First monospecific bloom of the harmful raphidophyte *Chattonella antiqua* (Hada) Ono in Alexandria waters related to water quality and copepod grazing

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First monospecific bloom of the harmful raphidophyte *Chattonella antiqua* (Hada) Ono in Alexandria waters related to water quality and copepod grazing

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The massive red tide bloom of *Chattonella antiqua* that occurred in Alexandria waters during late August/early September 2006 was monospecific, of very high density, and of wide spatial distribution, and was accompanied by mass fish and invertebrates mortalities. During the bloom, surface water temperature ranged between 26.5 and 28.5 °C and salinity between 23 and 27 psu. The bloom started under very high nitrate and ammonia levels; where the uptake of ammonia seemed to be faster than that of nitrate, and the bloom seemed to avoid the copepod grazing. Mass fish and invertebrates mortality was observed. Few cells of *Heterosigma* species have been reported for the first time in the Alexandria waters. Several environmental constraints (including physical factors), nutrient loading, copepod grazing and comparison with other data for *C. antiqua* blooms in Alexandria waters are also discussed.

Keywords: *Chattonella antiqua*; Raphidophytes; HAB species; Copepod grazing; Alexandria; Mediterranean Sea

1. Introduction

The toxic raphidophycean *Chattonella antiqua* (Hada) Ono was first reported in the Seto Inland Sea, Japan, in 1964 [1]. It caused several episodes of mass mortalities in cultured yellowtails during 10 major blooms between 1970 and 1987 [2]. In recent years [3, 4], there has been an apparent global increase in novel blooms of the *Chattonella* with wild fish mortality [5, 6].

The *C. antiqua* morphology, life cycle, autecology, ecophysiology, and toxin production have been reported in several studies, mostly based on isolates from Japanese waters [7–15]. It has been reported that *Chattonella* species do not produce fish-killing toxins at all times. It is likely that toxicity varies among strains or species and is affected by external environmental variables [16].

Although red tide occurrence in the Eastern Harbour of Alexandria, Egypt is dated back to almost 50 years ago [17], the phenomenon received limited attention until the beginning of the 1990s [18–25]. The first survey to follow up the different stages of the red tide outbreaks

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west of Alexandria (Mex Bay) was carried out during 1992 [26]. Labib studied the same area [21–23] on monthly sampling and reported several periods of water discoloration.

Since 1998, there has been evidence of an increase in the frequency of the red tide outbreaks, intensity, magnitude, and the number of harmful/harmless causative species (24 species) in Alexandria waters during the warm seasons [27–31]. This indicates that their geographical extent seems to be of a rising trend. Most of the red tides in the Eastern Harbour of Alexandria are originated in the west in Mex Bay (10 km to the west of the harbour), and have been transferred by local diffusion driven by hydrographical processes [32].

Chattonella antiqua has a very short history in Alexandria waters. It was reported in the harbour for the first time in September 1998, together with other causative red tide species [27, 30]. Since then, blooms of *C. antiqua* became recurrent in Alexandria waters, particularly in the summer and autumn [31, 33], with maximum cell densities of 1.8×10^6 cells l^{-1} [29], and are sometimes associated with massive mortality of fish, crabs, and other marine organisms.

The overall objective of the data presented here on the occurrence of the massive monospecific *C. antiqua* bloom in Alexandria waters in late August–early September 2006 (derived basically from daily sampling) is to clarify the short-term variability of the bloom, the effects of environmental physical and chemical variables, species interaction, and copepod grazing. In the mean time, the aim is to compare it with other data recorded during previous *C. antiqua* blooms in Alexandria waters and with other results derived from culture experiments.

In order to investigate the red tide outbreaks in Mex Bay, after 14 years of the last detailed survey carried out there during 1992, a monitoring program was carried out from May to October 2006, which represents the typical period of red tide occurrence in Alexandria waters.

2. Materials and methods

2.1 *Study area*

Sampling was carried out from four stations covering about 28 km along the coastal area off Alexandria from Mex Bay in the west to Montaza area in the east. The covered stations are: Mex Bay, Kayet Bey, Eastern Harbour, and Montaza area, respectively (figure 1). It is known that different land-based sources discharge their water directly into the study area. The Umum drain discharges about $6.5 \times 10^6 \, m^3 \, d^{-1}$ of drainage water from Lake Maryout into in Mex Bay, and the Maadia outlet discharges $6 \times 10^6 \, m^3 \, d^{-1}$ from Lake Edku in Abou Qir area, east of Alexandria. The discharged waters contain agricultural, industrial, and urban wastes. The Eastern Harbour of Alexandria is located in the central part of the city, and it is subjected to increasing eutrophication processes since the 1970s. Due to the nature of water circulation, the harbour is affected by the water discharged from Mex Bay.

However, recently, remedial actions have been taken by the Alexandria Governorate to close outfalls along Alexandria beaches and the main sewer of Alexandria (Kayet Bey) at the western side of the harbour. The load of the closed outfalls has been diverted into Lake Maryout. This in turn discharges its waters into Mex Bay through Umum drain, as mentioned.

2.2 *Sampling and laboratory analysis*

Sampling in Mex Bay was carried out between the 2 July and 27 August, almost every 2 d to follow the development of the bloom. Then, between the 29 August and 5 September, sampling was carried out daily at the four mentioned sampling stations.
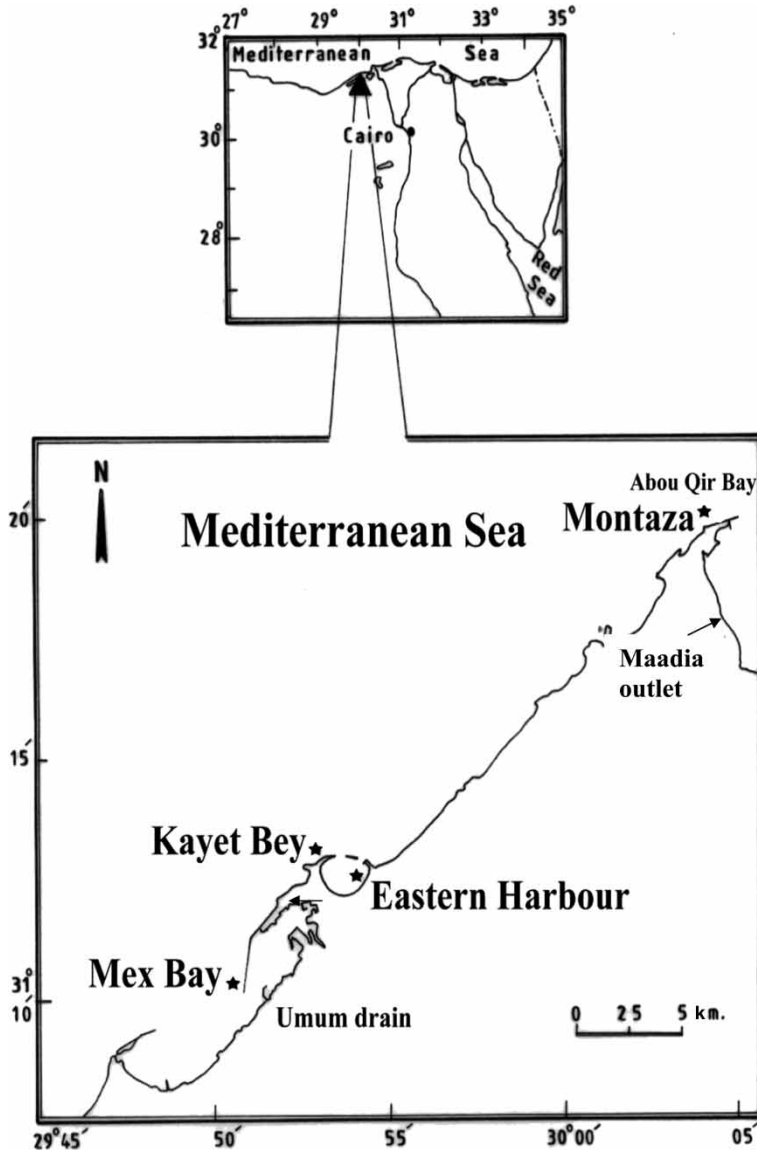
Figure 1.   Study area and location of sampling stations.

The following parameters were measured: surface water temperature (by a thermometer accurate to $\pm 0.1\,°C$), salinity (using a calibrated salinity refractometer, S/Mill), dissolved inorganic nutrients (nitrite, nitrate, ammonia, silicate, and phosphate), dissolved oxygen, organic matter, and chlorophyll *a* [34]. Temperature and salinity were measured also at the bottom depth in the Eastern Harbour only. In the Kayet Bey area, salinity and chlorophyll *a* were the only measured parameters.

Samples for chlorophyll *a* determination were filtered through a Whatman GF/F filter and kept frozen until analysis. Fresh phytoplankton samples were first examined for identification under a research microscope according to Tomas [35], and then preserved in buffered neutral formalin (4%), a few drops of Lugol's acid solution were added and they were counted [36].

Zooplankton samples (30 l) were collected from the Eastern Harbour during the 1st–4th September using vertical hauls of a plankton net ($55 - \mu$m mesh size and 50-cm mouth diameter). The samples were preserved in 7% formaldehyde, then examined for quantitative taxonomic analyses, and the abundance was calculated as organisms m^{-3} (org. m^{-3}).

Statistical analyses were performed by a one-way ANOVA or Student's test [37]. Differences are termed significant when $p < 0.05$.

The bloom index (BI) for *C. antiqua* was calculated as:

$$\mathrm{BI} = \sum_i \frac{N_{it}}{N_i \max} \times 100,$$

where N_{it} is the cell density of *C. antiqua* (i) at time t, and $N_i \max$ is the the maximum cell density of *C. antiqua* (i) during the period concerned [38].

3. Results

3.1 *Physical and chemical conditions*

The physical and chemical parameters during the bloom period are shown in figure 2. The surface water temperature ranged normally between 26.5 and 28.5 °C, with a slight increase observed on the 4th September, as affected by an air temperature of 31 °C.

Salinity exhibited a wide range of variation. At the beginning of the bloom in Mex Bay on 29th August, it reached 28.5 psu and then sharply decreased to 23 psu at the bloom peak on the 1st September, followed by an increase (27 psu) on the 4th September. A similar variability pattern was observed in Kayet Bey, with the lowest value (26.5 psu) on the 2nd–3rd September. The bloom peaks in the Eastern Harbour and Montaza were associated with a higher salinity (35.8 and 37.5 psu, respectively) compared with other stations.

The bottom temperature and salinity measurements in the Eastern Harbour indicated thermo-haline stratification in the water column with 1–1.5 °C, and 2.5–3.5 psu between the warmer-less saline surface layer and above the bottom.

The oxygen content of the water reflected the trend of the bloom. Oxygen levels of 3.5–5.62 ml l^{-1} (80.09–122.44% saturation) were measured at the beginning of the bloom. Then, concentrations increased as the bloom developed with its maximum in Mex Bay on the 31st August and 1st September (11.5 and 9.5 ml l^{-1}, 247.63% and 187.01% saturation, respectively), and it was around 9 ml l^{-1} (204.55% saturation) on the last day in both the harbour and Montaza.

Nitrite concentrations never exceeded 1.8 μM. The lowest (0.27 and 0.35 μM) appeared in the Eastern Harbour and Montaza on the 2nd and 4th September, respectively. Nitrate was very high at the beginning of the bloom in Mex Bay (about 13 μM), and severely dropped during its peak (about 75% loss). However, the replenishment resulting from water-discharge input was very fast. The same trend was observed in the Eastern Harbour and Montaza, with minimum nitrite values of 0.55 and 0.65 μM on the 2nd and 3rd September, respectively.

Ammonium concentrations were higher than nitrate concentrations along the whole area. The bloom triggered in Mex Bay with 22.5 μM, and its peak consumed >75% of ammonium. The concentration increased to 8.5 μM, despite the partial dissipation of the bloom on the 4th September. Its concentration in the Eastern Harbour and Montaza, during the bloom peaks was 1.8–2.5 and 0.35–1.8 μM, respectively.

Phosphate concentration in Mex Bay was relatively high a day before the bloom peak (5.56 μM), losing about 60% on the 1st September while ranging between 1.5 and 2.5 μM
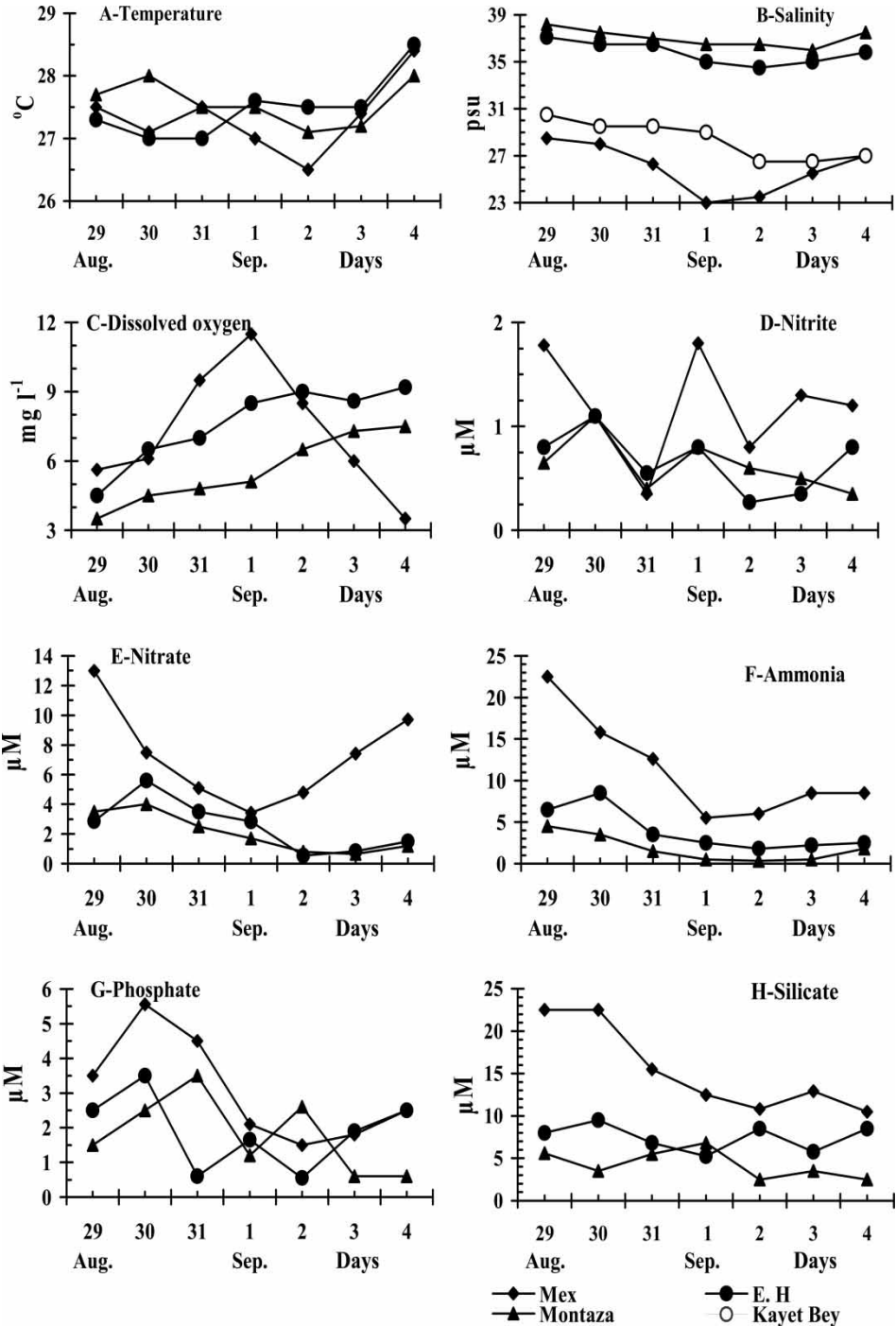
Figure 2. Measured parameters. A: temperature (°C); B: salinity; C: dissolved oxygen (mg l^{-1}); D: nitrite (μM); E: nitrate (μM); F: ammonia (μM); G: phosphate (μM); H: silicate (μM) during the bloom periods.

at the end of the bloom. The same trend was observed in the Eastern Harbour and Montaza, with minimum values around 0.6 μM. Replenishment was observed due to a new supply of freshwater discharge.

Silicate concentrations, due to the monospecific nature of the bloom, were very high along the whole area, particularly in Mex (maximum 33.2 μM). However, a marked decrease was noticed during the bloom peak, with values ranging between 2.5 and 12.5 μM.

3.2 *Bloom dynamics*

3.2.1 Description of the bloom.   Water discoloration suddenly occurred in Mex Bay on August the 30th and then extended towards both east and west directions. The water turned brown-red on the 1st September, covering the entire bay. The spreading visible layer (about 2 km in width) coming from the entire bay was seen extending mainly to the east (Kayet Bey). It entered the Eastern Harbour by the 31st August, and then the bloom developed so rapidly that the discoloured water occupied the entire Eastern Harbour from the 2nd–4th September. The bloom was visible along the coastal area off Alexandria and appeared relatively less dense in the Montaza area (about 18 km from the Eastern Harbour). On the 5th September, the coloration of the water disappeared as suddenly as it had appeared under an abnormally warm strong south wind (average velocity $> 10\,\mathrm{m\,s^{-1}}$ and air temperature of 31–32 °C). The concentrations of chlorophyll *a* and *C. antiqua* are shown in figure 3.

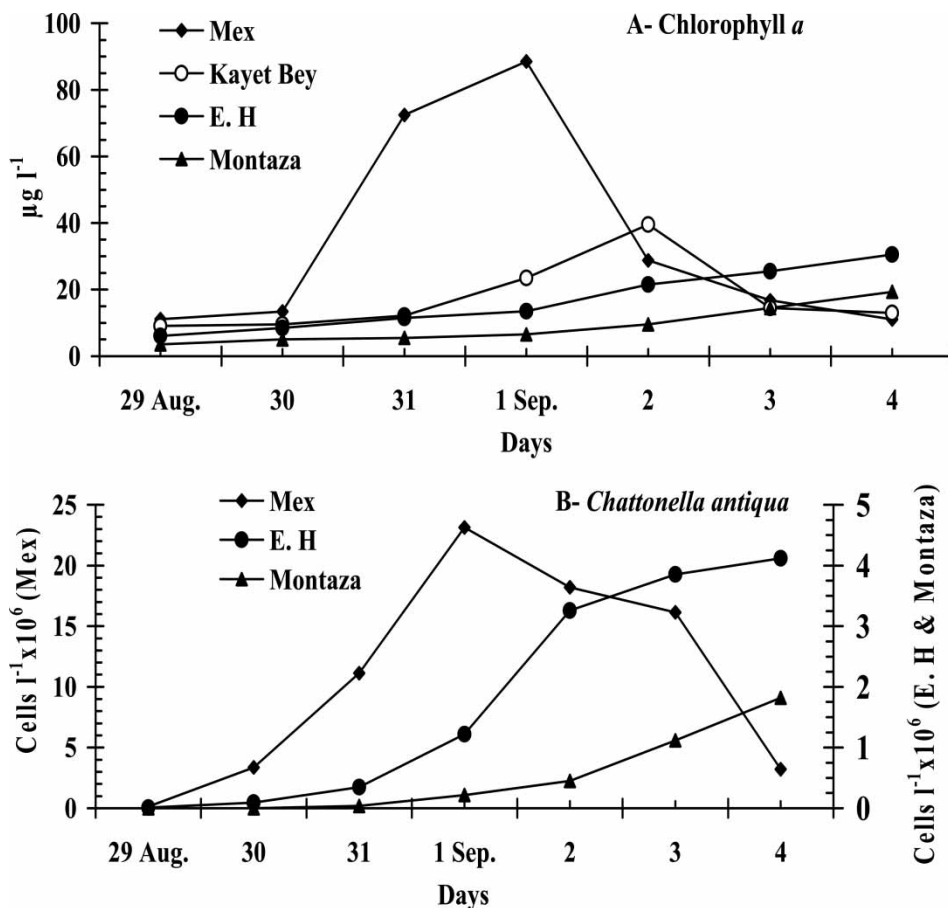
Figure 3.   Measured parameters. A: (mg l^{-1}); B: chlorophyll *a* concentration (μg l^{-1}); C: density of *Chattonella antiqua* (cells l^{-1} × 10^6) during the bloom periods.

As the raphidophycean *C. antiqua* (length 60–120 µm, width 35–50 µm) was the causative species, the bloom was monospecific. However, the bloom progressed differently in the stations studied. It started in Mex Bay on the 29th August and developed so rapidly that 3.36×10^6 cells l^{-1} of *C. antiqua* gathered at the surface on the next day (Chl *a* 13.4 µg l^{-1}). There was a steady increase in the abundances on the 1st September (23.13×10^6 cells l^{-1}) increasing Chl *a* to 88.5 µg l^{-1}. This was followed by gradual decrease, and the bloom lost about 20% on the 2nd September. The bloom was partially visible during the next 2 d, with 3.23×10^6 cells l^{-1} on the 4th September.

In the Eastern Harbour, *Chattonella antiqua* attained 1.22×10^6 cells l^{-1} on the 1st September (Chl *a* 13.5 µg l^{-1}). The bloom peak appeared on the 4th September (4.12×10^6 cells l^{-1}, Chl *a* 30.5 µg l^{-1}), and on the same day it was at its highest density in Montaza area (1.82×10^6 cells l^{-1}, Chl *a* 19.3 µg l^{-1}).

The bloom was accompanied by a mass mortality of fish and invertebrates. Symptoms of anoxic conditions were also observed in dead fish, manifested as a yellowish coloration of the body and gills. The common fish species were rabbit fish (*Siganus* sp.), striped sea bream (*Lithognathus marmyrus*, *Sargus* sp.), black goby (*Gobius* spp.), and red crab (*Portunus pelagicus*).

3.2.2 Accompanying phytoplankton species.

Different phytoplankton species were observed during the bloom. *Skeletonema costatum* (Grev.) Cleve represented the main component of the community at the bloom beginning, with its highest count of 0.31×10^6 cells l^{-1} in Mex Bay. The phytoplankton composition during the bloom development included: *Nitzschia longissima* (Breb) Ralfs, *Guinardia delicatula* (Cleve) Hasle and *Thalassiosira subtilis* (Ostenfeld) Gran. They attained the highest count of 0.13×10^6 cells l^{-1}, 0.39×10^6 cells l^{-1}, and 0.14×10^6 cells l^{-1}, respectively, in Mex. *Pseudo-nitzschia* sp., *N. longissima* and *Rhizosolenia setigera* Brightwell showed a count of (0.25×10^6, 0.15×10^6 and 0.18×10^6 cells l^{-1}, respectively) in the Eastern Harbour, *S. costatum* and *N. longissima*. In Montaza, the former species was at 1.12×10^6 cells l^{-1} on the major peak day. Microflagellates gained 0.45×10^6 cells l^{-1} on the major bloom peak in Mex Bay. Dinoflagellates included nine species, among them, *Alexandrium ostenfeldii* (Paulsen) Balech & Tangen, *Gymnodinium catenatum* Graham, and *Scrippsiella trochoidea* (Stein) Loeblich III reaching densities between 0.018×10^6 and 0.027×10^6 cells l^{-1} in Mex; *Prorocentrum triestinum* Schiller (0.035×10^6 cells l^{-1}) in the Eastern Harbour; *P. minimum* (Pavillard) Schiller and *P. triestinum* (0.004×10^6 and 0.018×10^6 cells l^{-1}, respectively) in Montaza.

Few cells of a *Heterosigma* species appeared in Mex, Kayet Bey and the Eastern Harbour. Their density did not exceed 150 cells l^{-1}. This is the first report of *Heterosigma* in Alexandria waters.

The BI was calculated to follow the development of the bloom. Results of Mex Bay (table 1) reflected the rapid development of the bloom between the 29th and 30th August (23.55-fold increase), which was then sharply reduced to 3.13-fold with the bloom maintenance until its major peak on the 1st September. Meanwhile, the calculation indicated that the bloom dissipation was also very fast, and the bloom lost about 30.16–86.03% of its maximum on the 3rd–4th September.

Similar results could be found in the Eastern Harbour (table 2) during the 29th–30th August (10-fold increase), which then gradually decreased between the 2nd and 3rd September, indicating a slow development. The sharp development of the bloom in Montaza lagged one day and was seen between the 30th and 31st August (20.5-fold increase). The bloom progressed there between the 1st and 3rd September at an almost constant rate (around a 2.5-fold

Table 1.   Bloom index (BI) of *Chattonella antiqua* in Mex Bay between 2 July and 1 September 2006.

Days	BI	Days	BI
2 July	0.168	5 August	0.167
6 July	1.148	6 August	0.131
9 July	0.0	8 August	0.507
11 July	0.012	10 August	0.542
14 July	0.040	13 August	1.427
18 July	0.0	14 August	2.094
20 July	0.002	17 August	0.070
23 July	0.280	20 August	0.100
25 July	0.673	24 August	0.200
26 July	0.075	27 August	0.321
28 July	0.069	29 August	0.616
30 July	0.249	30 August	14.510
31 July	0.424	31 August	48.110
1 August	0.249	1 September	100.0
3 August	0.217		

Table 2.   Bloom index (BI) of *Chattonella antiqua* in the Eastern Harbour (EH) and Montaza between the 29th August and 4th September 2006.

Station	Days						
	29 August	30 August	31 August	1 September	2 September	3 September	4 September
EH	0.22	2.21	8.5	29.61	79.01	93.57	100
Montaza	0.055	0.11	2.25	12.1	24.73	61.54	100

Table 3.   Copepod abundance in the Eastern Harbour (org. m^{-3}).

Species	Days			
	1 September	2 September	3 September	4 September
Acartia sp.	427	140	78	54
Copepod nauplii	740	233	171	116
Copepodite stages	466	78	178	86
Eutrepina sp.	350	140	31	54
Oithona sp.	700	272	31	47
Paracalanus parvus	580	171	54	23
Total copepods	3420	1244	596	538

increase). However, these results must remain tentative, and other factors such as accumulation, movement, and others must be considered.

The accompanied copepods found in the Eastern Harbour between the 1st and 4th September are given in table 3. There was a steady decrease by days with the bloom development.

4.   Discussion

Varying forcing factors, including physical dynamics, climate change, nutrient load, and grazing seem, to affect the current massive raphidophyte bloom of *Chattonella antiqua*. The bloom occurred in Mex Bay under physical conditions that are in line with those previously documented during red tide occurrences in Alexandria waters [24, 25, 29–31, 33]. The previous studies indicated that: (1) due to the daily input of discharging waste waters, the bay has often

become strongly stratified in the summertime; (2) the regional surface temperature varied by nearly a degree in the entire bay; and (3) salinity can change rapidly and dramatically, and frequently varies by 10 psu or more between the surface and above the bottom layers during stratification events.

The establishment of a thermo-haline stratification of the water column and the weak wind period, which strengthen the stratification [39], accelerated the processes of the bloom formation. The conclusion that such stratification is a crucial triggering factor to the development of harmful algal blooms (HABs) was well documented [40], as is the fact bloom cessation is favoured by increased turbulence, which has a negative impact on their massive cell densities [41]. As *C. antiqua* exhibits a daily vertical migration [42, 43] and crosses deep physical gradients, the stratification could also be beneficial for its blooming formation [44]. The vertical movement of cells under such conditions undoubtedly has another functional significance in minimizing grazing losses, besides allowing cells to obtain nutrients at depth and light at the surface.

From 1998 until 2005, 10 recurrent blooms of *C. antiqua* have occurred in Alexandria waters, with densities varying between 0.31×10^6 and 1.8×10^6 cells l^{-1}. A connection with the physical and chemical parameters measured during these blooms is given in table 4. The current bloom was characterized by a monospecific nature, very dense population, and wide spatial distribution that was never previously recorded for any other *C. antiqua* blooms in the Alexandria waters. The bloom was accompanied by a massive fish and invertebrate mortality, and this was the second case of marine mortality recorded west of Alexandria since 2001 [29]. However, the mortality factors, whether gill clogging or release of toxins, are still unclear.

A wide salinity variation was observed during the bloom period; yet, the major bloom peaks usually appeared under the lowest salinity values (23–27 psu).

Blooms of *C. antiqua* occurred in Alexandria waters in a temperature range of 20–30 °C, and a salinity range of 23–38.5 psu, thus indicating its eurythermal and euryhaline nature, which agrees with Yamatogi *et al.* [45] for salinity. Temperatures of >15 °C, are normally reported during *Chattonella* blooms in Japanese coastal waters [9]. Comparison of the results

Table 4. Physical and chemical parameters with *Chattonella antiqua* blooms in Alexandria waters between 1998 and 2006.

Date	Temperature (°C)		Salinity (psu)		Nutrient concentrations at surface (μM)				Cells ml^{-1}
	Sur.	Bott.	Sur.	Bott.	NO$_3$	NH$_4$	SiO$_4$	PO$_4$	
30 September 1998*	26.0	24.5	37.0	38.2	0.55	–	1.6	0.8	540
27 July 1999*	25.8	24.8	35.4	37.2	0.8	–	1.8	1.3	850
21 May 2000*	21.0	19.0	35.0	36.8	1.4	–	0.5	1.3	1140
24 September 2000*	26.0	25.5	34.8	36.0	1.5	–	1.0	3.2	650
7 Oct 2000*	24.5	24.0	38.5	39.0	1.8	–	0.7	0.9	310
13 May 2001†	25.0	–	27.0	–	6.9	–	–	7.6	1800
21 September 2001†	26.0	–	32.0	–	4.6	–	–	4.1	890
3 October 2001†	24.0	–	30.0	–	1.5	–	–	1.5	450
21 August 2004§	27.0	–	34.0	–	1.18	0.41	6.9	0.6	1200
14 August 2005§	28.2	–	36.5	–	0.9	0.58	0.71	4.5	490
1 September 2006¶	27.3	–	23.0	–	3.43	5.5	12.5	2.1	23 130
4 September 2006¶	27.0	–	35.8	–	1.5	2.5	8.5	2.5	4120
4 September 2006¶	27.5	–	37.5	–	1.2	1.8	2.5	1.5	1820

Note: – denotes no measurement.
*Mikhail [27].
†Mikhail [29, 30].
§Mikhail *et al.* [31].
¶Present study.

Table 5. Optimal values and minimum–maximum temperature and salinity values for growth in culture experiments of *Chattonella antiqua* and other *Chattonella* species isolated from different areas.

Species	Temperature (°C)	Salinity (psu)	Area	Reference
C. antiqua	25*	25–41*	Harima-Nada, Seto Inland Sea, Japan	[12]
C. antiqua	25* 24.4–30.6† 20–25†	– – –	Hiroshima Bay, Japan	[8]
C. verruculosa	15* 5–25†	25* 15–35†	Hiroshima Bay, Japan	[74]
C. marina	20–25*	20–30*	Kagoshima Bay, Japan	[75]
C. marina	10–30*	15–45*	Boston Bay, Australia, Seto Inland Sea, Japan	[76]
C. antiqua	30* 15–32.5‡	32* 16–36‡	Isahaya Bay, Japan	[45]
C. marina	30* 12.5–32.5‡	24* 16–36‡		
C. subsalsa	20–30*	15–25*	Delaware Inland Bays, USA	[46]

Note:–denotes no measurement.
*Optimum growth range.
†Total growth range.
‡Growth range.

obtained from culture experiments of *C. antiqua* and other *Chattonella* species is given in table 5.

A nutrient-replete environment, such as Mex Bay, is expected to sustain quasi-continuous dense blooms. The bay is a highly eutrophic ecosystem, with major nitrogen and phosphorus inputs from agricultural and urban sources, estimated (in tons per month) as 6.6–50.7 NO_3, 16–320.8 NH_4, and 12.4–33.4 PO_4 [26]. Previous studies attributed the increases in phytoplankton biomass and the shift of communities towards bloom-forming species observed over the past decade to these anthropogenic nutrient inputs.

Nutrient concentrations barely decrease, thus limiting the development of the bloom, which started in Mex Bay in the presence of very high NO_3 and NH_4 concentrations, comparable with those observed with the *C. antiqua* bloom in 2001 in the Kayet Bey area, which is directly affected by waters discharging from Mex [29]. The uptake of NH_4 seems to be faster than that of NO_3. Under experimental conditions, Nakamura and Watanabe [12] concluded that NO_3 and NH_4 stimulate the growth of *C. antiqua*, while NH_4 concentrations $>150\,\mu M$ severely inhibit its growth. The half saturated constant for NH_4 is comparable with that of NO_3, and the maximum uptake rate of NH_4 is about 2.2 times greater than that of NO_3, which indicates a much more rapid uptake of NH_4 [13]. Zhang *et al.* [46] observed that NH_4 inputs might promote the bloom formation of *C. antiqua* much more than NO_3, and that the blooms produced a much higher biomass, when NH_4 was used as a nitrogen source than when growth was supported by NO_3. On the other hand, NO_3 is the preferred inorganic nitrogen source for other raphidophytes, such as *Heterosigma* species [47]. High NO_3 concentrations were also associated with a bloom of *Chattonella* cf *verruculosa* [48].

Phosphate concentrations during the bloom peaks (1998–2006) ranged between 0.6 and 7.6 μM, and almost 50% of these peaks occurred at $PO_4 > 2\,\mu M$. These results underline the importance of PO_4 availability, which is in agreement with Nakamura [14] and Zhang *et al.* [46] for *Chattonella*. Since blooms of *C. antiqua* are considered to begin after the germination of cysts [49], the presence of nutrient-rich surface water, actively supplying the vegetative cells in the water column, seems to play an important role in supporting a relatively rapid growth at the bloom starting period.

The toxicity of *C. antiqua*, if present, could be regulated by environmental factors [50]. A negative correlation was found between salinity and cell toxicity; the highest toxin production was observed by Haque [51] at 25 psu, when the maximum cell division rate occurred, and the toxin yields decreased sharply at salinities exceeding 30 psu.

The present major peak of *C. antiqua* was about 45 times greater than that of its first bloom in late September 1998 and about 15 times greater than the values observed in May 2001, which were the highest of the entire period. Such an extremely high dense population could also enhance its harmful effect and/or toxicity. White [52] postulated that a population of 0.1×10^6 cells l^{-1} of *C. antiqua* can cause yellowtail death, which is known to be very sensitive to this species. The toxicity of *C. antiqua* was hard to detect until the cell density reached approximately 1.95×10^6 cells l^{-1}, as fish did not die at a low density but rather showed abnormal movements for 30–45 min, recovering gradually and swimming normally within a few hours [10].

Interactions between HAB species and other algae may be an important survival strategy for some HAB species. It has long been argued that production of allelopathic exudates allows some harmful species to outcompete co-occurring phytoplankton [53, 54]. These interactions between *Chattonella* spp. and the dinoflagellate *Alexandrium catenella* were observed by Iwasaki [55].

Skeletonema costatum in Mex Bay dominated the community during most of July, producing peaks between 1.2×10^6 and 6.15×10^6 cells l^{-1} in the fourth week. The present *C. antiqua* bloom was preceded by increased numbers of *S. costatum*, which, during the bloom peak, contributed 37.3% of the total standing crop in Montaza. The relation between blooms of *S. costatum* prior and/or in combination with other red tide species in the harbour and elsewhere was previously reported [25, 56, 57], as well as between *S. costatum* and *Heterosigma* (*H. akashiwo*, Hada, reverse relationship) by Han [58]. *S. costatum* probably produces stimulants for growth of red tide species [55]. However, the mode of interaction underlying the apparent temporal relationship between these species is unknown [59].

The success of *Rhizosolenia* and *Nitzschia* species to grow well under the present bloom with relatively limited nutrients at times agrees with other observations for the occurrence of *Dactyliosolen fragilissimus*, *Guinardia striata*, as well as *Nitzschia rigida* with other red tide species in Alexandria waters [19, 20], and elsewhere [60]. The bloom of these species and microflagellates in stratified-low nutrient waters was reported [61, 62].

Concerning the occurrence of *Heterosigma* sp. with blooms of *C. antiqua* in Alexandria waters, Peperzak [4], who mapped the regional expansion of raphidophyte blooms and locations of associated fish kills in European coastal waters, pointed out that new occurrences of *Chattonella* are increasing, and *Heterosigma akashiwo* is usually present. The presence of *Heterosigma* sp. under the present physical bloom condition agrees with that recorded for *H. akashiwo* by Mori *et al.* [63] (15–25 °C, 12–28 psu), Yamochi [64] (18–26 °C), Ono *et al.* [65] (10–30 °C), Haque and Onoue [66] (20–30 psu), and Zhang *et al.* [46] (16–30 °C, 10–30 psu).

Zooplankton abundance during the bloom period in the harbour did not exceed 3420 org. m^{-3}, with a tendency to decrease with the bloom development. The present bloom seems to be unaffected by the copepod grazing, and *C. antiqua* displayed a significant advantage with respect to a copepod (bottom-up) control on its growth. Uye [67] reported an inverse relation between copepod communities and *C. antiqua* during its bloom, while the grazing pressure could be important with its initial stage. The density of *Paracalanus parvus*, which feeds on *C. antiqua* [39], dropped sharply at the bloom peak on the 4th September. Low microzooplankton grazing rates have been measured for *Chattonella* during a number of blooms, as well as in culture experiments with mesozooplankton grazers such as copepods [68]. Hansen *et al.* [5] reported that zooplankton grazing did not appear to have a major impact on the bloom of

Chattonella cf. *verruculosa*, thus reflecting zooplankton avoidance of the *Chattonella* bloom or high death rates of zooplankton exposed to the bloom.

Generally, a low copepod grazing pressure during massive red tide blooms could be explained by several causes, such as (1) a rejection by some grazers to toxic species [69], (2) the ability of a HAB species to build up dense populations [70], (3) the ability to produce grazing-inhibiting compounds that either deter grazers or are lethal to grazers [71], and (4) the production of more toxin under stress, that kill predators and the competing algal species [54]. One or more of these factors may be involved to explain the inverse relationship between *C. antiqua* and copepod grazing in the present study. It is reported that *Chattonella subsalsa* blooms resulted in fish mortality and/or a myriad of deleterious effects in zooplankton [72].

The correlation matrix showed water temperature and salinity to be the dominant parameters clearly affecting the BI variability ($r = 0.312$ and -0.859, respectively, significant at <0.05 level, $n = 32$, ANOVA), and salinity correlated negatively with (BI). The multiple regression equation ($BI = 217.67 - 1.52 \times temperature - 4.88 \times salinity$) explains that these two parameters could explain about 74% of the (BI) variability. Temperature is a principal factor affecting cyst germination and vegetation [73], which in turn may control the onset of *C. antiqua* blooms. The cyst-germination process is sensitive to temperature; no germination occurs at $10\,°C$ or lower; 20–$25\,°C$ is the suitable range; and the optimum is at $22\,°C$ [7]. The excitement occurs when the temperature rises to ~ 20–$22\,°C$ [39].

The present monitoring programme in Mex Bay has revealed new records of *Heterosigma* species that have not previously been reported in this area, thus prompting questions such as: Why do more and more new red tide species or new records occur? What are the changeable triggering factors that affect these phenomena? An increase in the number of intensive monitoring programmes is needed, together with the prevention of waste-water discharge directly into the coast of the city. The routes of entry for exotic red-tide species (ballast water, water currents, etc.) still need to be determined. The present study stresses the importance of studying the ecology of these organisms in their original sites. Yet, the role of cyst germination in the bloom onset, and models of transport and dispersion, must be considered. Such information is essential for effective management and mitigation of red-tide blooms.

Acknowledgements

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