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Biochemical Content (fatty acids, sterols, lipophilic vitamins, total protein, MDA, GSH, GSSG) of *Noctiluca scintillans* in the Southeastern Black Sea

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Abstract

Noctiluca scintillans reached the highest abundance (5105 ind/m^3) in April; and April was an important period in the Black Sea ecosystem because of the seasonal thermocline. While the relationship between temperature and abundance was important (P<0.05, r^2=-0.8), the relationship was not important between salinity and abundance (P<0.05, r^2=-0.06); and Chlorophyll-a (Chl-a) and abundance (P<0.05, r^2=-0.2). It was determined that total lipid was 0.5% in wet weight (WW). It was shown that the main Saturated Fatty Acids (SFA) were 16:0 (24%) and 18:0 (6%), main Monounsaturated Fatty Acids (MUFA) were 18:1ω-9*c* (22%) and 16:1ω-7 (4%), main Polyunsaturated Fatty Acids (PUFA) were 18:2ω-6c (19%) and 20:4ω-6c (8%). Phytosterols were the most important sterols in *N. scintillans*; and stigmasterol was 60 µg/gr and β-sterol was 8µg/gr as the most important ones in phytosterols. α-tocopherol was the highest amount (10.8 µg/g) in lipophilic vitamins. It was found that Malondialdehyde (MDA) was 65 µg/g, total protein was 10 mg/g, Glutathione (GSH) was 10565 µg/g and GSSG) was 49 µg/g. Consequently, PUFA and α-tocopherol were the most important biochemical parameters and α-tocopherol may reduce toxicity in blooms of *N. scintillans* because of inhibitory effect of α-tocopherol on lipid oxidation. Additionally, we can say that *N. scintillans* is a resistant dinoflagellate against oxidative stress**.**

Keywords: PUFA, phytosterols, toxicity, lipid oxidation

Güneydoğu Karadeniz'de *Noctiluca scintillans*'ın biyokimyasal içeriği (yağ asitleri, steroller, lipofilik vitaminler, toplam protein, MDA, GSH, GSSG)

Özet

Noctiluca scintillans Nisan ayında en yüksek bolluğa ulaşmıştır ve mevsimsel termoklinin de Nisan ayında oluşmasından dolayı, Nisan ayı Karadeniz ekosisteminde önemli bir dönemdir. Sıcaklık ve bolluk arasındaki ilişki önemli iken (P<0.05, r^2=-0.8), tuzluluk ve bolluk (P<0.05, r^2=-0.06), Chl-a ve bolluk (P<0.05, r^2=-0.2) arasındaki ilişki önemli değildir. Toplam lipit yaş ağırlıkta (WW) %0.5 olarak belirlenmiştir. Temel doymuş yağ asitlerinin (SFA); 16:0 (%24) ve 18:0 (%6), temel tekli doymamış yağ asitlerinin (MUFA); 18:1ω-9*c* (%22) ve 16:1ω-7 (%4), temel çoklu doymamış yağ asitlerinin (PUFA);18:2ω-6c (%19) ve 20:4ω-6c (%8) olduğu görülmüştür. *N. scintillans*' daki en önemli steroller fitosterollerdi ve fitosterollerden, stigmasterol 60 µg/gr ve β-sterol 8 µg/gr dı. α-tokoferol en yüksek miktara (10.8 µg/g) sahip lipofilik vitamindi. Bunu D_2 (3.5 µg/g) ve K_2 (0.2 µg/g) vitaminleri takip etmiştir. *N. scintillans*'da Malondialdehit (MDA) 65 µg/g, toplam protein 10 mg/g, Glutatyon (GSH) 10565 µg/g ve Glutatyon disülfit (GSSG) 49 µg/g olarak

bulunmuştur. *N. scintillans'*daki en önemli biyokimyasal parametreler PUFA ve α-tokoferoldur. Sonuç olarak, α-tokoferolun lipit oksidasyonunu engelleyici etkisinden dolayı, α-tokoferolun *N. scintillans* bloomlarında toksititeyi azalttığı ve *N. scintillans'*ın oksidatif strese karşı dayanıklı bir dinoflagellat olduğu da söylenebilir.

Anahtar Kelimeler: PUFA, fitosteroller, toksisite, lipit oksidasyonu

Introduction

Noctiluca scintillans (Macartney) Kofoid & Swezy is a large-size heterotrophic dinoflagellate. It is reported widely as a "red tide" organism in temperate, subtropical and tropical seas and is well known for its luminescence. *Noctiluca* does not produce toxins but it is reported as a harmful algal with oxygen depletion, gill clogging, and high ammonia levels on the basis of mass mortalities of finfish in farms associated (Okaichi and Nishio, 1976). It has been reported that *N. scintillans* may act as a vector of phycotoxins to higher trophic levels, by feeding on toxigenic microalgae (Escalera *et al.,* 2007). The blooms generally occur from spring to summer in many parts of the world, and can cause extreme events as increasing of the cells concentrate near the surface (Elbrächter and Qi, 1998; Miyaguchi *et al.,* 2006; Baek *et al.,* 2009; Padmakumar *et al*. 2010). The blooms are resulted often in a strong pinkish red or orange in some places as Australia (Hallegraeff, 1991), Thailand (Sriwoon *et al.* 2008), Gulf of Mannar, India (Gopakumar *et al*., 2009) Japan, Hong Hong, China (Huang and Qi, 1997), Turkey including the Bosphorus, Marmara Sea,Dardanelles (Unsal *et al.* 2003, Turkoglu *et al*., 2004, Turkoglu and Buyukates, 2005, Turkoglu, 2010; Turkoglu and Erdogan, 2010; Turkoglu, 2013), the Black Sea (Porumb,1992; Turkoglu and Koray, 2002; 2004) and in the Southeastern coast of the Black Sea (Kopuz *et al.,* 2014). *N. scintillans* blooms are known to occur after diatom blooms ((Kiørboe, 1998; Tiselius and Kiorobe, 1998; Dela-Cruz *et al*., 2002) because availability of phytoplankton as prey is one of the important factors for the variation in abundance of *N. scintillans* (Elbrächter and Qi, 1998). Therefore, we used Chl-a concentration as the basic criteria of the phytoplankton biomass. However, the abundance of *N. scintillans* shows temporal variations from seasons to years, and its blooms have been reported to correlate with environmental factors. However, the initial trigger of the largescale bloom formations have not specifically been attributed to the any particular condition. The cause of the large-scale bloom formation in coastal embayments is still controversial (Miyagucci *et al*, 2006).

N. scintillans is an opportunistic omnivorous dinoflagellate and has a rapid reproduction capability, together with polyphagous feeding behavior enables outbursts in populations under favorable conditions, frequently dominating the >200 µm fraction of plankton (Uhlig and Sahling, 1990; Shanks and Walters 1996; Elbrachter and Qi, 1998). The diet of the species consists of a broad spectrum of prey, including phytoplankton, nauplii and eggs of zooplankton (acartia), anchovy, organic detritus, and bacteria (Hattori, 1962; Sekiguchi and Kato, 1976; Schaumann *et al*., 1988; Kirchner *et al*., 1996; Elbrachter and Qi, 1998; Quevedo *et al.,* 1999), thus enabling the species to prey on items theoretically higher up in the trophic web. It is one of the common and numerous components of the heterotrophic plankton in the Black Sea (Erkan *et al.*, 2000; Kovalev *et al.,* 2001; Feyzioglu and Sivri, 2003; Özdemir and Ak, 2012; Mikaelyan *et al*., 2014) and has an important role on the pelagic ecosystem of the Black Sea (Oguz *et al.,* 2001 a, b). Thus, we thought that to obtain information about some

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biochemical parameters of *N. scintillans* would be useful to create source. - We especially researched these parameters in the period when there was the most abundance. Biochemical parameters as lipids are important biochemical compounds in trophic transfer in marine food webs - because they are rich in carbon with a very high energy value (Parrish, 1988). They can be used as biomarkers in ecology (Budge *et al*., 2006; Litzow *et al.*, 2006). Especially, PUFA, including docosahexaenoic acid (20:6ω3, DHA), eicosapentaneoic acid (20:5ω3, EPA) and α-linolenic acid (18:3ω3, ALA) etc., are among the crucial classes of fatty acids. They are critical structural components and precursors of signaling molecules, which are involved in many diverse biological and biochemical processes, and important to maintain physiological functions in consumers (Ackman *et al.* 1980; Sargent *et al*., 1987; Caramujo *et al.*, 2008; Lund *et al.*, 2008). Additionally, proteins play extremely important roles in most biological processes of living beings, such as enzymatic catalysis, transport and storage, coordinated motion, mechanical support, immune protection, generation and transmission of nerve impulses, and control of growth and differentiation (Zaia et al., 1998). MDA level constitutes a good marker of peroxidative damage to cell membranes (Gil *et al.*, 2002). Thus, it is used as an indicator of lipid peroxidation. In addition, it is a highly reactive three carbon dialdehyde produced as a byproduct of PUFA peroxidation (Janero, 1990) and also during arachidonic acid (ARA) metabolism for the synthesis of prostaglandins (Marnette, 1999). GSH - plays an important role in protecting these from oxidative damage (López-Barea and Gómez-Ariza, 2006). GSH is the most abundant antioxidant in aerobic cells and is synthesized from l-glutamate, l-cysteine, and l-glycine in two ATP requiring steps catalyzed by the enzymes g-glutamylcysteine ligase and GSH synthetase. The cysteine thiol moiety gives antioxidant properties of GSH. The thiol is oxidized by cellular pro-oxidants, such as free radicals and reactive aldehydes, to form GSSG (Owen and Butterfield, 2010).

To our knowledge, this paper is the first report about biochemical content of *N. scintillans*. The main objective of this study is to investigate some biochemical parameters as lipophilic vitamins, fatty acids, sterols, MDA, GSH and GSSH in *N. scintillans*, which has a wide range of food, almost non-predator and causes blooms; also, to determine the relation between abundance and environmental conditions.

Materials and Methods

Study Area and Sampling

The study was performed in the Southern part of the Black Sea at a coastal station with coordinates 40^0 57' 12" N - 40^0 9' 30" E (Figure 1). *N. scintillans* samples were collected with a 200 µm mesh Hydro-Bios net with a mouth diameter of 110 cm. The vertical haul was used from the entire oxic layer starting from a depth of 130 m up to the surface layer. Samplings for abundance were made on a single day for every corresponding month between March 2012 and February 2013 aboard KTU's research vessel Yakamoz. The water depth was 250 m. Water samples were taken using Nansen bottles on the surface layer. Temperature and salinity values were measured with a conductivity-temperature-depth-oxygen CTD profiler (CTD, General Oceanic Idronaut, 316).

Abundance of *N. scintillans*

N. scintillans cells were counted under BH_2 stereo microscope. The abundance was calculated as the number of individuals in m^3. In the basis, the volume of filtered water and the total number of individuals were used (Harris *et al.*, 2000).

Chlorophyll-a Analysis

Water samples were collected from the surface layer for Chlorophyll-a (Chl-a) assay. The collected samples were filtered through 0.45 µm cellulose acetate membrane. The filtered samples were immediately frozen at -20ºC and kept until analysis. Before the analysis, Chl-a was extracted from the filtered samples using 90% acetone, and was assayed with spectrometric method (Parsons *et al*., 1984).

Lipid Extraction

The samples were homogenized with tissue fragmentation buffer. The homogenate was centrifuged at 5000 rpm for 5 min at 4°C. After centrifuging, the supernatant part was used for GSH, MDA, and GSSH. The remaining homogenate pellets were extracted in hexane: isopropanol (3:2 v/v) (Hara and Radin, 1978) and used by centrifuging for the ADEK vitamin and fatty acid analysis.

Derivatization and Analysis of Fatty Acids

An aliquot was taken from the supernatant part of the sample pellet and 5 mL 2% H_2SO_4 in methanol was added. The mixture was vortexed and then kept in the oven for 12h (50°C). Then, after being cooled to room temperature, 5 mL of 5% NaCl was added and then it was vortexed. Fatty Acid Methyl Esters (FAME) were extracted with 2×5 ml hexane. FAME were treated with 5 ml 2% $KHCO_3$ solution and then the hexane phase was evaporated by the nitrogen flow; and then, samples were taken to auto sampler vials by dissolving in 1 ml fresh hexane (Christie,1990)..

The FAME were analyzed on a SHIMADZU GC 17 Ver. 3 Gas Chromatography (Kyoto, Japan). The GC column was 25 m of long Machery-Nagel (Germany) capillary column with an inner diameter of 0.25 µm and a 25 micron-thick film was used. The column temperature was kept at 120-220°C, the injection temperature was kept at 240°C, and the detector temperature stayed constant at 280°C. The column temperature program was adjusted as 120-220°C, and the temperature increase was determined to be 5°C/min until 200 and 4°C/min from 200-220°C. It was kept at 220°C for 8 min and the total duration was set as 35 min. Nitrogen gas was used as the carrier gas. Before the analysis of the FAME, mixtures of standard fatty acid methyl esters were injected and the residence time of each fatty acid was determined. After this process, the necessary programming was made and the fatty acid methyl ester mixtures of the samples were analyzed (Christie, 1990).

Derivatization and Analysis of Lipophilic Vitamins and Sterols

The 5 mL supernatant was taken to 25 mL tubes with caps and 5mL KOH: methanol (1: 10 v/v) was added. The tubes werevortexed and then they were kept in the oven at 85°C for 15 min. They were taken and cooled to room

temperature, and 5 mL of pure water was added and mixed. -Lipophilic molecules were extracted with 2×5 mL hexane. The hexane phase was evaporated with nitrogen flow. It was dissolved in 1 mL acetonitrile: methanol (50 + 50% v/v) and then was taken to auto sampler vials and was analyzed.

Sterols were analyzed on a Shimadzu HPLC, by using LC-10 ADVP UV as the visible pump; SPD-10AVP as the detector; CTO- 10ASVP, as column oven; SIL-10ADVP as auto sampler, unit DGU-14A as degasser and Class VP software (Shimadzu, Kyoto Japan). The acetonitrile: methanol (60+40% v/v) was used in the mobile phase. The mobile phase flow rate was 1 mL. UV detector and the Supelcosil LC 18 (15×4.6 cm, 5 µm; Sigma, USA) column were - used. Wave lengths of detection were 326 nm for vitamin A, 202 nm for vitamin E and 265 nm for vitamins D, K (Katsanidis and Addis, 1999).

Analysis of MDA, GSH, GSSG and Total Protein

The supernatant part was used for MDA, GSH and GSSG. 1mL perchloric acid (10% v/v) was added to 1 mL supernatant. After centrifuged, MDA, GSH and GSSG were analyzed on a Shimadzu HPLC. MDA was analyzed according to Karatepe *et al.*, 2004. GSH was analyzed according to Yilmaz *et al.*, 2009. Peaks were identified by comparison of retention times and spectra (multiwave length scan) of the standard and the samples. Total protein amount in *N. scintillans* was measured by method of Lowry *et al.*, 1951.

Statistics

The data obtained were analyzed using the Analysis of Variance (ANOVA) Method. Used software was STATISTICA 8.0 in case there were any significant differences.

Results and Discussion

Hydrography, Chl-a, and the Abundance of *N. scintillans*

Samplings of temperature, salinity, Chl-a, and the abundance of *N. scintillans* were carried out during March 2012-February 2013 (Figure 2). It was determined that *N. scintillans* reached the highest abundance in April during the sampling period (March 2012-February 2013). Therefore, biochemical content of *N. scintillans* was investigated in April.

During the sampling period (March 2012-February 2013) for hydorography, temperatures of sea surface fluctuated from 9°C in March to 28 °C in August; and a seasonal thermocline was observed to start at a depth of 20 m in April when *N. scintillans* was sampled for biochemical content. It was observed that the temperature of the sea surface increased (12ºC) with the increase of the temperature in April. Water temperature at a depth of 40 m decreased to 7°C and the cold intermediate layer (CIL) observed during certain periods in the Black Sea dominates the region. CIL is characterized by temperatures less than 8ºC (Alkan *et al.*, 2013). In the same sampling period, salinity of the sea surface was 18 ppt and it reached 19 ppt in 100 m depending on the depth. Agirbas *et al*. (2014) reported that the thickness of the CIL was observed to be larger (65-130 m) in the coastal station of the Southeastern Black Sea (Trabzon coastline) and the salinity varied greatly within the range of 18 to 21‰ in the CIL during March 2010-December 2010. Measurements at Southeastern Coastal Site of the Black

Sea (off Trabzon) took a decade (2001-2011) and were documented by Alkan *et al*. (2013). They indicated that the temperature had the lowest values in the range of 7-10ºC during March and the highest values as 25-29ºC during July-August for all the years. And also, the surface mixed layer is characterized by salinity values between 17.5-18.0 psu (Alkan *et al*., 2013).

The mean integrated concentration of Chl-a during the sampling period (March 2012-February 2013) was 1.08 µg/L in the surface layer. Chl-a was the lowest value in April (0.54 µg/L) and while Chl-a was 1.41 µg L-1 in March, it decreased sharply in April and increased again to 1.65 µg L-1 in May (Figure 1). Chl-a concentration was used as the basic criteria of the phytoplankton biomass and researched intensively seasonal, annual and monthly variations (Vedernikov and Demidov, 1993; Oguz *et al*., 2002; Kopelevich *et al*., 2002; Moncheva, 2003; Agirbas *et al*., 2014). The Black Sea is characterized with two seasonal peaks of Chl-a in Autumn and Spring (Lalli and Parsons, 2004). Intense concentrations of Chl-a occurs during autumn and two subsequent secondary peaks in late winter-early spring (February-March) and early summer (June) (Alkan *et al*., 2013).

During the sampling period (March 2012-February 2013), *N. scintillans* was the most abundant (5105 ind/m^3) in April, following March (1358 ind/m^3) and February (888 ind/m^3) (Figure 2). Although the relation between the temperature and the abundance was important (P<0.05, r^2=-0.8), the relation between the salinity and the abundance was not important (P<0.05, r^2=-0.06). Also, the relation between Chl-a and the abundance was not important (P<0.05, r^2=-0.2). Ozdemir and Ak (2012) reported that the rate of *N. scintillans* was 57%, 98% and 85%, respectively, in April, May and June, in all of the zooplankton species in the Southeastern Black Sea (Trabzon Coast). But they did not give individual amounts. In their study, abundance of *N. scintillans* had the highest rate in May. But, only two species represented zooplankton abundance. In the study. the abundance of *N. scintillans* was 57% in April, but the abundance rate was given in five zooplankton species. Also, the influence of the environmental factors should not be forgotten. Therefore the abundance of *N. scintillans* can change in the same season. An unusual blooming of *N. scintillans* was observed in April, 2011 in the same place by Kopuz *et al*. (2014).

Biochemical Content

In marine ecosystems, lipids provide the densest form of energy which is transferred from algae to vertebrates via zooplankton. Also, they contain essential fatty acids and sterols which are considered to be important drivers of ecosystem health and stability. Fatty acids and sterols are also susceptible to oxidative damage leading to cytotoxicity and a decrease in membrane fluidity. The physical characteristics of biological membranes can be defended from the influence of changing temperature, pressure, or lipid peroxidation by altering the fatty acid and sterol composition of the lipid bilayer. The influence of essential lipids, lipid oxidation, and membrane composition on food web structure and function will become increasingly important in the context of global warming and ozone depletion (Parrish, 2013). In the present study, the biochemical parameters as fatty acids, sterols and lipophilic vitamins have been investigated.

Table 1 represents the FA composition (percent of total FA) of *N. scintillans* from dinoflagellate in April, 2012. The total lipid amount was determined as 0.5% in Wet Weight (WW). Some fatty acids, or their derivatives, are thought to work synergistically with other known toxins such as domoic acid (Wang and Shimizu, 1990; Wang

et al., 1993), diarrhetic shellfish poison (DSP) (Lawrence *et al.*, 1994), or the neurotoxin brevetoxin (Boer *et al.*, 2012). Interestingly, de Boer *et al.* (2012) found that two essential fatty acids, i.e. the C_{20} PUFA, ARA and EPA, were more toxic than the C_{18} PUFA octadecatetraenoic acid (OTA, 18:4ω-3). Since ARA and OTA have the same number of double bonds, and ARA is more toxic than EPA, the degree of unsaturation does not seem to be a key determinant of toxicity level (Parrish 2013).EPA with 2% and DHA with 3% were the two PUFA mainly detected in April, 2012. Fatty acid composition of algal lipids varies widely with species, habitat, light, salinity, pollution and environmental conditions (Kim *et al.*, 1996; Ratana-Arporn and Chirapart, 2006) and in most studies 16:0 is predominant (Khotimchenko *et al.*, 2002; Li *et al.*, 2002; Gressler *et al.*, 2010; Leblond *et al.*, 2003). Dinoflagellates are the major contributors to the marine food web and are second only to diatoms as primer producers of organic matter in the Ocean (Tappan, 1980). Dinoflagellates can form extensive blooms known as red-tide in coastal areas (Hallegraeff, 1993). Therefore, they are a major source of essential long-chain highly unsaturated fatty acids (HUFA), sterols, and other nutrients to other marine species. They are also an important source of organic matter in marine sediment (De Leeuw *et al*., 1983). HUFA are high in dinoflagellates (Holz, 1981; Nichols *et al*., 1984). Also, feeding preference of *N. scintillans* would modify the quantity and distribution of ω-3 polyunsaturated fatty acids in the ambient environment (Zhang *et. al.* 2015; Zhang et. al., 2016). Morever, altering the amount and ratio of some HUFA (DHA and EPA) available to higher trophic levels, such as copepods, fish larvae or hydromedusae (Zhang et. al., 2016).

Mansour *et al.* (1999) reported that saturated fatty acids were dominated by 16:0 and its concentration ranged from 9.0% to 24.8% in terms of the total fatty acids in different marine dinoflagellates species. They indicated that MUFA and PUFA's level changed according to the species. While they existed at high levels in some species, they existed at low levels in some species. In this study, the major MUF A were oleic acid (18:1ω-9) in *N. scintillans* (22%) and it was the second most abundant compound. The same result was given by Leblond *et al.* (2003) and they determined that 16:0 was the first dominant and, 18:1ω-9 was the second dominant fatty acid in *Karenia brevis* (dinoflagellate). But, Holz (1981) and Parrish *et al*. (1994) reported that MUFA was not usually abundant in dinoflagellates. Mansour *et al.* (1999) observed that the elevated content (11.8%) of in *Gymnodinium sp.* (dinoflagellate) was unexpected. The fatty acid profile can distinguish among algal classes but it may be limited use for the identification of species within the same algal class (Holz, 1981; Volkman, 1989). Viso and Marty (1993) determined the profiles of fatty acids of nine different marine algal groups, and they were even able to define the species-specific lipid compositions.

Free sterols are dominated in sediments of the Black Sea and the sterols are clearly of dinoflagellate origin (Boon *et al.*, 1979). The sterols are essential for all eukaryotes. They are components of the membranes and have a function in regulation of the membrane fluidity and permeability. Sterols also play an important role as precursors of many steroid hormones including vitamin D and brassinosteroids as well as for a wide range of secondary metabolites such as saponins and glycolalkaloids (Piironen *et al*., 2000). Also, sterols are a source of oxygenated lipids: the oxysterols which are the sterols bearing a second oxygen function. These compounds have a variety of biological properties including cytotoxicity and effects on specific enzymes (Parrish, 1991). Phytosterols are present in small amounts, and two common examples are the stigmasterol and the sitosterol (Abidi, 2001). Phytosterols are bioactive compounds, which can be found in a great variety of plant-based foods

(Brufau *et al.*, 2008). Phytosterols were the most important sterols in *N. scintillans*. Stigmasterol was 60 µg/g and β-sterol was 8 µg/g from phytosterols.

In our study, it was shown that α- tocopherol (vitamin E) had the highest amount (10.8 µg/g) in lipophilic vitamins, following D_2 (3.5 µg/g) and K_2 (0.2 µg/g). α-tocopherol is an important antioxidant, and antioxidants play a major role in reducing or preventing the lipid peroxidation (Aitken *et al.*, 1989). One of the most effective antioxidants is α-tocopherol as it breaks the free radical chain reaction by forming a relatively stable tocopheroxyl radical (Selley *et al*., 1991). α-tocopherol is identified as the major naturally occurring tocopherol in the lipids of marine organisms (Ackman and Cormier, 1978; Parazo *et al*., 1998). It is essential for integrity and optimal function of reproductive, muscular, circulatory, nervous and immune systems (Hoekstra, 1975; Sheffy and Schultz, 1979; McDowell, 2000). The most important determinant of vitamin E requirements is the dietary concentration of unsaturated fatty acids as PUFA are highly susceptible to auto-oxidation (Nacka al *et al.*, 2001). Animals ingesting high levels of PUFA require high concentrations of vitamin E to protect tissue lipids from free radical attack (Debier *et al*., 2002; Lammi-Keefe and Jensen, 1984; Machlin, 1991). PUFA and α- tocopherol prevent the occurrence of oxidative stress in the marine organisms, and a correlation between increased dietary PUFA and α- tocopherol requirement has been found in blue tilapia (Roen *et al.* 1990) turbot (Stephan *et al.* 1995), carp (Runge *et al.*, 1992) and Atlantic salmon (Waagboe 1991). High level α-tocopherol probably reflects higher degree of antioxidant protection required ω-3 PUFA-rich organisms (Hamre and Lie 1995). It has also an accelerating effect on reproduction (Kahn-Thomas and Enesco, 1982; Shalaby *et al*., 2004). This effect could be possibly attributed to the well-known antioxidant effect of α-tocopherol (Shalaby *et al*., 2004). Therefore, higher α-tocopherol amount than the other lipophilic vitamins may cause blooms in suitable temperature condition, because of reproduction accelerating effect of the α-tocopherol in *N. scintillans*. Additionally, the most important reason of indirect toxicity of *N. scintillans* may be the inhibitory effect of α-tocopherol on lipid oxidation.

In this study, the MDA level was found as 65 µg/g, the total protein amount was found as 10 mg/g, the GSH level was found as 10565 µg/g and the GSSG level was found as 49 µg/g in *N. scintillans*. MDA was used as an indicator of lipid peroxidation, and protein carbonyl content, which provides an indication of protein oxidative damage. It is a highly reactive three carbon dialdehyde produced as a byproduct of polyunsaturated fatty acid peroxidation (Janero, 1990) and also during arachidonic acid metabolism for the synthesis of prostaglandins (Marnette, 1999). MDA can combine with several functional groups on molecules including proteins, lipoproteins, RNA and DNA (Sevilla *et al*., 1997). GSH is a key contributor to the cellular antioxidant defense system and to the maintenance of the intracellular redox milieu for the preservation of thiol disulfide redox states of proteins. GSH is also involved in cellular signaling, regulation and redox activation of transcription factors, and thioldisulfide exchange reactions. GSH oxidation to GSSG are resulted in intracellular redox imbalance as reflected in a decreased GSH-to-GSSG ratio (GSH/GSSG), a condition often associated with oxidative stress (Dalle-Donne *et al*., 2007). In our study, GSH/GSSG was measured as 216 µg/g in *N. scintillans*. It was shown that MDA level was lower than GSH, and GSH/GSSG was quite high. We can say that *N. scintillans* is a resistant dinoflagellate against oxidative stress. Fidan *et al.* (2008) showed that GSH, as natural antioxidant, levels were found to be the same as MDA due to increase and decrease of oxidative stress in *Carassius carassius* in Eber Lake. MDA level increases when the natural antioxidant system is insufficient (Şahan *et al.*, 2003). The

increase of its level in winter could be because of the cold conditions of the fish environment, and the lipid catabolism occurs in cold seasons to get much more energy for their metabolisms (Fidan *et al*., 2008). Because, Fidan *et al.* (2008) indicated that MDA levels varied during the seasons and the highest level was in winter and the lowest level was in spring and summer seasons. They observed that the highest GSH level was in winter season.

Conclusion

N. scintillans is one of the most important heterotrophic dinoflagellates in the Black Sea ecosystem. It creates blooms. But it is not toxic. In this study, some biochemical parameters of *N. scintillans* were investigated in the most abundant periods (April) of *N. scintillans* together with biological and physiological factors. We determined that April was an important period in the Black Sea because seasonal thermocline occurred and the abundance of *N. scintillans* reached the highest amount in April. Consequently, we found a negative relationship between the temperature and the abundance of *N. scintillans*. However, the between the salinity, Chl-a and abundance of *N. scintillans* was not a relationship. Additionally, we can say that PUFA, α-tocopherol and GSH were the most important parameters in bloom of *N. scintillans*. α-tocopherol and GSH may reduce toxicity in *N. scintillans* because of inhibitory effect of α-tocopherol on lipid oxidation, and key role in the cellular antioxidant defense system of GSH and *N. scintillans* is a resistant dinoflagellate against oxidative stress. Future studies are required to establish new methods for a few cell. In addition, effect of biochemical content on the bloom formation of *N. scintillans* should research with annually series. However, it is difficult to provide enough sample amounts for biochemical analyses on *N. scintillans* and enough sample amount can provide in most abundant or bloom periods. Therefore we thought that the study would contribute to future studies about biochemical content of *N. scintillans*. Moreover, the study is the first known record about biochemical content of *N. scintillans*.

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Table 1. Fatty acid composition of *N. scintillans*

Fatty Acids	**%**
16:0	24.34±0.91
C18:0	6.11 ±0.56
C23:0	1.03 ±0.08
∑SFA	31.48±0.43
16:1ω-7	3.96±0.18
18:1ω-9*t*	1.03±0.43
18:1ω-9*c*	21.99±1.34
20:1ω-9	0.83±0.06
∑MUFA	27.81±1.49
18:2ω-6t	1.68±0.08
18:2ω-6c	18.49±2.26
18:3ω-3c	7.12±0.69
20:4ω-6	8.13±0.76
20:5ω-3 (EPA)	2.21±0.06
22:6ω-3 (DHA)	3.08±0.11
∑PUFA	40.71±2.22

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∑HUFA	13.42±0.81

Figure 1. Sampling station in the Southeastern Black Sea.

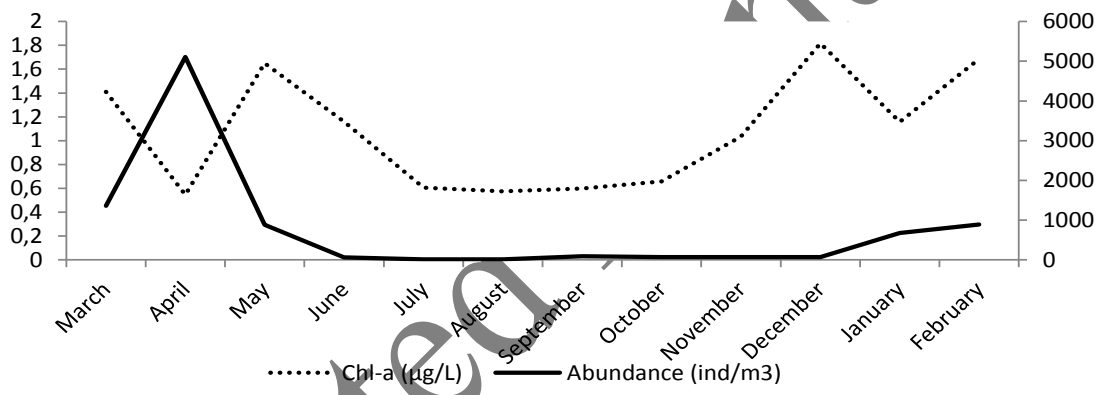

Figure 2. Abundance and Chl-a of *N. scintillans* (ind/m^3) during the sampling periods