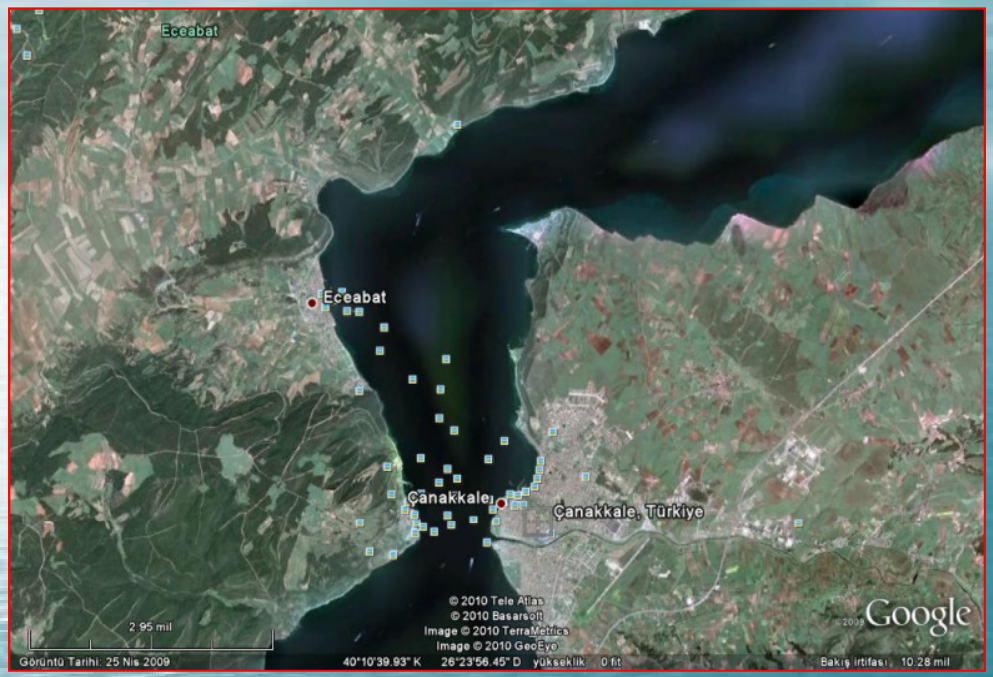
Recurrent Blooms of a Common Heterotrophic Dinoflagellat *Noctiluca Scintillans* (Macartney) in the Sea of Marmara (Çanakkale Strait)



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Main Scope of the Study



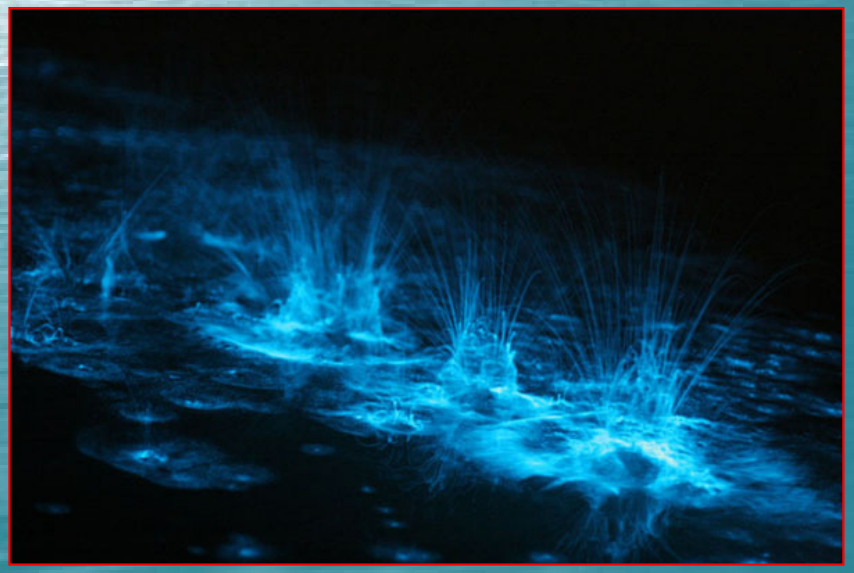
➢To explain the bloom density and cell volume of *N.scintillans* connected to eutrophication of the Dardanelles.

➢To exhibit the bloom circumstances and the reasons of dinoflagellate *N.scintillans* along with environmental conditions in the Dardanelles.



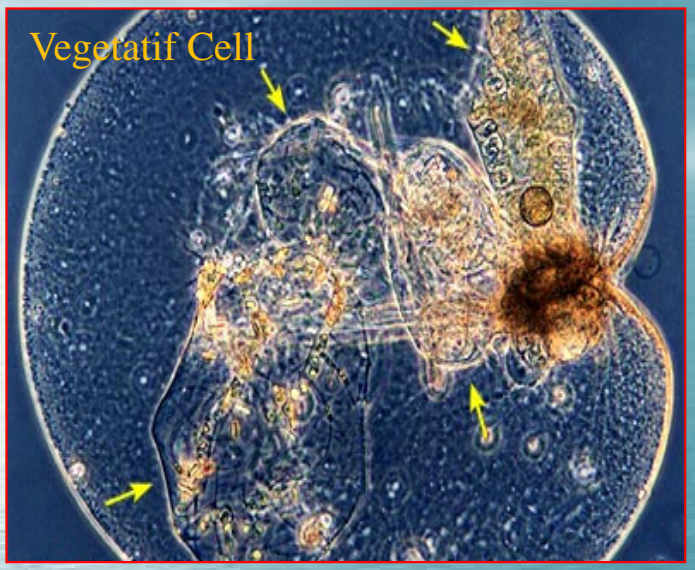
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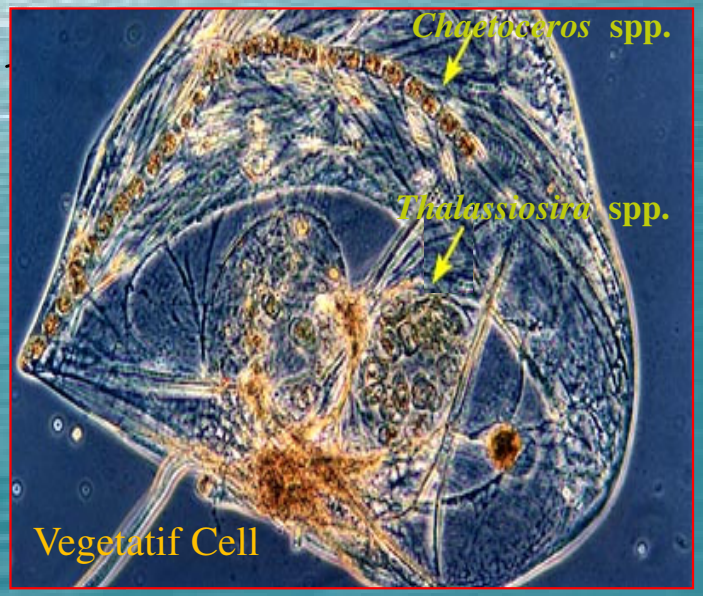




➢***Noctiluca scintillans***, commonly known as the **Sea Sparkle**, and also published as *Noctiluca miliaris*, is a free-living non-parasitic marine-dwelling species of dinoflagellate that exhibits bioluminescence. The bioluminescent characteristic of *N. scintillans* is produced by a luciferin-luciferase system located in thousands of spherically shaped organelles, or "microsources", located throughout the cytoplasm of this single-celled protist. Nonluminescent populations within the genus *Noctiluca* lack these microsources (*Eckert and Reynolds, 1967*).

Introduction





➢*N. scintillans* is a heterotrophic (non-photosynthetic) organism that engulfs its food (phagotrophic) which primarily consists of plankton, including diatoms and other dinoflagellates, as well as fish eggs and bacteria (Fukuyo et al. 1990; Hallegraeff 1991; Taylor et al. 1993; Steidinger & Tangen 1996).

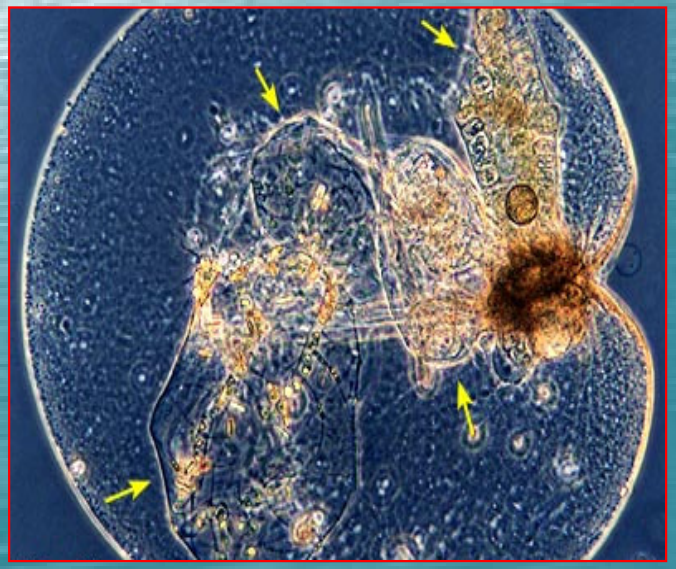
➢**Diatoms are often found in the vacuoles** (internal membrane-bound storage compartments) **within these single-celled creatures** (*Thomas and Titelman, 1998*).

➢The diatom *Chaetoceros* spp. and *Thalassiosira* spp. has been noted in the literature as a favored food source of these organisms (Fukuyo et al. 1990; Hallegraeff 1991; Taylor et al. 1993; Steidinger & Tangen 1996).

➢**Like this underside figure,** centric structure of the cell demage due to feeding by spine diatoms such as *Rhizosolenia* spp.

Introduction





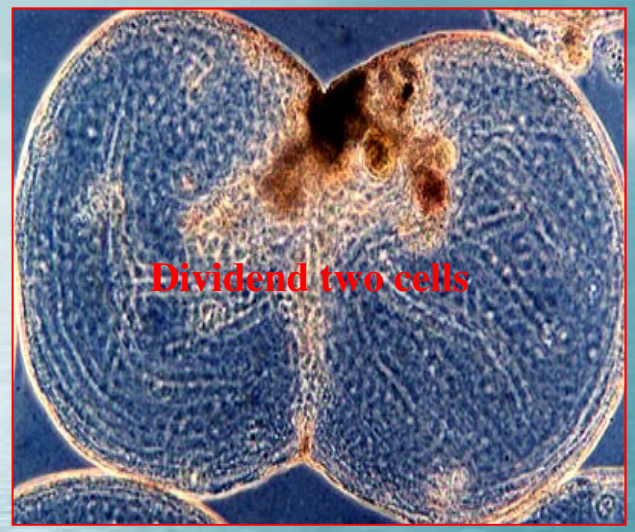
➢***Noctiluca scintillans*** **is an unarmored marine planktonic dinoflagellate species distributed world wide (cosmopolitan) in cold and warm waters** (*Dodge 1982; Fukuyo et al. 1990; Hallegraeff 1991; Taylor et al. 1995; Steidinger & Tangen 1996*).

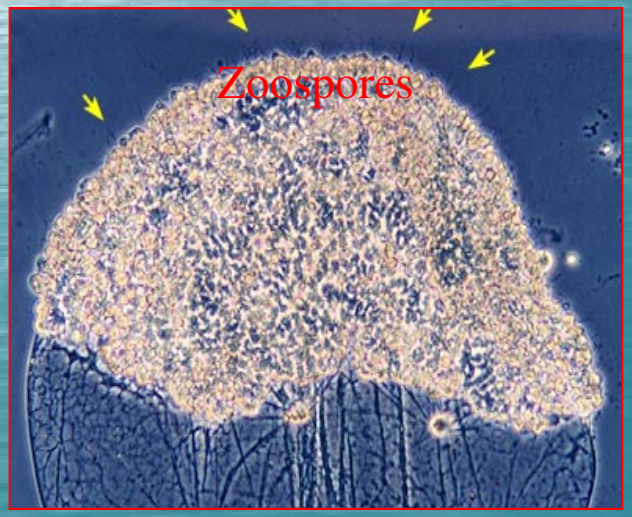
➢***N. Scintillans*** **populations are prevalently found in neritic and coastal waters of tropical and subtropical regions** (*Dodge 1982; Fukuyo et al. 1990; Hallegraeff 1991; Taylor et al. 1995; Steidinger & Tangen 1996*).

➢Not all blooms associated with *N. scintillans* are red. The color of *N. scintillans* is in part derived from the pigments of organisms inside the vacuoles of *N. scintillans*. For instance, green tides result from *N. scintillans* populations that contain green-pigmented prasinophytes (Chlorophyta) that are living in their vacuoles (*Housmann et al., 2003*).

➢ **It is known that this species is also especially one of the dominant dinoflagellates in Turkish Strait System** (*Turkoglu, 2010; Turkoglu and Erdogan, 2010*)**.**

Introduction



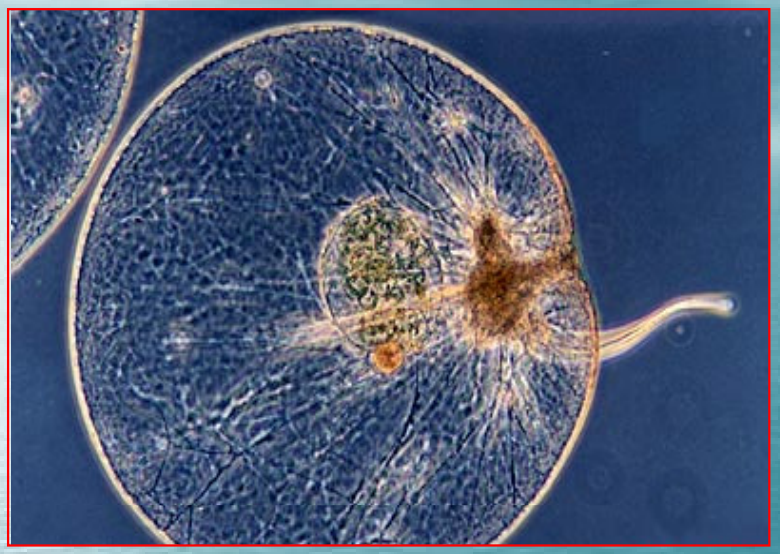


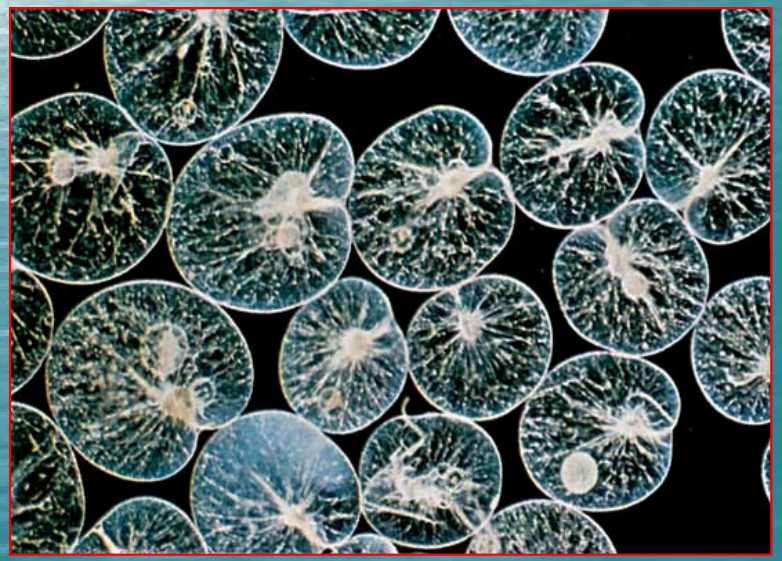
➢**Toxic blooms of *N. scintillans* have been linked to massive fish and marine invertebrate kills.**

➢Although this species does not produce a toxin, it has been found to accumulate toxic levels of ammonia which is then excreted into the surrounding waters possibly acting as the killing agent in blooms (Okaichi & Nishio 1976; Fukuyo et al. 1990).

➢***N. scintillans* reproduces asexually by binary fission and also sexually via formation of isogametes (Zingmark 1970).**

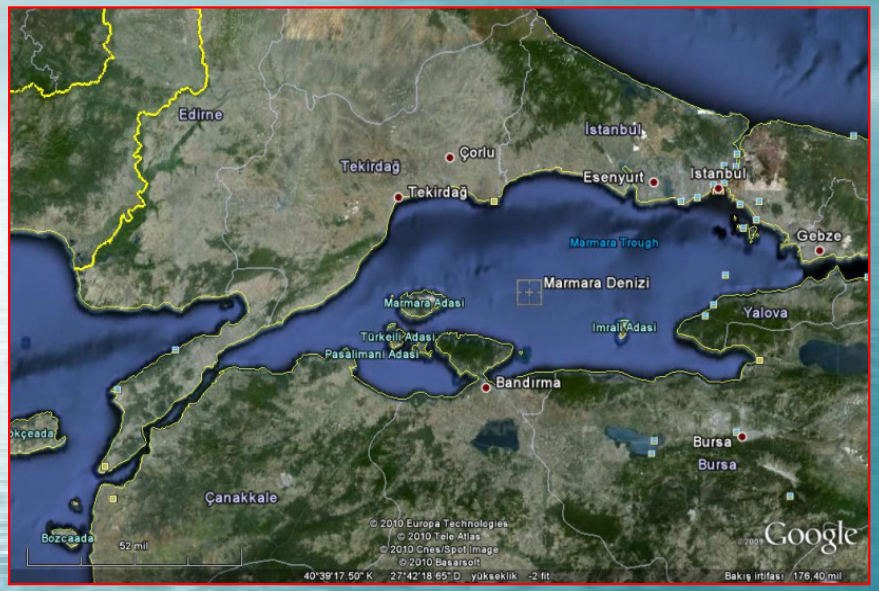
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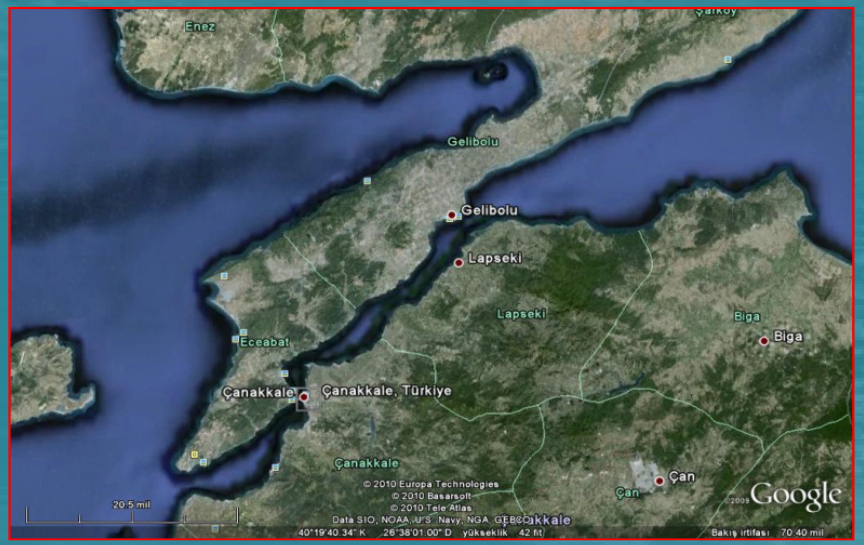
- The size of the single-celled *N. scintillans* ranges from 200 to 2000 µm in diameter, assuming the generally spherical shape.
- ***N. scintillans* lacks the armor plates possessed by other types of dinoflagellates.**
- And, unlike many other dinoflagellates, the chromosomes of the *Noctiluca* are not clearly-visible and condensed throughout its lifecycle.

Identification of the study Area (Dardanelles)

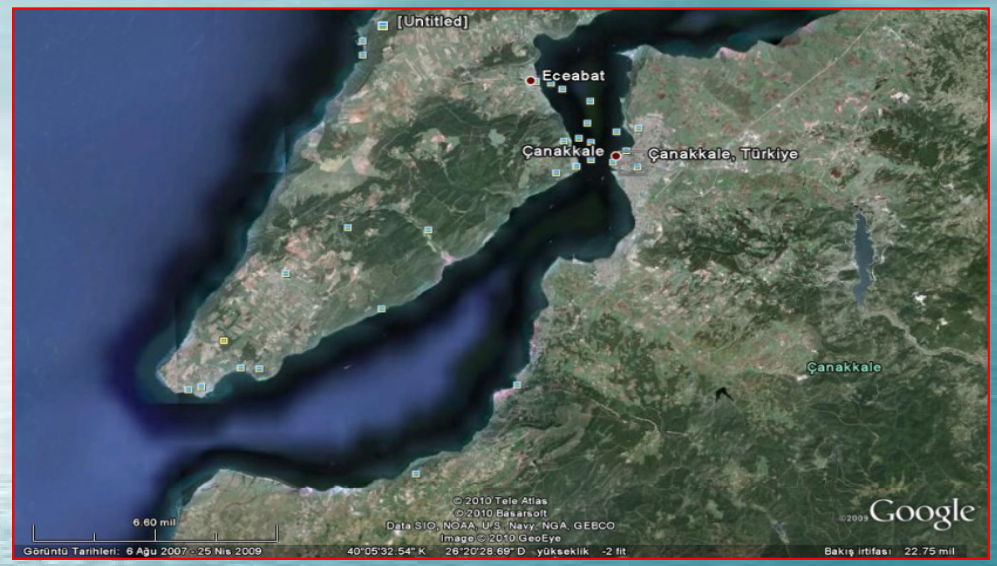


- **The Dardanelles is a part of the Turkish Strait System and located between the Aegean Sea and the Sea of Marmara.**
- It has two flow system reverse to one another; one of the currents derives from the Aegean Sea, where the water density is high (38-39 ppt), and the second one comes from the Sea of Marmara, characteristically low in density (22-26 ppt).

- The width of the Strait varies from 1.35 to 7.73 km.
- **The average depth of the Strait is approximately 60 m with the deepest part reaching more than 100 m (**Unsal *et al.* 2003; Turkoglu *et al.* 2004a, 2006; Baba *et al.* 2007**).**



Identification of the study Area (Dardanelles)



- Its NE/SW trend is interrupted by a north-south bend between Eceabat and Canakkale. This bend is also the narrowest part of the Dardanelles.
- **In addition to the first bend, there is a second bend called "Nara Cape".**

- The narrowing of the Dardanelles leads to different surface temperature and salinity values in the northeast and southwest of the Nara Cape.
- **The surface waters in the southern part of the Dardanelles were also more saline especially in the spring and winter seasons compared to other seasons** (Unsal et al., 2003; Turkoglu et al., 2004a, 2006).

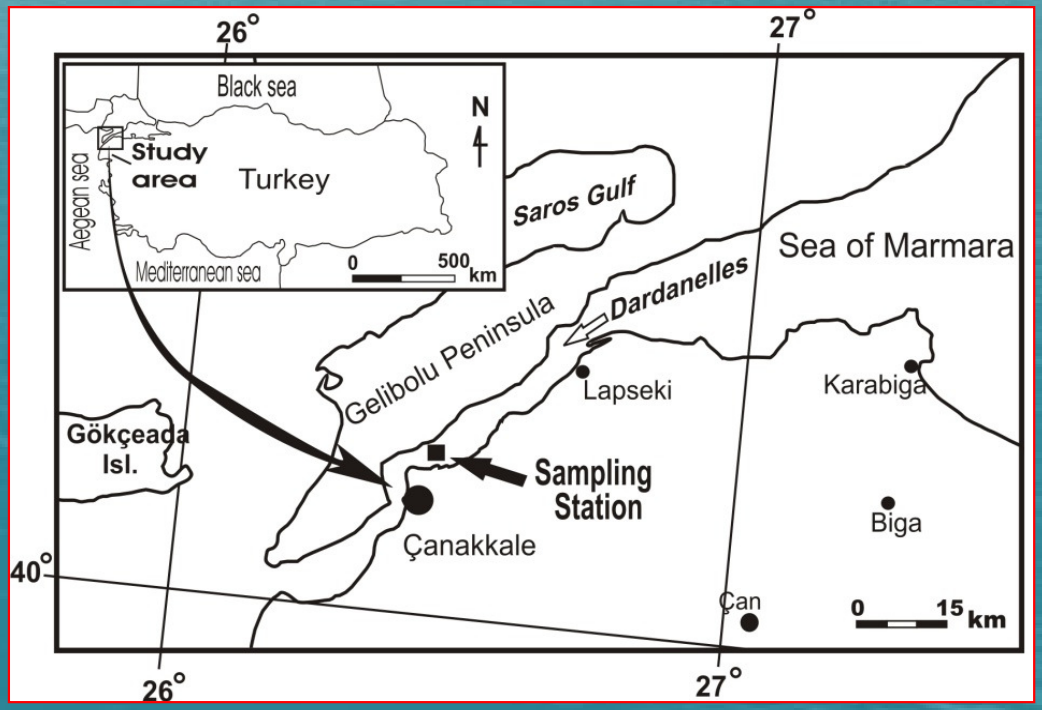


Material and Method
(Sampling Station)



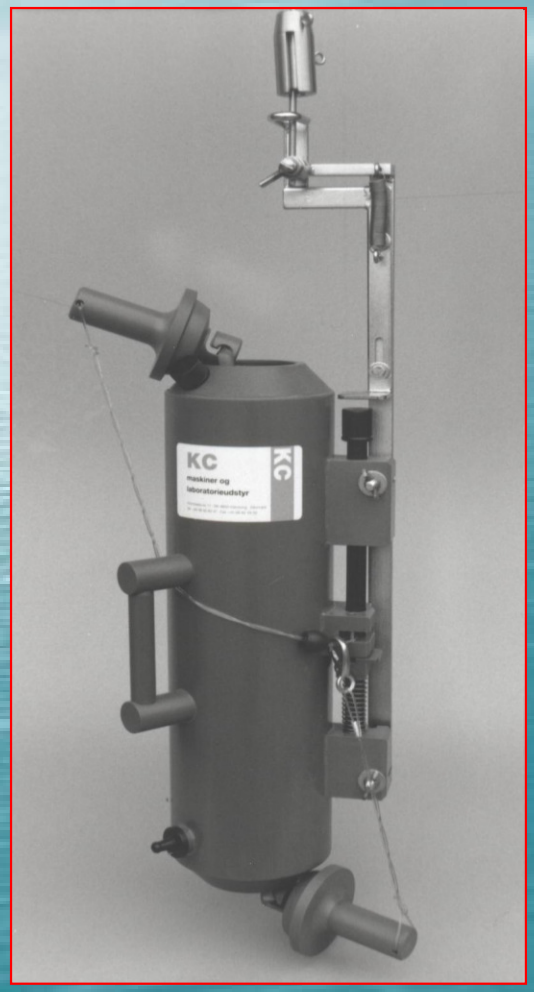
- This station has around 80 m depth.
- **It is an topographical downwelling area due to the Nara Cape.**

- ***The location of the sampling station*** (40°12¹42¹¹.12 N – 26°26¹29¹¹.18 E) ***is given in Fig. 1.***



➢ *Fig. 1. Dardanelles (Çanakkale Strait, Turkey) and study area (sampling station)*

Material and Method
(Collection of Water Samples)



- *Water samples* for nutrient analyses, phytoplankton enumeration, and chlorophyll a estimation were collected by a Niskin Sampling Bottle from different depths (0.5, 10, 25, 50 and 75 m) three time a month (at 10-day intervals) between March 2001 and January 2004.

Material and Method
(In situ Measurements - CTD)





- CTD parameters (Sea temperature, salinity, pH, and dissolved oxygen) were measured in situ using an YSI 6600 Model Multiple Probe System.

Material and Method
(Nutrient Analysis)



- Water samples for nutrient analyses were kept frozen until analysis.

- Analysis for nitrite+nitrate, inorganic phosphate and silicate (SiO_4) were measured using a Technicon model auto-analyzer according to Strickland & Parsons (1972).

Material and Method
(Chlorophyll a Analysis)



- Chlorophyll a samples were filtered through GF/F glass fiber filters.
- The filters were folded into aluminum foil and immediately frozen for the laboratory analysis.

➢**Chlorophyll a was analyzed spectrophotometrically after extraction by 90% acetone (*Strickland and Parsons, 1972*).**

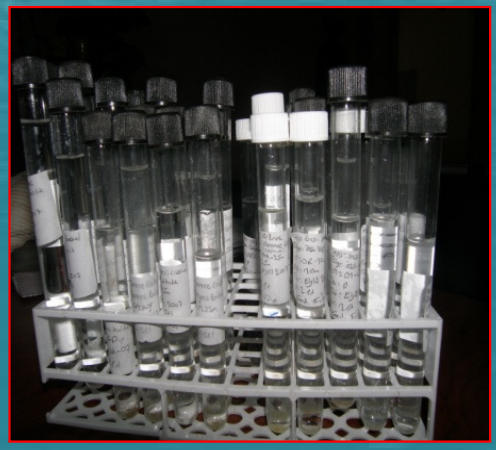
Material and Method
(Quantitative analysis of phytoplankton)





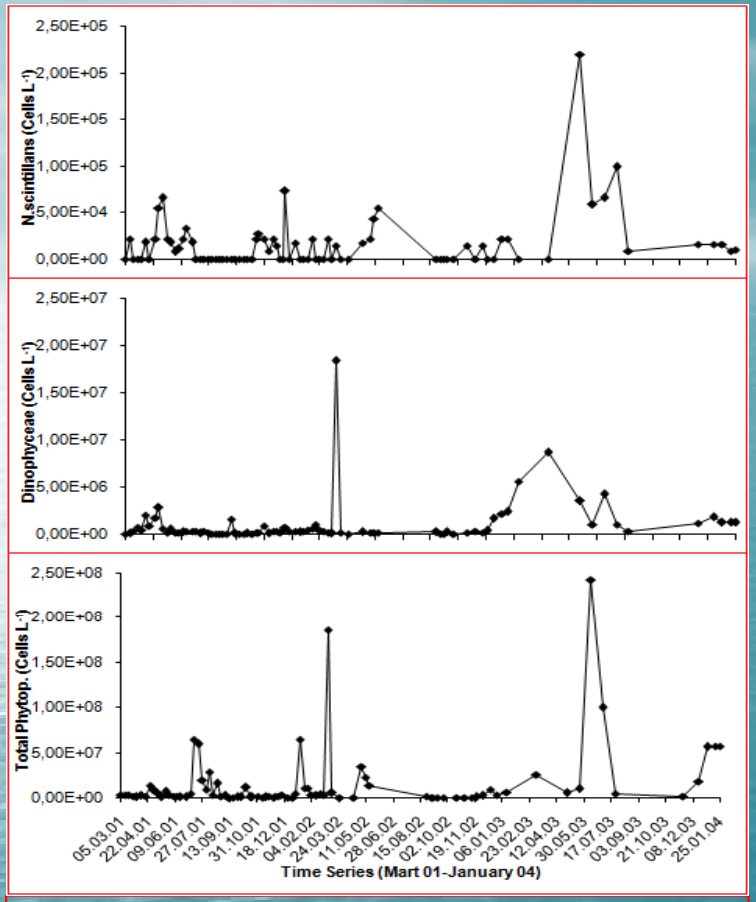
- For quantitative analysis of phytoplankton, samples were preserved with 2% buffered lugol formation.
- **Microscopic analysis was conducted within a week of the collection.**

- Sampling Glass, Sedimentation Chambers, Sedgwick Rafter Counting Slides were used for enumeration of *N.scintillans* (Guillard 1978; Hasle 1978; Venrick 1978).





Temporal Variations in Phytoplankton



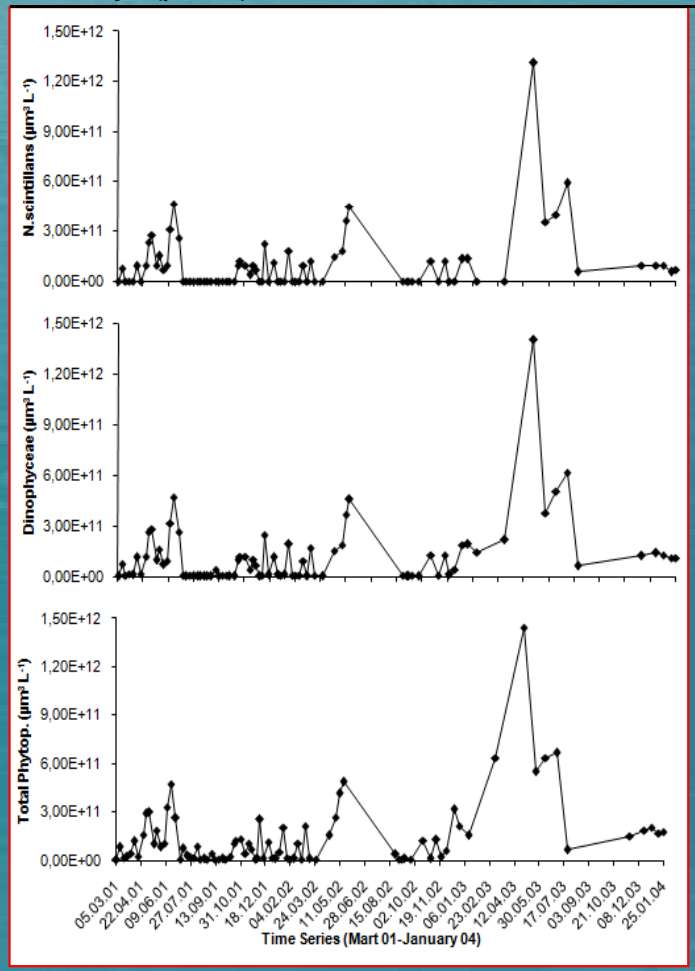
➢**Fig. 2. Temporal distributions of *Noctulica scintillans*, dinoflagellates and total phytoplankton cell density in the Dardanelles between March 2001 and January 2004**

➢Cell density of *N.scintillans* variated between 0.00E+00 and 2.20E+05 cells L^{-1}, cell volume variated between 0.00E+00 and 1.31E+12 μm^3 L^{-1}.

➢Cell density of phytoplankton variated between 1.00E+05 and 1.84E+07 cells L^{-1}, phytoplankton cell volume variated between 2.51E+09 and 1.44E+12 μm^3 L^{-1} due to very excessive algal blooms such as *E.huxleyi* in summer 2003.

➢**Table 3.** Descriptive statistics phytoplankton data groups in the Dardanelles in period of March 2001 and January 2004

	N	Minimum	Maximum	Mean	SD
N.scintillans (Cell L^{-1})	83	0,00E+00	2,20E+05	1,59E+04	3,01E+04
Dinophyceae (Cell L^{-1})	83	1,00E+04	1,84E+07	9,66E+05	2,34E+06
Total Phyto (Cell L^{-1})	83	1,00E+05	2,43E+08	1,53E+07	3,68E+07
N.scintillans (μm^3 L^{-1})	83	0,00E+00	1,31E+12	9,81E+10	1,84E+11
Dinophyceae (μm^3 L^{-1})	83	1,50E+08	1,40E+12	1,16E+11	1,94E+11
Total Phyto (μm^3 L^{-1})	83	2,51E+09	1,44E+12	1,43E+11	2,14E+11



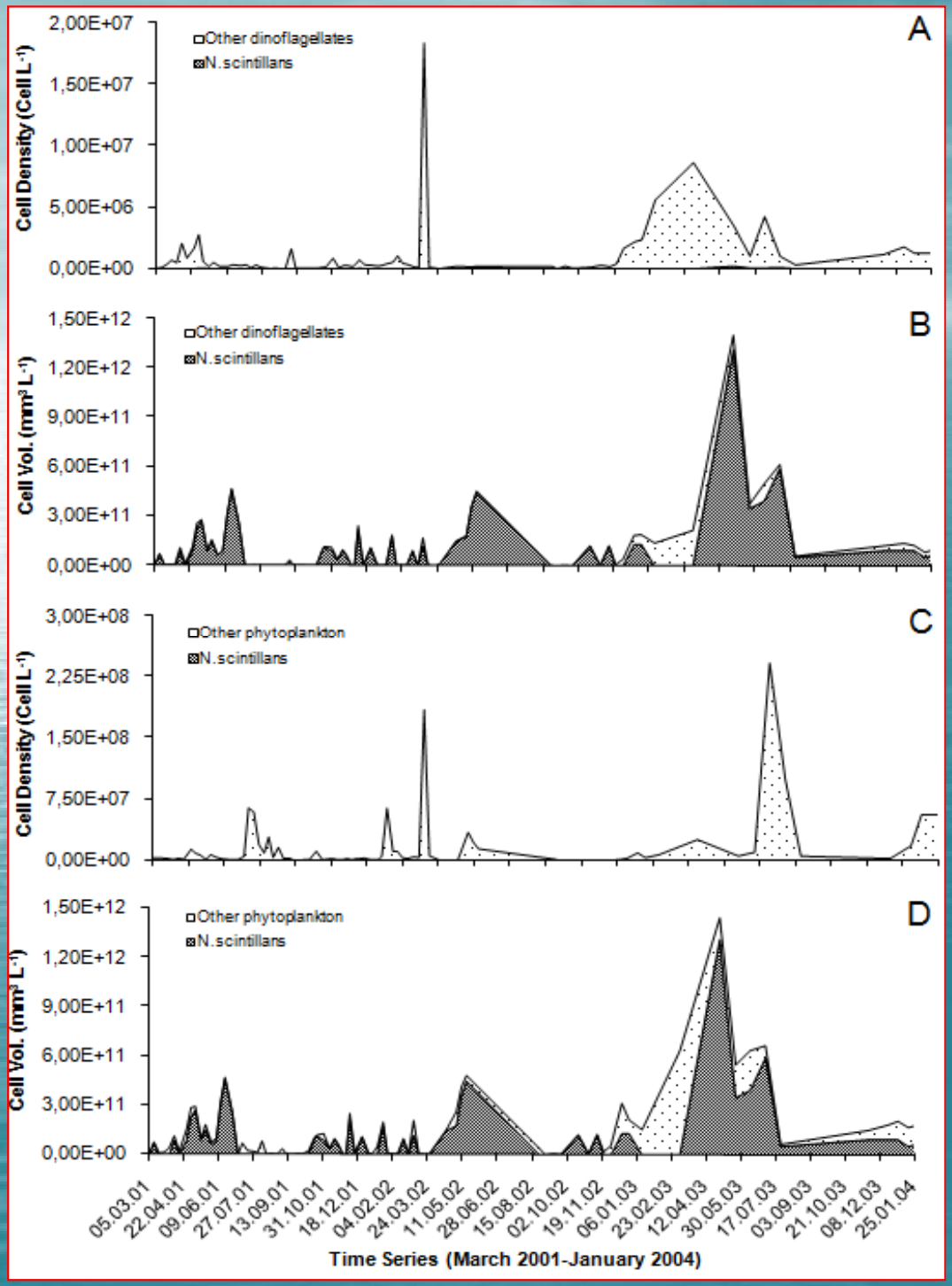
➢**Fig. 3. Temporal distributions of *Noctulica scintillans*, dinoflagellates and total phytoplankton cell volume in the Dardanelles between March 2001 and January 2004**

➢**March and June period** were the first *very excessive bloom period* in view of *N.scintillans* during the year.

➢**The second bloom period is the period of October and December in the Dardanelles.**

➢During the year, although maximum values of cell density and cell volume were observed in November and December, production potential in the period of March and June was higher than in the period of November and December.

➢**However, *N.scintillans* didn't demonstrate an important growth between July and October.**

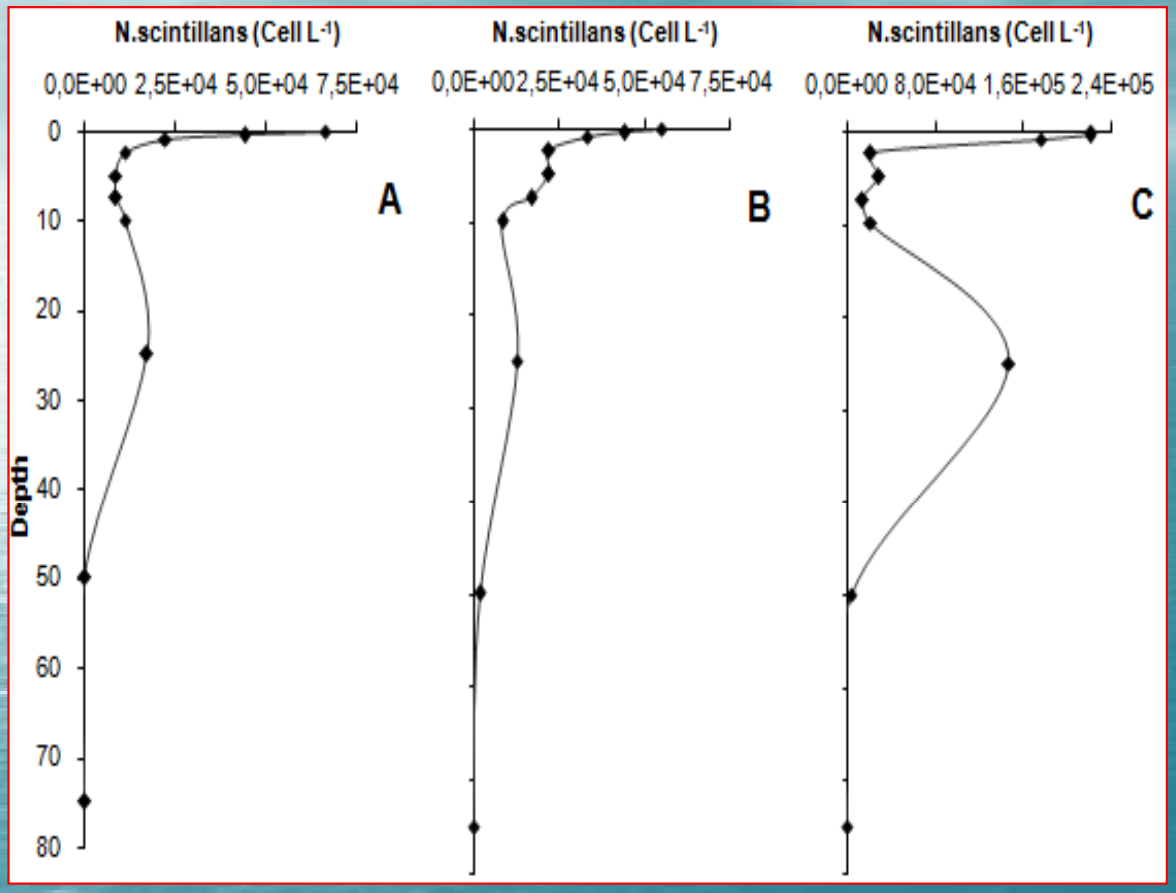


Temporal Contribution of *N.scintillans* to Phytoplankton

- Although there was no important contribution of *N.scintillans* to both dinoflagellates and total phytoplankton in view of cell density,
- **there was the very important contribution of *N.scintillans* to both dinoflagellates and total phytoplankton in view of cell volume.**

- ***Fig. 4. Variations in temporal contribution of Noctulica scintillans cell density (A) and bio-volume (B) to total dinoflagellate and total phytoplankton cell density (C) and bio-volume (D) in the Dardanelles between March 2001 and January 2004***

Vertical Distribution of N.scintillans



➢*Fig. 5. Vertical distribution of Noctulica scintillans cell density in excessive bloom periods (A: 08 May 2001; B: 17 May 2002; C: 01 May 2003) in the Dardanelles between March 2001 and January 2004*

➢**Vertical distribution of *Noctulica scintillans* cell density in excessive bloom periods** (A: 08 May 2001; B: 17 May 2002; C: 01 May 2003) **revealed that this species decreased with depth in the Dardanelles. However, in addition to high density in the superficial layers, there was increase tendency at 25 meter depth.**

Contribution of N.scintillans to Phytoplankton

➢***Table 1.*** *Rational contributions (%) of dinoflagellat N. scintillans to Dinophyceae and total phytoplankton in surface waters (0.5 m) of the Dardanelles in period of March 2003 and January 2004*

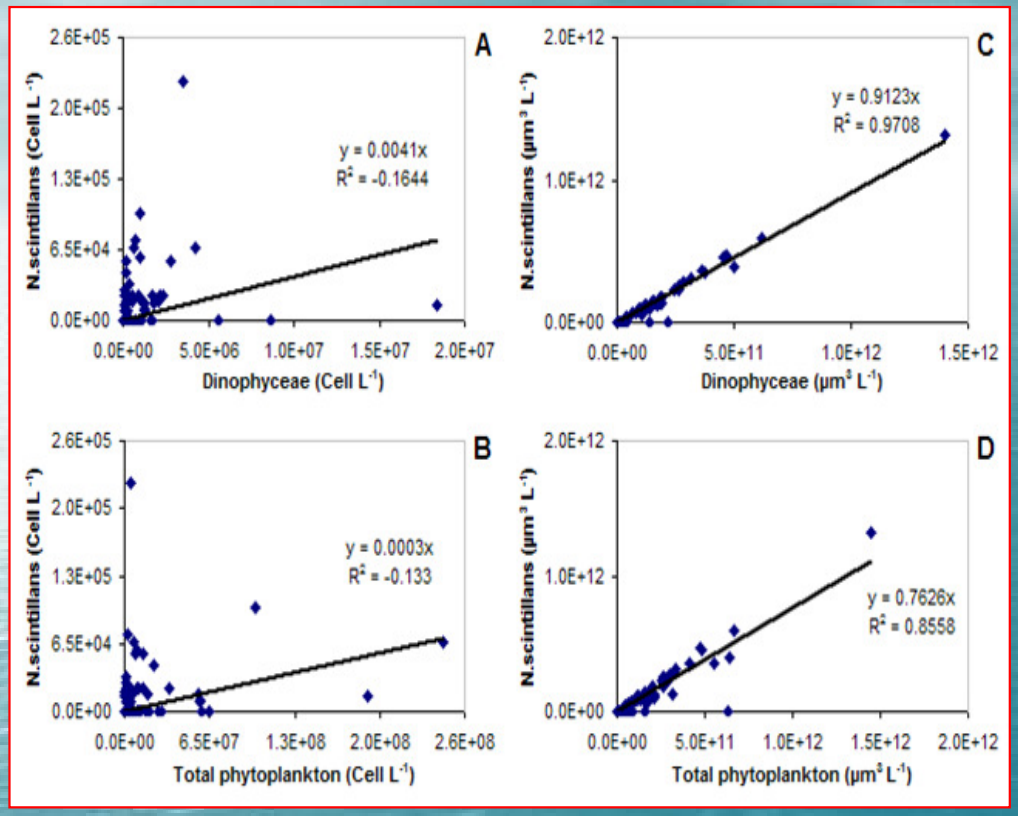
Date	*N.scint.* Cell l^{-1}	Other Dinophy	Other Phyto	Dinophy % Density	Tot.Phyto % Density	Dinophy. % Vol	Tot.Phyt % Vol
14.01.03	0,0E+00	5,61E+06	6,73E+06	0,00	0,00	0,00	0,00
07.03.03	0,0E+00	8,65E+06	2,54E+07	0,00	0,00	0,00	0,00
01.05.03	2,2E+05	3,28E+06	5,04E+06	6,29	4,19	93,75	90,90
23.05.03	5,9E+04	9,71E+05	9,90E+06	5,70	0,59	93,15	63,73
12.06.03	6,6E+04	4,19E+06	2,43E+08	1,55	0,03	78,71	61,83
03.07.03	9,9E+04	9,21E+05	9,99E+07	9,71	0,10	95,86	88,73
24.07.03	8,8E+03	3,05E+05	5,14E+06	0,86	0,01	86,99	80,30
21.11.03	1,6E+04	1,14E+06	2,21E+06	1,35	0,70	76,37	62,70
19.12.03	1,6E+04	1,77E+06	1,80E+07	0,88	0,09	67,68	51,01
02.01.04	1,6E+04	1,24E+06	5,62E+07	1,25	0,03	74,84	45,45
16.01.04	8,8E+03	1,24E+06	5,61E+07	0,70	0,02	41,91	25,45
25.01.04	1,1E+04	1,25E+06	5,62E+07	0,85	0,02	51,03	30,99

➢**Contribution of *N.scintillans* to total Dinophyceae and phytoplankton cell density was lower** (0-33.3 and 0-3.70% respectively) **than contribution to total Dinophyceae and phytoplankton cell volume** (0-99.5 and 0-99.0%, respectively).

➢This situation was supported by correlation data.

➢ **01 May, 2003 was one of the most important excessive bloom period.**

Correlation Data



➢***Fig. 6.*** *Relationships between N. scintillans cell density and dinoflagellates (A), total phytoplankton cell density (B) and between N. scintillans cell volume and dinoflagellates (C), total phytoplankton cell volume (D) in the Dardanelles in the period of March 2001 and January 2004. For each regression, the coefficients of determination (R^2) and the process of equating (y) are shown*

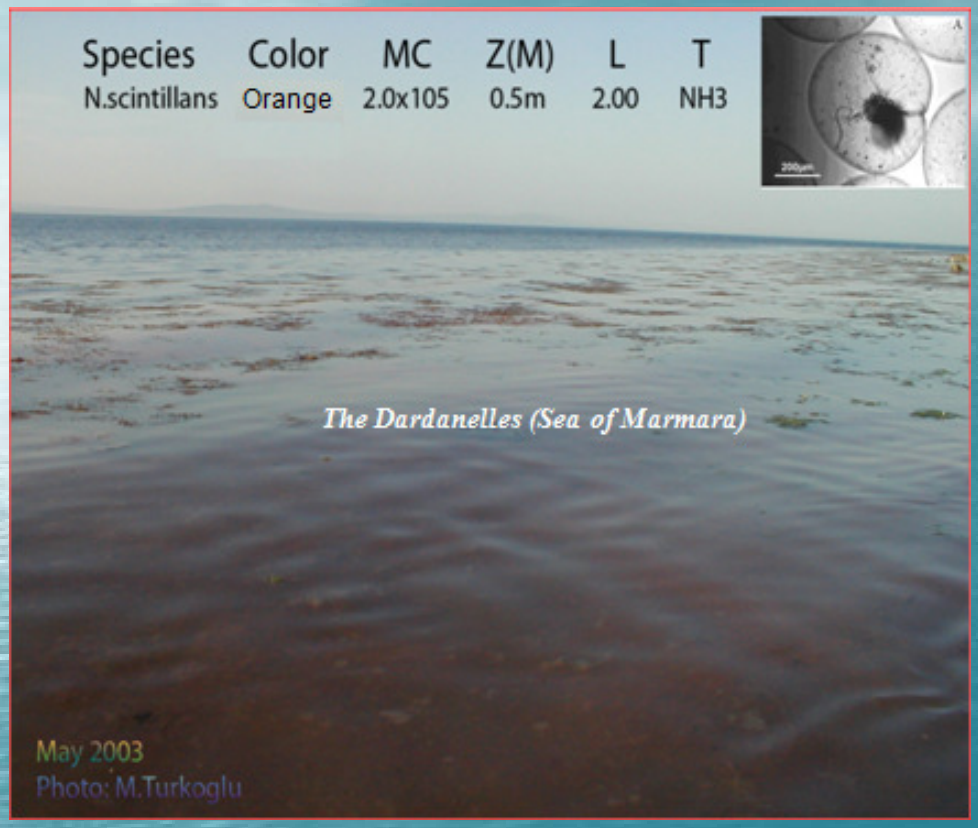
➢**Although there was no correlation (R=-0.1644 and -0.133) in view of cell density,**

➢there was an important positive correlation (R=0.971 and 0.856) in view of cell volume between both dinoflagellates and total phytoplankton with *N.scintillans*.

➢***Noctiluca scintillans*** **blooms are also known to occur after diatom blooms (*Dela-Cruz et al., 2002*).**

➢Therefore, there are generally negative correlations between abundance of phytoplankton especially diatoms and abundance of *N.scintillans* (*Huang and Qi, 1997*) .

Orrance Color Appearance by *N.scintillans* in May, 2003

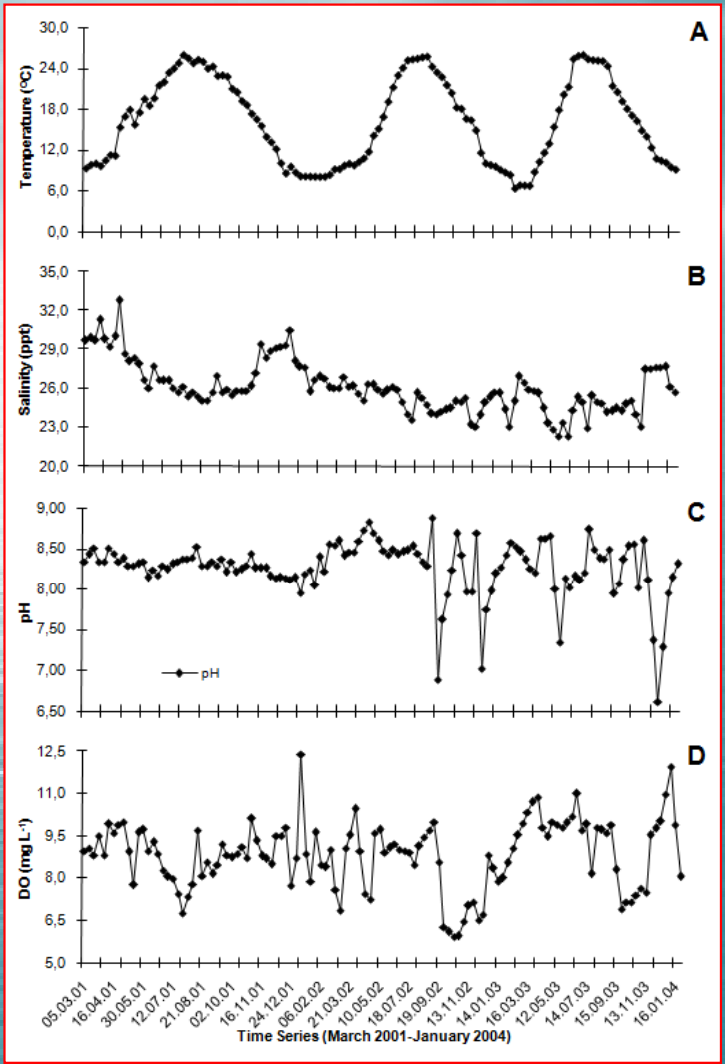


➢**Fig. 7.** *Heterotrophic dinoflagellate species Noctulica scintillans responsible to excessive algal bloom (small picture) and its bloom color image (big picture) in Lapseki coastal area of the Dardanelles in the Sea of Marmara in the early summer period (MC: Maximum cell L^{-1}, Z: Depth which shows maximum cell density, L: LLoyd cluster index, T: Harmful effect type)*

➢**<u>Except for May 2003,</u>** there was not any orange color appearance in every spring season (especially in May) in the period of 2001 and 2004, but May 2003 period was the most important proliferation period (2.20E+05 cells L^{-1}) in just before *Ehux* bloom during the June 2003, in the Dardanelles (*Turkoglu, 2008*).



Physicochemical Variations



➢***Fig. 8.*** *Temporal distribution of temperature (A), salinity (B), pH (C) and dissolved oxygen (D) in the Dardanelles between March 2001 and January 2004*

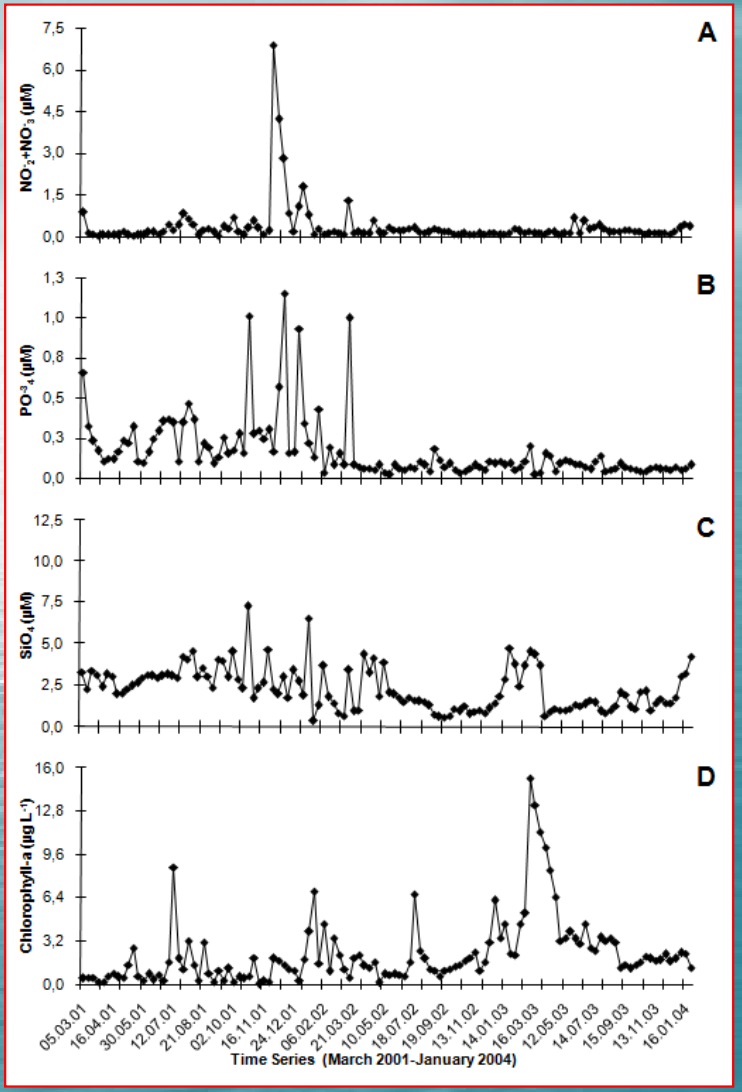
➢***Table 2. Descriptive statistics different data groups in the Dardanelles in period of March 2001 and January 2004***

	N	Min	Max	Mean	SD
Temperature (ºC)	83	6,75	26,00	16,00	6,18
Salinity (ppt)	83	22,28	32,80	26,56	2,06
pH	83	6,61	8,88	8,24	0,36

➢***Table 3.*** *Relationships (correlation) between different data groups in the Dardanelles in period of March 2001 and January 2004 (Red colored numbers reveal important correlations)*

Correlations	Temp.	Salinity	pH	DO	NO^{-}_{2}+NO^{-}_{3}	PO^{-3}_{4}	SiO_4	Chl-a	*N.scint.*	Dinophy.	Tot.Phy	N.scint.	Dinop.	Tot.Phy
Temp.	1,000													
Salinity	-0,456	1,000												
pH	0,051	0,095	1,000											
DO	-0,202	0,293	-0,004	1,000										
NO^{-}_{2}+NO^{-}_{3}	-0,132	0,225	-0,047	0,093	1,000									
PO^{-3}_{4}	-0,089	0,279	0,045	0,060	0,360	1,000								
SiO_4	0,010	0,313	0,189	0,049	0,053	0,386	1,000							
Chl-a	-0,190	-0,212	-0,072	-0,027	-0,017	-0,110	-0,003	1,000						
N.scint-D	-0,019	-0,151	0,019	0,225	-0,070	-0,028	-0,168	0,057	1,000					
Dinop-D	-0,257	-0,063	0,038	-0,053	-0,093	-0,146	-0,141	0,320	0,149	1,000				
Tot.Phy-D	0,022	-0,208	-0,013	0,050	-0,068	-0,153	-0,039	0,182	0,195	0,576	1,000			
N.scint-V	0,024	-0,203	0,029	0,191	-0,114	-0,112	-0,167	0,042	0,954	0,143	0,200	1,000		
Dinop-V	-0,026	-0,216	0,011	0,203	-0,126	-0,135	-0,163	0,147	0,948	0,242	0,248	0,986	1,000	
Tot.PhytV	-0,057	-0,237	-0,027	0,228	-0,138	-0,155	-0,125	0,309	0,893	0,326	0,313	0,928	0,9694	1,000

Physicochemical Variations



➢***Fig. 9.*** *Temporal distribution of nitrite+ nitrate (A), phosphate (B), silicate (C) and chlorophyll-a (D) in the Dardanelles between March 2001 and January 2004*

➢***Table 3.*** *Descriptive statistics different data groups in the Dardanelles in period of March 2001 and January 2004*

	N	Min	Max	Mean	SD
$NO^-_2+NO^-_3$ (µM)	83	0,05	6,89	0,43	0,93
PO^{-3}_4 (µM)	83	0,02	1,15	0,22	0,22
SiO_4 (µM)	83	0,35	7,23	2,49	1,34
Chl *a* (µg L^{-1})	83	0,03	15,21	1,77	2,16

➢***Table 3.*** *Relationships (correlation) between different data groups in the Dardanelles in period of March 2001 and January 2004 (Red colored numbers reveal important correlations)*

Correlations	Temp.	Salinity	pH	DO	$NO^-_2+NO^-_3$	PO^{-3}_4	SiO_4	Chl-a	*N.scint.*	Dinophy.	Tot.Phy	N.scint.	Dinop.	Tot.Phy
Temp.	1,000													
Salinity	-0,456	1,000												
pH	0,051	0,095	1,000											
DO	-0,202	0,293	-0,004	1,000										
$NO^-_2+NO^-_3$	-0,132	0,225	-0,047	0,093	1,000									
PO^{-3}_4	-0,089	0,279	0,045	0,060	0,360	1,000								
SiO_4	0,010	0,313	0,189	0,049	0,053	0,386	1,000							
Chl-a	-0,190	-0,212	-0,072	-0,027	-0,017	-0,110	-0,003	1,000						
N.scint-D	-0,019	-0,151	0,019	**0,225**	-0,070	-0,028	-0,168	0,057	1,000					
Dinop-D	**-0,257**	-0,063	0,038	-0,053	-0,093	-0,146	-0,141	0,320	0,149	1,000				
Tot.Phy-D	0,022	-0,208	-0,013	0,050	-0,068	-0,153	-0,039	0,182	0,195	0,576	1,000			
N.scint-V	0,024	-0,203	0,029	0,191	-0,114	-0,112	-0,167	0,042	0,954	0,143	0,200	1,000		
Dinop-V	-0,026	-0,216	0,011	0,203	-0,126	-0,135	-0,163	0,147	**0,948**	**0,242**	**0,248**	**0,986**	1,000	
Tot.PhytV	-0,057	**-0,237**	-0,027	**0,228**	-0,138	-0,155	-0,125	**0,309**	**0,893**	**0,326**	**0,313**	**0,928**	**0,9694**	1,000

Discussion and Conclusion

➢***The occurrence of N.scintillans red tide has frequently been observed in the Dardanelles, Turkish Straits System for several decades (Turkoglu, 2005; Turkoglu, 2010; Turkoglu and Erdogan 2010).***

➢*Noctiluca scintillans blooms are often observed as reddish or orange patches in Turkish waters in May-July in calm weather (Fig. 7).*

➢***In May 2003, extensive bloom of N.scintillans suggests that Sea of the Marmara is one of the eutrophicated and very turbid aquatic system.***

➢*In the Sea of Marmara, high concentrations of phytoplankton food source (Fig. 2-4) that likely result from environmental conditions such as well-mixed nutrient-rich waters (Fig. 9) and seasonal circulation factors (Fig. 8) are implicated in population blooms of N. scintillans, known as "red tides".*

➢**On the other hand, it is possible that this species can be exhibit a more higher growth rate for a certain alga or a few special algae than others** *(Zhou et al., 1994)***.**

➢*It is known that availability of phytoplankton as prey is also one of the important factors for the variation in abundance of N. scintillans (Elbrachter and Qi, 1998). Like in this study results (Figs. 2 and 3), Noctiluca scintillans blooms are also known to occur after diatom blooms (Dela-Cruz et al., 2002). Huang and Qi (1997) reported a negative relationship between the abundance of N. scintillans and chlorophyll a during blooms like in correlation results of this study.*

➢***It is known that toxic blooms of N. scintillans have been linked to massive fish and marine invertebrate kills. Although this species does not produce a toxin, it has been found to accumulate toxic levels of ammonia which is then excreted into the surrounding waters possibly acting as the killing agent in blooms (Okaichi & Nishio 1976; Fukuyo et al. 1990).***

Discussion and Conclusion

➢***It is known that N. scintillans is a nonphotosynthetic heterotrophic and phagotrophic dinoflagellate species. Therefore, there is any chloroplast in cytoplasm and the cytoplasm is mostly colorless.***

➢*Reddish or orange color appearance in the Sea of Marmara are thought to be related to gut content which contains some phytoplankton species such as dinoflagellate Prorocentrum spp. and diatoms D.fragilissimus, Leptocylindrus spp. and Thalassiosira spp. The presence of such photosynthetic symbionts can responsible to appear various color such as pink, reddish, orange and green in color of cytoplasm (Sweeney 1978).*

➢***Sometimes, in some tropical regions such as Indonesia, Malaysia, and Thailand, the watercolor is however green due to the presence of green prasinophyte endosymbionts (****Sweeney 1978; Dodge 1982; Fukuyo et al. 1990; Hallegraeff 1991; Taylor et al. 1995; Steidinger & Tangen 1996****).***

➢*Therefore, gut content and thereby it's optical characteristic will be different each other due to different phytoplanton species fed by N.scintillans both in different seasons and different ecosystems.*

Suggestions

- **Such algal blooms must be monitored by various methods such as in situ and remote sensing in aquatic systems, especially in fisheries aquaculture areas.**
- It is known that intense blooms of *Noctiluca scintillans are characterised by a high reflectance for wavelengths higher than 580 nm and a sharp increase in reflectance from 520 to 580 nm (Van Mol1 et al., 2007).*
- **Because of its strong colour, it should be possible to discriminate *Noctiluca scintillans optically from other components in the sea. Thus, such blooms must be mapping by using optical sensor detection method (Van Mol1 et al., 2007).***
- *As stated by Van Mol1 et al. (2007), detection by remote sensing is possible if the concentration is sufficiently high and if the spatial resolution of the sensor is fine enough to resolve patches.*
- ***Moreover, I suggest that It can be possible to detect N.scintillans by optical sensors mounted on ships and Turkish and international airplanes is possible in Turkish waters and the Mediterrenean Sea.***

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