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Winter bloom of coccolithophore *Emiliania huxleyi* and environmental conditions in the Dardanelles

Muhammet Turkoglu

ABSTRACT

Following a summer bloom of coccolithophore *Emiliania huxleyi* (Lohmann) Hay & Mohler, 1967, in 2003, a winter bloom was observed for the first time between late December 2003 and early January 2004 in the Dardanelles. Microscopic observations showed that the cell dimensions of *E. huxleyi* (*Ehux*) varied from 9.85 to 13.50 μm in diameter (mean: 11.20 ± 1.38 μm). While *Ehux* revealed a relatively small population density (1.60×10^4 cells L^{-1}) in early December 2003, the bloom started in middle December 2003 (7.86×10^6 cells L^{-1}) and then peaked in early January 2004 (5.03×10^7 cells L^{-1}) in the superficial layer. The peak dramatically decreased in late January 2004 (7.50×10^6 cells L^{-1}). *Ehux* was the dominant species and represented about 90.0% of the phytoplankton assemblage. The bloom started flourishing after the diatom and dinoflagellate blooms under nitrogen depletion and moderate light, temperature and salinity conditions. Water temperature (10.31 ± 1.14°C) and salinity values (27.05 ± 0.88 ppt) were usually stabile. Surface chlorophyll-a concentrations ranged from 1.23 to 2.32 μg L^{-1} during the bloom. The ratios of N:P (mean: 4.12 ± 2.22) and Si:P (40.35 ± 16.25) of the bloom period were lower than those of the non-bloom periods.

Key words | algal blooms, Dardanelles, *Emiliania huxleyi*, environmental factors, winter

Muhammet Turkoglu (corresponding author)
Fisheries Faculty, Hydrobiology Department,
Canakkale Onsekiz Mart University,
Terzioglu Campus,
17100 Canakkale,
Turkey
Tel.: +902862180018-1572
Fax: +902862180543
E-mail: *mturkoglu@comu.edu.tr*

INTRODUCTION

Except for the polar oceans, *E. huxleyi* (*Ehux*) is one of the most abundant coccolithophores occurring globally in the entire oceans in early summer periods. This species drifts freely and prefers the surface layers of the oceans. It has received considerable attention since it tends to produce massive blooms under favorable conditions (Balch *et al.* 1991, 1992; Tyrrell & Taylor 1995; Nanninga & Tyrrell 1996; Hattori *et al.* 2004; Smyth *et al.* 2004; Turkoglu 2008). In summer, high surface irradiance, shallow stratification with a mixed layer depth of about 10–20 m, anomalies in salinity and temperature, and low phosphate and silicate concentrations favor the bloom of this species (Egge & Heimdal 1994; Tyrrell & Taylor 1995; Nanninga & Tyrrell 1996; Smyth *et al.* 2004; Zeichen & Robinson 2004).

In recent years, satellite images have revealed surprising bright expanses of water in some marine systems such as the eastern Bering Sea in the middle of winter. Similar bright waters occur in summer and have been identified as blooms of the coccolithophore *Ehux*. However, *Ehux* blooms are an unlikely cause of the bright waters in winter because hostile conditions should prevent extensive phytoplankton blooms (Broerse *et al.* 2003).

However, Sorrosa *et al.* (2005) clearly showed that low temperature suppresses coccolithophorid growth but induces cell enlargement and stimulates the intracellular calcification that produces coccoliths. For instance, while coccolithophore *Ehux* grows at a wide temperature range (10–25°C), another coccolithophore *Gephyrocapsa oceanica* Kamptner, 1943 grows at a narrow temperature range (20–25°C) and cell size is inversely correlated with temperature (Sorrosa *et al.* 2005). At low temperature, the enlargement of chloroplasts and cells and the stimulation

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of coccolith production have been morphologically confirmed under fluorescent and polarization microscopes (Sorrosa *et al.* 2005). The uptake of ^{45}Ca by *Ehux* at 10°C has been greatly increased after a five-day lag and exceeded that at 20°C (Sorrosa *et al.* 2005). During the blooms, the numbers of *Ehux* cell densities usually outnumber those of other species, frequently accounting for about 80% or more of the total number of phytoplankton cells (Turkoglu 2008).

One important matter of blooms of *Ehux* is that it alters the ecological conditions of a marine system by acting as a source of organic sulfur (i.e. dimethyl sulfide) to the atmosphere (Balch *et al.* 1992; Burkill *et al.* 2002) and calcium carbonate to the sediments (Balch *et al.* 1996; Tekiroglu *et al.* 2001). Moreover, high cell densities can provoke the water to change to a milky white or turquoise color due to significant changes in the inherent optical properties of water (Brown & Yoder 1994; Cokacar *et al.* 2001, 2004; Smyth *et al.* 2004; Turkoglu 2008). Besides, as a consequence of blooms the coccolithophore are now receiving greater attention, as their role in the global sulfur and carbon cycles may influence the world's climate and their potential as nuisance bloom algae have implications for commercial fishing and the marine ecosystem (Jordan & Chamberlain 1997). Therefore, documenting the occurrence of blooms in time and space is essential to characterize the biogeochemical environment of a target region. Studies on coccolithophore *Ehux* by Turkoglu *et al.* (2004*b*,*c*) and Turkoglu (2008) have also indicated advancing of this species from the Black Sea region through the Sea of Marmara and the Dardanelles under favorable conditions.

Phytoplankton species succession in the Turkish Straits System which contains the Bosphorus, Dardanelles and Sea of Marmara shows similarities to the succession in the Black Sea (Koray *et al.* 2000; Turkoglu & Koray 2000, 2002). The time sequence of SeaWiFS images shows the development of the *Ehux* bloom in the Sea of Marmara in the early summer of 2003. In the images, the turquoise color indicates the regions with the highest coccolith accumulations. During the early summer bloom period, the cell density of *Ehux* increased from 3.58×10^7 to 2.55×10^8 cells L^{-1} in the superficial layer. Between 12 June and 25 June, *Ehux* exceeded 2.0×10^8 cells L^{-1} in the superficial layer (Turkoglu *et al.* 2004*b*,*c*; Turkoglu 2008). In addition to blooms of *Ehux* in the early summer periods, the system was also controlled by dinoflagellates such as *Ceratium* spp. and *Prorocentrum* spp. during the year and diatoms such as *Proboscia alata* (Brightwell) Sundström, 1986, *Pseudo-nitzschia pungens* (Grunow ex P.T. Cleve) Hasle, 1993 and *Dactyliosolen fragilissimus* (Bergon) G. R. Hasle, 1991 in winter, spring and late summer periods (Unsal *et al.* 2003; Turkoglu *et al.* 2004*b*–*d*). Researchers have reported harmful and toxic algal blooms such as *Dinophysis* spp. and *Gonyaulax* spp. that potentially threat the region (Unsal *et al.* 2003; Turkoglu *et al.* 2004*b*–*d*).

The main target of this study is to exhibit the bloom circumstances and the reasons of coccolithophore *Ehux* along with environmental conditions in winter period in the Dardanelles. Environmental characteristics, hydrographic structure, inorganic nutrients and chlorophyll-a have been investigated in relation to the blooms of the Dardanelles. This study reports the winter bloom of *Ehux* in the Dardanelles and interactions of this species with other phytoplankton species in response to environmental parameters for the first time.

MATERIALS AND METHODS

The Dardanelles is a very important water passage connecting the Aegean Sea to the Sea of Marmara. It has two current systems; one of the currents derives from the Aegean Sea, where the water density is high, and the second one comes from the Sea of Marmara, characteristically low in density. Aegean Sea water is typically flowing from the southwest (SW) to northeast (NE) below the Sea of Marmara water. Its NE/SW trend is interrupted by a north–south bend between Eceabat and Canakkale. In addition to the first bend, there is a second bend called the "Nara Cape". The width of the Strait varies from 1.35 to 7.73 km and the narrowest part is located between Canakkale and Kilitbahir. 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.* 2004*c*, 2006; Baba *et al.* 2007). However, the depth of the study area in the Dardanelles is 80 m. The location of the sampling station (40°09′00″N 26°24′00″E) is given in Figure 1.

Water samples for nutrient analyses, phytoplankton enumeration and chlorophyll-a estimation were collected

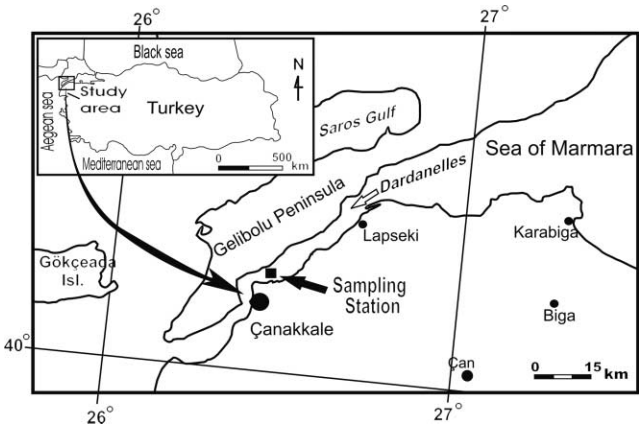


Figure 1 | Map of the sampling station in the Dardanelles.

by a Niskin Sampling Bottle from depths of 0.5, 10, 25, 50 and 75 m in the Dardanelles (40°09′N, 26°24′E) (Figure 1) in a 10-day interval between December 2003 and January 2004. Sea temperature, salinity, pH and dissolved oxygen (DO) were measured *in situ* using an YSI 6600 Model Multiple Water Analysis Probe.

Water samples for nutrient analyses were kept frozen until analysis. Analysis for nitrite + nitrate ($NO_2^- + NO_3^-$), inorganic phosphate (PO_4^{-3}) and silicate (SiO_4) were measured using a Technicon model auto-analyzer according to Strickland & Parsons (1972).

Chlorophyll-a samples were filtered through GF/F glass fiber filters. The filters were folded into aluminum foil and immediately frozen for laboratory analysis. Chlorophyll-a was determined spectrophotometrically after extraction by 90% acetone (Strickland & Parsons 1972).

For the quantitative analysis of phytoplankton, samples were preserved with 2% buffered formalin (v/v) and microscopic analysis was conducted within a week of collection. Utermöhl Sedimentation Chambers and Neubauer and Sedgwick Rafter Counting Slides were used in combination for enumeration of the phytoplankton species, depending on the dimensions and concentrations of the organisms (Guillard 1978; Hasle 1978; Venrick 1978).

Pearson correlation analysis and paired samples' *t* test were conducted using SPSS 11.5 (SPSS 2003). In some cases linear regression relationships were also obtained. All variables except pH were previously $\log_{10}$ transformed to improve linearity, normality and homogeneity of variances (Quinn & Keough 2002).

RESULTS

Physical variables

Vertical profiles of temperature and salinity suggested that there were two different water masses during the *Ehux* winter bloom (Figure 2(A, B)). During the winter period, salinity values in the upper layer between 0 and 10 m, an intermediate layer between 10 and 50 m and a lower layer between 50 and 75 m varied between the values of 25.72–27.77, 26.28–37.67 and 38.31–38.75 ppt, respectively (Figure 2(B)). Both seasonal reverse thermocline and halocline interfaces were clear due to the two different

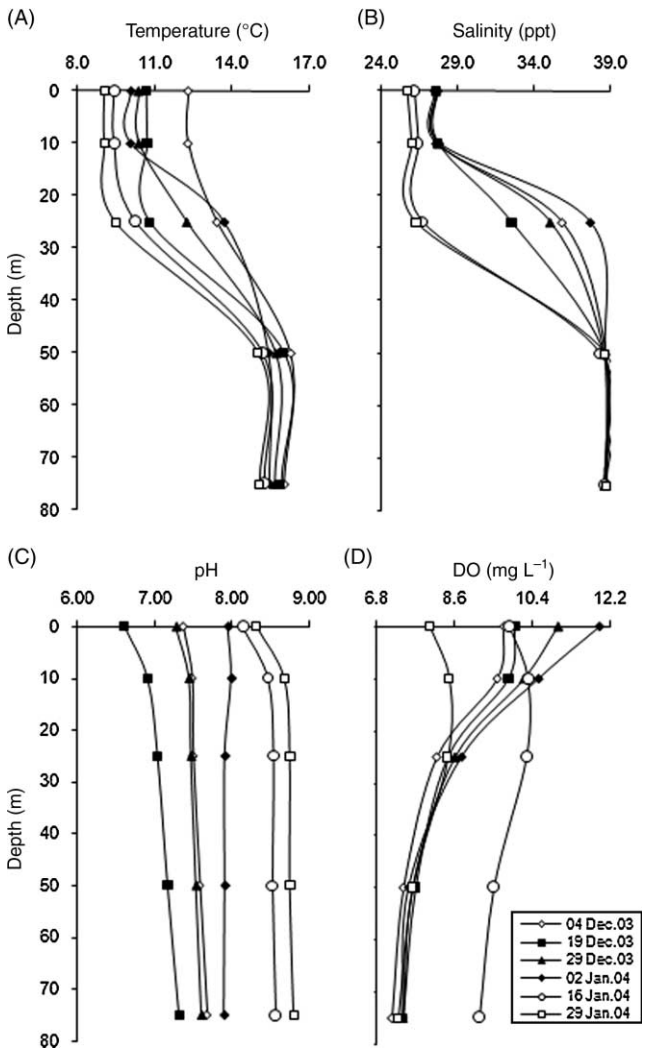


Figure 2 | The vertical profiles of temperature (A), salinity (B), pH (C) and dissolved oxygen (DO) (D) in winter in the Dardanelles.

Table 1 | Descriptive statistics of biological, physical, and chemical data groups in the surface layer (0.5 m) of the Dardanelles

	N	Min	Max	Mean	SD
Temperature (°C)	6	9.06	12.30	10.31	1.14
Salinity (ppt)	6	25.72	27.67	27.05	0.88
pH	6	6.61	8.31	7.61	0.64
DO ($mg\,L^{-1}$)	6	8.04	11.95	10.10	1.31
$NO_2^- + NO_3^-$ (μM)	6	0.10	0.44	0.26	0.14
PO_4^{-3} (μM)	6	0.05	0.08	0.06	0.01
SiO_4 (μM)	6	1.40	4.25	2.51	1.16
N:P	6	2.00	7.33	4.12	2.22
Si:P	6	24.00	58.50	40.35	16.25
Chl-a ($\mu g\,L^{-1}$)	6	1.23	2.32	1.94	0.43
Dinoflagellates ($cell\,L^{-1}$)	6	1.65×10^5	4.41×10^6	1.77×10^6	1.41×10^6
Diatoms ($cell\,L^{-1}$)	6	3.93×10^5	1.20×10^7	4.20×10^6	4.29×10^6
E. huxleyi ($cell\,L^{-1}$)	6	1.60×10^4	5.03×10^7	2.08×10^7	1.86×10^7
E. huxleyi cell diameter (μm)	35	9.85	13.50	11.25	1.45

Notes: *N*, sampling number; Min, minimum value; Max, maximum value; SD, standard deviation.

water layer systems and formed between 25 and 50 m in the non-bloom period (before bloom and after bloom) and between 10 and 50 m in the bloom period of *Ehux* (Table 1 and Figures 2(A, B)).

During the *Ehux* winter bloom, pH changed from 6.61 to 8.67 in the upper layer and from 7.17 to 8.79 in the lower layer (Figure 2(C)). DO values in both the upper (8.04–11.95 $mg\,L^{-1}$) and the lower layer (7.16–9.17 $mg\,L^{-1}$) of the Dardanelles revealed high saturation (Figure 2(C)). DO concentrations gradually decreased from the upper superficial layer to the lower layer during the winter bloom.

Nutrient behaviors

Vertical profiles of inorganic nutrients showed that the concentrations in the upper layer were generally lower than those in the lower layer during winter bloom conditions (Figure 3(A–C)). There was a decrease in concentration of $NO_2^- + NO_3^-$ (0.11–0.28 μM) at 10 m in the vertical profile. Under 10 m, $NO_2^- + NO_3^-$ gradually increased with depth (0.16–0.61 μM), while PO_4^{-3} was generally resistant to change with depth (0.05–0.09 μM) except that there was a small increase at 25 m (0.05–0.12 μM). SiO_4 concentrations changed between 1.40 and 4.25 μM in the upper layer during the winter *Ehux* sampling period, but they were below concentrations of 2.00 μM during the *Ehux* bloom except for the value recorded on 2 January 2004 (3.03 μM) when a peak value in cell density of *Ehux* was observed. However, they increased slowly with depth (2.21–3.50 μM) except for the vertical SiO_4 profile recorded on 29 January 2004. SiO_4 decreased with depth on this date (3.05–4.25 μM) (Figure 3(C)).

The ratios of N:P were significantly lower (2.00–7.33) (Figure 3(D)) than the assimilatory optimal of the Redfield ratio (N:P = 16.0). On the other hand, there was a decreasing profile in the ratios of Si:P between the lower layer and the upper layer (Figure 3(E)). The ratios of N:P ranged from 1.83 to 10.90 (Figure 3(D)), while the ratios of Si:P ranged from 17.1 to 62.2 in both the upper layer and the lower layer during the bloom conditions (Figure 3(E)).

Succession of *E. huxleyi* and other phytoplankton groups

Results of microscopic observation clearly showed that cell dimensions of *Ehux* varied between 9.85 and 13.50 μm in diameter (mean: 11.20 ± 1.38 μm) (Table 1). Although there was an important development of the *Ehux* bloom (Figure 4(A)) in the Dardanelles, there was no apparently turquoise or bright color change in the system in early winter (December, 2003) and mid-winter

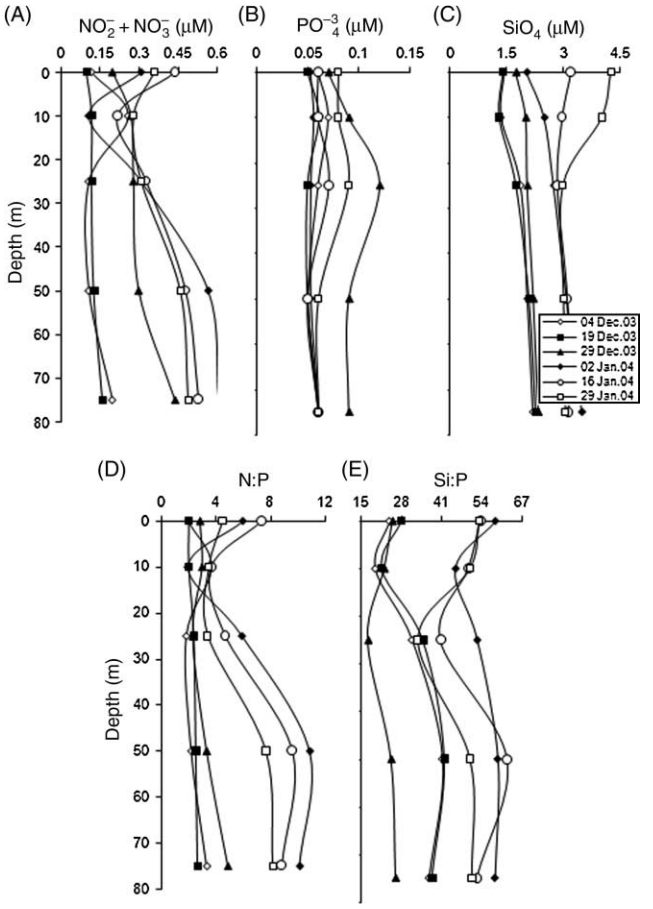


Figure 3 | The vertical profiles of $NO_2^- + NO_3^-$ (A), PO_4^{-3} (B), SiO_4 (C), N:P (D) and Si:P ratios (E) in winter in the Dardanelles.

(January, 2004). The algal bloom started to improve towards the middle of December, then reached a maximum value (5.03×10^7 cells L^{-1}) (Figure 4(A)) in early January (2 January 2004) and afterward lost out its advantage in late January (7.50×10^6 cells L^{-1} versus 1.20×10^7 cells L^{-1}) (Figure 4(C)).

Vertical profiles of *Ehux* (Figure 4(A)) revealed that its cell density increased from 1.60×10^4 to 5.03×10^7 cells L^{-1} in the upper layer. In early January, *Ehux* exceeded 5.00×10^7 cells L^{-1} in the upper layer. However, the density dramatically decreased with depth. For instance, the cell concentration declined from 5.03×10^7 in the upper layer to 1.76×10^5 cells L^{-1} in the lower layer (Figure 4(A)). The cell density of dinoflagellates and diatoms showed that there was no effect of *Ehux* on the development of dinoflagellates, although there was an effect of *Ehux* on the development of diatoms. There were also high densities of dinoflagellates (1.0×10^6–4.41×10^6 cells L^{-1}) (Figure 4(B)) contributed by *Prorocentrum* spp. and *Ceratium* spp. in the upper layer during the *Ehux* winter bloom period. However, diatoms were at minimum density values just before and at the time of the *Ehux* winter bloom peak value (Figure 4(C)). Afterwards, diatoms such as *Leptocylindrus* spp., *P. pungens*, and *D. fragilissimus* started to increase due to the loss of *Ehux* bloom effort and their excessive cell densities exceeded a value of 1.00×10^7 cells L^{-1} in the upper layer in late January (Figure 4(C)). The density of diatoms reached 2.10×10^7 cells L^{-1}, which was the maximal bloom value in the sub-surface (10 m) during the bloom period (Figure 4(C)).

Ehux was found to be the dominant species, accounting for more than 90.0% of the phytoplankton assemblage in the middle of the bloom (Table 2). Diatoms (5.92%) and dinoflagellates (3.99%) were other important

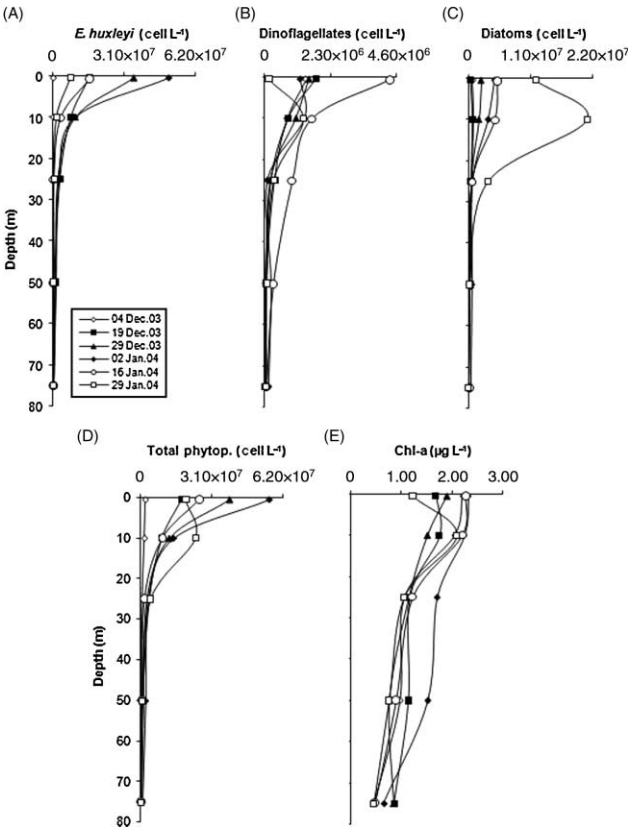


Figure 4 | The vertical profiles of *E. huxleyi* (A), dinoflagellates (B), diatoms (C), total phytoplankton (D) and chlorophyll-a (E) in winter in the Dardanelles.

Table 2 | Rational contributions (%) of dinoflagellates, diatoms and coccolithophore *E. huxleyi* to total phytoplankton in the different water layers of the Dardanelles

Date	Stratification	Depth	Dinophyceae			Bacillariophyceae			*E. huxleyi*		
			04.12.03	*19.12.03*	*29.12.03*	*04.12.03*	*19.12.03*	*29.12.03*	*04.12.03*	*19.12.03*	*29.12.03*
Dec. 03	Upper layer	0.5	65.43	9.97	3.99	33.84	2.19	5.92	0.72	87.49	90.09
		10	45.14	8.80	8.80	53.95	7.48	15.20	0.91	83.06	76.00
	Intermediate layer	25	26.35	8.91	8.38	73.65	13.18	22.72	0.00	77.33	68.90
	Lower layer	50	13.98	5.63	7.22	81.10	23.94	31.20	0.00	70.42	61.58
		75	13.25	19.08	20.06	86.75	37.57	53.19	0.00	43.35	26.75
			02.01.04	*16.01.04*	*29.01.04*	*02.01.04*	*16.01.04*	*29.01.04*	*02.01.04*	*16.01.04*	*29.01.04*
Jan. 04	Upper layer	0.5	2.24	17.27	0.84	8.28	20.05	61.02	89.49	62.67	38.14
		10	2.78	17.33	5.65	24.20	48.79	87.87	66.09	33.88	6.49
	Intermediate layer	25	3.83	50.99	7.71	21.53	35.90	84.11	71.90	13.11	8.18
	Lower layer	50	11.85	43.91	17.85	28.89	38.95	70.99	55.63	17.14	11.16
		75	21.97	33.33	34.55	54.59	55.56	65.45	23.44	11.11	0.00

taxonomic groups during the *Ehux* bloom (Table 2). While the largest contribution to diatoms came from species of *Leptocylindrus* spp., *P. pungens* and *D. fragilissimus,* the largest contribution to dinoflagellates came from species of *Prorocentrum* spp. (especially *Prorocentrum micans* Ehrenberg, 1834, the most opportunist one) and *Ceratium* spp. such as *Ceratium furca* (Ehrenberg, 1834) Claparède et Lachmann, 1859 and *Ceratium fusus* (Ehrenberg, 1834) Dujardin, 1841 according to their cell density order.

While cell densities of the diatoms and dinoflagellates in the superficial layer varied between 1.65×10^5 and 4.41×10^6 cells L^{-1} and between 3.93×10^5 and 1.2×10^7 cells L^{-1}, respectively, their densities in the sub-surface layer varied between 8.33×10^5 and 1.66×10^6 cells L^{-1} and between 7.07×10^5–2.1×10^7 cells L^{-1}, respectively. Except for the 29 January 2004 sampling date on which *Ehux* algal bloom highly decreased (7.5×10^6 cells L^{-1}), the cell density of diatoms was below 5.0×10^6 cells L^{-1} in the superficial layer (0.5 m) due to the fairly high *Ehux* bloom (1.60×10^7–5.03×10^7 cells L^{-1}), and was above 1.0×10^7 cells L^{-1} both in the surface layer (0.5 m) and in the sub-surface layer (10 m) in the last of the bloom (Figure 4(C)) due to the more dramatic decrease of the *Ehux* bloom (Figure 4(A)). However, the highest diatom production was in the surface and sub-surface layers due to a sufficient amounts of nutrients, especially silicate (>3.00 μM) (Figure 3(A–C)) and the dramatic decrease of the *Ehux* bloom (Figure 4(A)). This tendency of diatoms in the vertical profile was roughly similar to the vertical profile of the dinoflagellates which were less than diatoms (Figure 4(B, C)).

Phytoplankton chlorophyll-a

Chlorophyll-a concentrations ranged from 1.23 to 2.32 μg L^{-1} (1.94 ± 0.43) in the upper layer where there was an *Ehux* bloom (Table 1 and Figure 4(E)). However, chlorophyll-a maximal values were also observed in the sub-surface layer (10 m) due to diatom and other blooms at this depth during the bloom period (Figure 4(E)).

Comparative interactions

In regard to comparative interactions, analysis revealed that there was no significant relationship between *Ehux* and PO_4^{-3} (Figure 5). Dinoflagellates showed strong negative correlations with SiO_4 in the surface layer (Table 3). Additionally, strong positive relations were observed between dinoflagellates and PO_4^{-3} in the surface layer. Diatoms were strongly correlated with temperature, pH, PO_4^{-3} and SiO_4 in the interface layer (Table 3).

Because there were no high cell densities of *Ehux* in the bloom period, nutrients ($NO_2^- + NO_3^-$, PO_4^{-3} and SiO_4) and their proportional relations (N:P and Si:P ratios) were more correlated with dinoflagellates and diatoms rather than with *Ehux* in both the surface and the deep layer (Table 3).

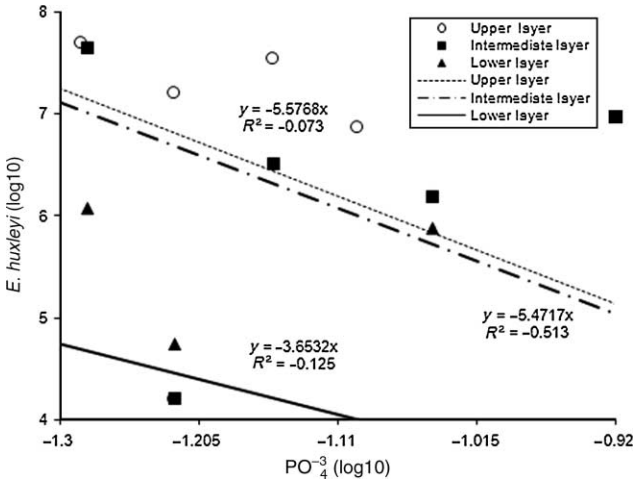


Figure 5 | Relationships between *E. huxleyi* and PO_4^{-3} in the upper layer (0.5 m), intermediate layer (25 m) and lower layer (50 m) of the Dardanelles. For each regression, the coefficients of determination (R^2) and the process of equating (y) are shown.

However, negative relationship between *Ehux* and PO_4^{-3} was not statistically significant (Table 3 and Figure 5) due to the fact that PO_4^{-3} was assimilated by high diatom production during the long time period before the bloom of *Ehux*. Therefore, there were lower concentrations of PO_4^{-3} in that period.

In the upper layer, positive correlations between chlorophyll-a and *Ehux* ($R = 0.330$), between chlorophyll-a and dinoflagellates ($R = 0.350$) and between chlorophyll-a and total phytoplankton ($R = 0.247$) were more important than the correlation between chlorophyll-a and diatoms ($R = 0.090$) due to competition between diatoms and *Ehux* (Table 3).

DISCUSSION

Insufficient inorganic nutrients, especially reactive phosphate due to extensive utilization of them by diatoms just before the *Ehux* algal bloom, high irradiance and so high temperature, and a stable water column in terms of vertical mixing following the establishment of the seasonal thermocline were the characteristics of the *Ehux* summer bloom in the Dardanelles (Turkoglu 2008), confirming previous studies on *Ehux* blooms in the North Sea and NE Atlantic (Nanninga & Tyrrell 1996; Smyth *et al.* 2004; Zeichen & Robinson 2004). In general, it has been suggested that *Ehux* blooms follow the blooms of diatoms in marine system (Holligan *et al.* 1993; Uysal 1995; Turkoglu & Koray 2002, 2004; Broerse *et al.* 2003; Cokacar *et al.* 2004; Turkoglu *et al.* 2004*b*; Turkoglu 2005, 2008; Oguz & Merico 2006).

Table 3 | Pearson correlation coefficients for the relationship between physicochemical and biological data groups in the Dardanelles

	Dinop	Bacil	*Ehux*	Phyto
Upper layer				
Temperature	−0.111	−0.776	−0.265	−0.450
Salinity	−0.134	−0.840*	0.440	0.236
pH	0.051	0.827*	0.060	0.251
DO	0.183	−0.554	0.844*	0.727
$NO_2 + NO_3$	0.356	0.742*	0.169	0.364
PO_4^{-3}	−0.357	0.681	−0.338	−0.207
SIO_4	−0.088	0.957†	0.055	0.266
N:P	0.500	0.517	0.349	0.502
Si:P	0.116	0.724	0.323	0.495
Chl-a	0.350	0.090	0.330	0.247
Intermediate layer				
Temperature	−0.601	−0.599	0.190	−0.492
Salinity	−0.727	−0.617	0.463	−0.266
pH	0.469	0.682	−0.637	0.106
DO	0.901*	−0.193	−0.250	−0.218
$NO_2 + NO_3$	0.320	0.362	−0.178	0.239
PO_4^{-3}	−0.037	0.296	−0.208	0.046
SiO_4	0.357	0.589	−0.386	0.251
N:P	0.232	0.043	0.082	0.195
Si:P	0.134	−0.045	0.123	0.131
Chl-a	−0.209	−0.307	0.471	0.160
Lower layer				
Temperature	−0.616	−0.475	0.093	−0.149
Salinity	−0.806	0.003	0.092	−0.062
pH	0.501	0.151	−0.497	−0.240
DO	0.729	−0.178	−0.326	−0.165
$NO_2 + NO_3$	0.795	0.749	0.202	0.473
PO_4^{-3}	−0.269	0.141	0.165	0.093
SiO_4	0.805	0.567	−0.005	0.279
N:P	0.871*	0.667	0.157	0.437
Si:P	0.758	0.276	−0.089	0.145
Chl-a	0.343	0.585	0.705	0.737

*Significant at $\alpha = 0.05$.
†Significant at $\alpha = 0.01$.
$N = 6$.

There was an extensive bloom of *Ehux* (5.03×10^7 cells L^{-1}) in spite of deficient irradiance and temperature. However, this bloom was lower than that of the 2003 summer bloom (2.55×10^8 cells L^{-1}) (Turkoglu 2008), probably due to insufficient irradiance and temperature (min–max: 9.06–12.30; mean: 10.31 ± 1.14°C). Notwithstanding, results of microscopic observations clearly showed that cell dimensions of *Ehux* in the winter bloom period (min–max: 9.85–13.50 μm in diameter; mean: 11.20 ± 1.38 μm) were bigger in diameter than the cell dimensions in the summer bloom period (min–max: 7.86–12.37 μm in diameter; mean: 9.05 ± 1.05 μm) in the Dardanelles (Table 4). According to the "paired samples' *t* test", the difference between winter and summer mean values in cell diameters of *E. huxleyi* was important at a level of "$p < 0.01$" (Table 4). Regarding correlations between *Ehux* and CTD parameters, there were some supportable relationships. *Ehux* were weakly related with temperature ($R = -0.265$), salinity ($R = 0.440$) and pH ($R = 0.060$), but strongly with DO ($R = 0.844$) in the upper layer (Table 3). However, a negative relationship between *Ehux* and temperature during the winter bloom was supported by Sorrosa *et al.* (2005). They clearly revealed that low temperature suppresses coccolithophorid growth but induced cell enlargement and stimulated the intracellular calcification that produces coccoliths. They also showed that *Ehux* grew at a temperature range between 10 and 25°C and its cell size was inversely correlated with temperature. At low temperature, the enlargement of chloroplasts and cells and the stimulation of coccolith production have been morphologically confirmed under fluorescent and polarization microscopes, respectively (Sorrosa *et al.* 2005).

In this study, although the maximum density of *Ehux* was lower (5.03×10^7 cells L^{-1}) in the 2003–2004 winter period (Figure 4(A)) than the maximum density in the 2003 summer period (2.55×10^8 cells L^{-1}) (Turkoglu 2008), the contribution of *Ehux* to the total phytoplankton in the peak of the bloom time (29 December 2003) was similar (90.09%) (Table 2) to the contribution of *Ehux* in the 2003 summer period (97.58%) (Turkoglu *et al.* 2004*b*; Turkoglu 2008). It is known that, during *Ehux* bloom periods, the contribution of *Ehux* to total phytoplankton usually outnumbers the contribution of other taxonomic groups to total phytoplankton, frequently accounting for about 80% or more of the total phytoplankton cell densities (Cokacar *et al.* 2004; Turkoglu *et al.* 2004*b*; Turkoglu 2008).

Nutrient behavior during the *Ehux* winter bloom was similar to the one that occurred during the summer period (Turkoglu 2008). It was reported that nutrient dynamics in the Dardanelles differed slightly due to different water masses (Polat & Tugrul 1995; Unsal *et al.* 2003; Turkoglu *et al.* 2004*a*,*c*,*d*; Turkoglu & Erdoğan 2007*a*,*b*; Turkoglu *et al.* 2007; Turkoglu 2008). Insufficient nutrient concentrations, especially silicate (1.40–2.03 μm) in the surface layer rather than in the deep layer (2.10–3.50 μm) was likely due to the utilization of nutrients by the early diatom blooms. However, diatoms were found in low densities in

Table 4 | Paired samples' *t* test between winter and summer mean values in cell diameters of *E. huxleyi*

		Paired samples' statistics							
		Mean	***N***	**Std. deviation**	**Std. error mean**				
Pair 1	Summer	9.053	35	1.052	0.178				
	Winter	11.201	35	1.383	0.234				
		Paired samples' correlations							
		N	**Correlation**	**Sig.**					
Pair 1	Summer and winter	35	−0.017	0.925					
		Paired samples' test							
		Paired differences							
		95% confidence interval of the difference							
		Mean	**Std. deviation**	**Std. error mean**	**Lower**	**Upper**	**t**	**df**	**Sig. (two-tailed)**
Pair 1	Summer–winter	−2.148	1.751	0.296	−2.750	−1.546	−7.256	34	0.000

the surface layer (0.5 m) and in the sub-surface layer (10 m), except for the sampling date of 29 January 2004. While the low diatom densities in the surface layer were affected by *Ehux* bloom, they were probably affected by insufficient irradiance in the sub-surface and deep layers (10–75 m). Consequently, although there was sufficient nutrient concentration high enough to tolerate for diatom growth in the lower layer during the *Ehux* winter bloom, the diatom growth was quite insufficient in the lower layer due to some ecological factors such as insufficient irradiance. However, just after the *Ehux* bloom, there was a high diatom production sourced by *Leptocylindrus danicus* P.T. Cleve, 1889 in the surface (0.5 m) (5.12×10^6–1.2×10^7 cells L^{-1}) and the sub-surface layer (10 m) (4.68×10^6–2.1×10^7 cells L^{-1}) between 16 January and 29 January 2004 (Figure 4(C)). Some researchers revealed that diatoms were favored when nitrogen was available at higher concentrations (Piehler *et al.* 2004) and large phytoplankton cells such as *L. danicus*, *D. fragilissimus* and *P. pungens* were better competitors for nitrate because of their larger specific storage volume (Dauchez *et al.* 1996; Kormas *et al.* 2002).

In contrast to diatoms, *Ehux* is known to tolerate low nutrients, especially low phosphate concentrations due to its alkaline phosphatase enzyme and this talent permits this group to outcompete other species (Balch *et al.* 1991; Paasche 2002). On the other hand, the study area was generally limited by nitrogen and the low N:P proportion in the bloom period was in conformity with previously reported values (Polat *et al.* 1998; Turkoglu *et al.* 2004*a*,*c*,*d*; Turkoglu & Erdoğan 2007*a*,*b*; Turkoglu *et al.* 2007; Turkoglu 2008).

Redfield *et al.* (1963) calculated that C:N:P proportions were in ratio of 106:16:1 in seawater. If N:P ratios in a marine system are generally below the normal value of 16:1, the system is limited by nitrogen (Stefanson & Richards 1963). However, if Si:N ratios in a system are below the value of 1:1, the system is limited by silicate. Since N:P proportions in the surface layer (min–max: 2.00–7.33; mean: 4.12 ± 2.22) were below the Redfield ratio (16:1) and Si:N ratios (min–max: 24.00–58.50; mean: 40.35 ± 16.25) were above the Redfield ratio (1:1), the Dardanelles was limited for nitrogen, but for phosphate and silicate. The fact that the system was limited by nitrogen has been accepted as the general situation not only for bloom periods but for no bloom periods as well in all water columns for two decade (Polat & Tugrul 1995; Polat *et al.* 1998; Turkoglu *et al.* 2004*c*,*d*, 2007; Turkoglu 2008). It is known that diatom increase in marine systems is likely to be limited by dissolved reactive silica when Si:N ratios are less than 1 according to the Redfield ratios (Redfield *et al.* 1963; Piehler *et al.* 2004) or N:Si ratios above 1 (Roberts *et al.* 2003).

CONCLUSIONS

This work is the first attempt to present temporal and vertical distribution of *Ehux* winter blooms and the interaction of this species with other phytoplankton groups in the same period in the Dardanelles. Previous studies have shown summer blooms of this species in the Sea of Marmara (Unsal *et al.* 2003; Turkoglu *et al.* 2004*a*–*c*; Turkoglu *et al.* 2007; Turkoglu & Erdogan 2007*a*,*b*; Turkoglu 2008) and in the Black Sea region since the 1980s (Moncheva & Krastev 1997; Mikaelyan 1997; Turkoglu & Koray 2002, 2004). Therefore, this study may also indicate advancing of this species from the Black Sea region through the Sea of Marmara and the Dardanelles under favorable conditions. This may be due to climate changes, in addition to the dramatic eutrophication of the system since the 1980s. Nowadays, this species composes not only extensive summer blooms but also winter blooms in the Sea of Marmara. Unfortunately, this species seems be able to create more extensive algal blooms in the near future. Further monitoring of the system in terms of anomalies in the temperature, salinity and nutrient changes as well as the phytoplankton species composition is needed for a better understanding of the ecological significance of this species in this system and its neighboring systems, the Black Sea and Northern Aegean Sea.

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