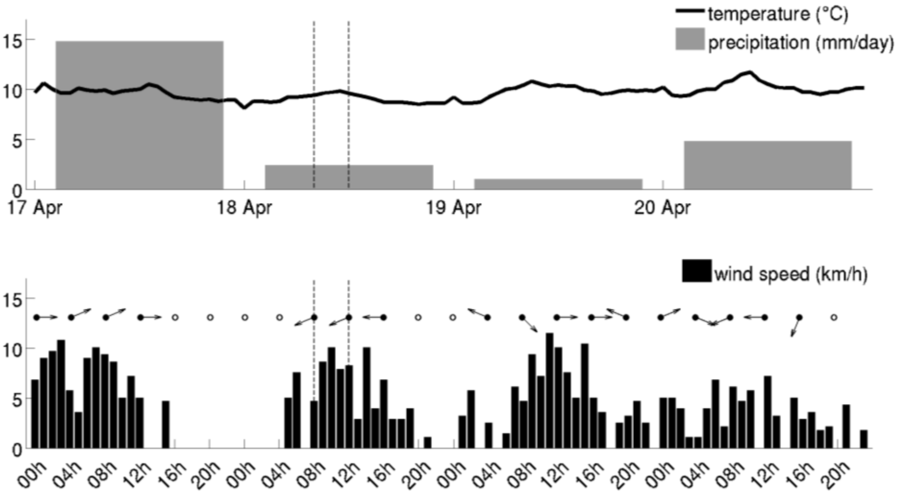

Figure 2. Meteorological factors during *N. scintillans* red-tide; precipitation (mm/day), air temperature (ºC), wind speed (km/hr) and wind direction, vertical dash lines denote the peak period of red-tide.

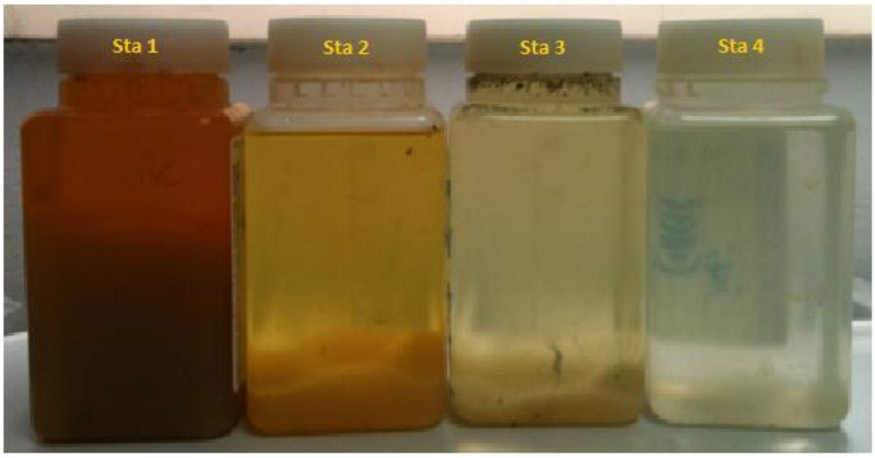

Figure 3. Displacement volume of *N. scintillans* in the sampling stations.

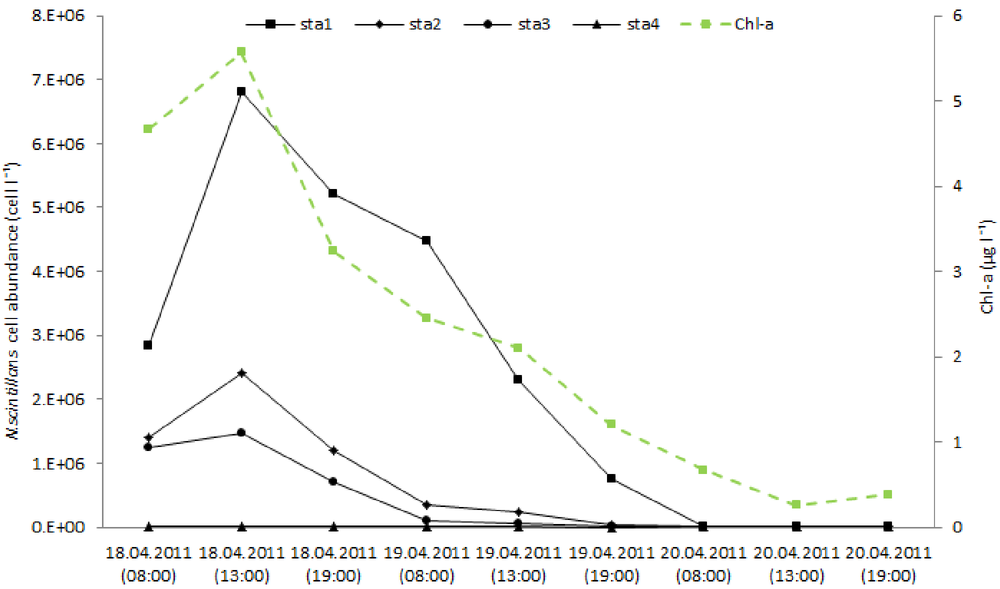

Figure 4. Abundance of *N. scintillans* in the sampling stations during bloom period and chl-*a* concentration in station 1.

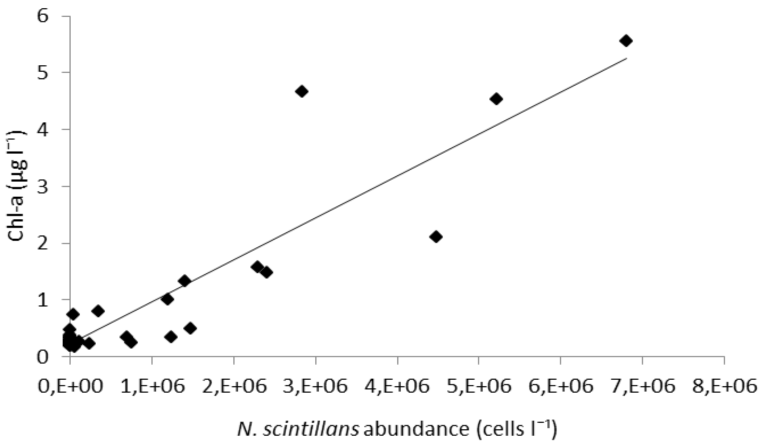

Figure 5. Relationship between *N. scintillans* abundance and chl-*a* concentration.

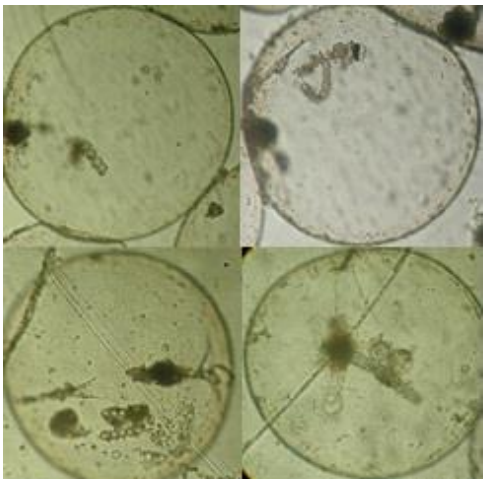

Figure 6. *N. scintillans* with diatom cells (*Melosira* spp.).

the range (10^5-10^8 cells/l) of red-tides in other regions (Table 1), but higher than previous studies reported in the Black Sea (Porumb, 1989; Harrison *et al*., 2011; Mikaelyan *et al*., 2014).

In spring, when *N. scintillans* is present throughout the photic zone, the presence of food and calm conditions are known as the key factors for the rapid increase in abundance (Harrison *et al*., 2011). High numbers of *N. scintillans* have been observed concurrently with high biomass of diatoms (Porumb, 1989; Kiørboe and Titelman, 1998; Dela-Cruz *et al*., 2002; Turkoglu, 2013) and high chl-*a* concentrations (Nakamura, 1998; Padmakumar, 2010). Our results are consistent with these previous works. In this study, the *N. scintillans* red-tide coincided with a diatom bloom and *N. scintillans* abundance positively correlated with chl-*a* concentration. *Melosira* spp. abundance during peak bloom period was 5×10^5 cells l^{-1} and rapidly decreased simultaneously with chl-*a* after end of the red-tide. The reduction in phytoplankton abundance (mainly diatoms, *Melosira* spp. constituted 80% of the phytoplankton density) was mainly controlled by *N. scintillans* grazing which was confirmed with the presence of *Melosira* spp. inside Noctiluca food vacuole. Our results suggest there is a top-down control of phytoplankton bloom by *N. scintillans.* The large size 425-809 µm in the peak of the bloom suggests good nutritional condition of *N. scintillans.*

The growth rate of *N. scintillans* is generally affected by temperature and salinity, but *N. scintillans* is known to be a eurythermal and euryhaline organism (Elbrächter and Qi, 1998). During bloom period relatively low sea-water temperature of 10.9°C was observed. Previous studies showed the optimum temperature (10-28ºC) and salinity (28-36 ‰) ranges for *N. scintillans* were wide and differed between regions (Table 1).The temperature in this study is within the optimum conditions reported in literature. However, salinity (15.04-17.7‰) was found lower than optimal ranges. It has been reported that when the salinity was lower than 27 ‰, *N. scintillans* densities were very low (Huang and Qui, 1997). On the other hand, Lirdwitayaprasit *et al*. (2006) reported that the blooms of *N. scintillans* were often found when salinity was range of 22-33‰ and sometimes 10-15‰. According to our data we suggest that multiple salinity strains of the species may exist. It is perhaps a 'low-salinity' strain capable of establishing populations in the southeastern Black Sea and proliferates when the food becomes more abundant. Our results also showed a decrease in the DO of the seawater during the peak period of blooms, probably as a result of large-scale respiration on the part of *N. scintillans*.

Several factors known to trigger *N.scintillans* blooms were present in this study. First, there was food availability indicated by the high number of *Melosira* spp. ($5x10^5$ cells l^{-1}) in the first day of the bloom. Second, there were optimum environmental conditions, indicated by the light wind and rain. In fact, in the day before the bloom was observed, there was relatively higher precipitation and almost no wind during several hours which may have initiated an increase in population. Precipitation can create a positively buoyant layer at the surface and weak winds do not generate sufficient turbulence to disrupt this salinity-induced stratified surface layer. These two factors may have combined to retain *N. scintillans* at the surface where it could proliferate. Other studies have shown the key role of wind in formation of *N. scintillans* blooms (Huang and Qi, 1997; Miyaguchi *et al*., 2006; Mikaelyan *et al*., 2014). The third mechanism was the ENE light winds that might have advected the N. scintillans towards the coast and increased the proximity of each cell. The proximity of the cells to each other has been also suggested as a necessary step to trigger red-tides, through swarmer-effects (Miyaguchi *et al*., 2006).

Previous studies have shown that the mass development of Noctiluca was mainly related to the temperature regime or low spring SST (Bityukov, 1969; Shiganova *et al*., 2008, Oguz and Velikova, 2010). A recent study by Mikaelyan *et al*. (2014) showed a relation between years with low wind speed and N. scintillans abundance.Mikaelyan *et al*. (2014) reported one of the most noticeable peaks in N. scintillans abundance also in 2011 in the northern Black Sea. In this study, wind was also low during the bloom period. Since *N. scintillans* has unique eurythermal characteristic, temperature does not seems as a key factor to trigger mass development. However colder sea surface temperatures could indicate deep mixing and the upwelling of nutrients, which can increase phytoplankton abundance and prey availability for *N. scintillans*. Other reasons for this red-tide might have been the absence of gelatinous predator (*Mnemiopsis leidyi*, etc.).

Satellite data is a useful tool to determine the spatial extension of the bloom. However, cloud-free data from the MODIS-AQUA and MERIS sensors were not available during the sampling period in the region of interest. During this study ammonia concentration were not measured. However, during and after red-tide, no dead fish and marine invertebrates were found in the red-tide area. This bloom could be described as harmless in Turkish waters. Nevertheless, their role in clearing the water of other plankton is thought to be an important element of the trophic chain. If the predators of *N. scintillans* do not exist in the water column, this type of blooms could cause losses of carbon transfer to the higher trophic levels. The occurrence of this anomalous event may indicate a shift in the planktonic food web as a result of natural variability or anthropogenic influences like eutrophication. Because of the potential for harm, HAB monitoring programme should be implemented to understand the occurrence and consequences of red-tide caused by *N. scintillans* and other HAB species along the Black Sea coast.

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