First occurrence of some *Alexandrium, Chattonella, Gymnodinium* and *Prorocentrum* species in Alexandria waters (Egypt)

*Première apparition de certaines espèces d'*Alexandrium, Chattonella, Gymnodinium *et* Prorocentrum *dans les eaux d'Alexandrie (Égypte)*

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ABSTRACT

Mikhail S. K. - First occurrence of some Alexandrium, Chattonella, Gymnodinium *and* Prorocentrum *species in Alexandria waters (Egypt).* Mar. Life, **13** *(1-2): 11-20.*

The paralytic shellfish poison producers (PSP), Alexandrium catenella, Alexandrium ostenfeldii, *the fish killers* Chattonella antiqua, Gymnodinium mikimotoi, *and the harmless dinoflagellate* Prorocentrum sigmoides, *can be found in the phytoplankton of the Eastern Harbour of Alexandria (Egypt). Although natural transport is responsible for the sudden appearance of these foreign invading species, the progressive eutrophication in Alexandrian waters shares the responsibility. Reduced salinity seems to contribute in part toward explaining the development of* C. antiqua *and* G. mikimotoi. *Their abundant peaks occurred within red tide bloom periods of different causative species, mainly diatoms, associated with limited fish and invertebrates mortality at times. They maintained typical environmental conditions enhancing their growth. The paper evidences increased intensity, geographical extent and toxicity of red tides in Alexandria waters.*

RÉSUMÉ

*Mikhail S. K. - [Première apparition de certaines espèces d'*Alexandrium, Chattonella, Gymnodinium *et* Prorocentrum *dans les eaux d'Alexandrie (Égypte)].* Mar. Life, **13** *(1-2) : 11-20.*

Les espèces productrices de toxines paralysantes (PSP), Alexandrium catenella, Alexandrium ostenfeldii, *les espèces responsables de l'épisode de mortalité massive de poissons,* Chattonella antiqua, Gymnodinium mikimotoi *et* Prorocentrum sigmoides *sont présentes dans le phytoplancton du Port Est d'Alexandrie (Égypte). Le transport par processus naturels est responsable de l'apparition de ces espèces étrangères ; l'eutrophisation progressive des eaux d'Alexandrie y participe également. La diminution de la salinité semble contribuer en partie au développement de* Chattonella antiqua *et* Gymnodinium mikimotoi. *Leurs pics d'abondance se produisent pendant les marées rouges dues à différentes espèces, principalement les diatomées, associées quelquefois à des phénomènes limités de mortalité de poissons et d'invertébrés. Les conditions environnementales typiques favorisant leur développement sont maintenues. L'article met en évidence l'accroissement en intensité, en superficie et la toxicité des marées rouges dans les eaux d'Alexandrie.*

INTRODUCTION

Dense phytoplankton blooms are known to develop in the Eastern Harbour of Alexandria (Zaghloul, Halim, 1992; Labib, 1994 a). Almost 40 years ago, large-scale red tide was reported for the first time (Halim, 1960). Considerable attention has been paid to monitoring red tide bloom outbreaks in the harbour during the last decade, the phenomenon being of regular occurrence in the warm seasons (Labib, 1994 b, 1996, 1997; Labib, Halim, 1995).

Although the phytoplankton community in the harbour is fairly well known, several new records are reported in this work. The type species of the genus *Alexandrium, A. minutum* Halim, was described from the Eastern Harbour (Halim, 1960). Four *Gym-*

nodinium species were known from the harbour: *G. sanguineum* (= *G. splendens*), *G. minor, G. simplex* and *G. catenatum* (Hassan, 1972; Ismael, 1993, 1998; Labib, 1997). From the six *Prorocentrum* species known from Egyptian Mediterranean waters, three are common and perennial in the harbour (*P. micans, P. minimum* and *P. triestinum*). The genus *Chattonella* was recorded for the first time from the harbour by Ismael and Halim (2000), but the species was not identified.

The present study is part of the monitoring programme carried out by the author in the Eastern Harbour of Alexandria (Egypt) during the three years survey from 1998 to 2000, the aim of which was to document the importance of short-time scale sampling as an adequate strategy to fully describe phytoplankton variability and ambient environmental conditions in a dynamic, eutrophic marine basin.

This paper reports the first occurrence of five phytoplankton species, the dinoflagellates, *Alexandrium catenella, A. ostenfeldii, Gymnodinium mikimotoi, Prorocentrum sigmoides* and the raphidophycean, *Chattonella antiqua* in the Eastern Harbour during 1998-2000. Except for *P. sigmoides,* the other four species, known to be occasionally harmful, caused limited fish and crab mortality in the Eastern Harbour.

The raphidophycean, *Chattonella antiqua* (Hada) Ono formed red tide blooms in the coastal waters of Japan (Ono, Takano, 1980), killing large numbers of farmed yellowtail fish. The species has been observed along the French Mediterranean coast (reviewed by Maestrini, 1991). Its occurrence (Imai *et al.*, 1993), morphology (Imai, 1990), autecology (Yamaguchi *et al.*, 1991; Khan *et al.*, 1995), and toxin production (Tanaka *et al.*, 1994) have been reported.

Gymnodinium mikimotoi Miya et Kominami ex Oda (Takayama, Matsuoka, 1991), an unarmoured dinoflagellate, is one of the most important red tide flagellates causing mass mortality in farmed shellfish populations in Japan (Matsuoka *et al.*, 1989; Yamaguchi, 1994). First reported as *Gymnodinium* sp. Type–'65 (Iizuka, Irie, 1966). It was later named *G. nagasakiense* (Takayama, Adachi, 1984). This species has been proposed as a synonym of *G. mikimotoi* (Takayama, Matsuoka, 1991). The European *Gyrodinium aureolum* is thought to be conspecific with *G. mikimotoi,* on the basis of the morphology and biochemistry of pigments (Hallegraeff, 1993). *G. aureolum* was first reported in European waters in 1966 (Braarud, Heimdal, 1970).

Alexandrium catenella (Wheddon et Kofoid) Balech (= *Protogonyaulax catenella, Gonyaulax catenella*) is a well-known paralytic shellfish poison producing dinoflagellate species (Fukuyo *et al.*, 1989; Yamamoto, Yamasaki, 1996). It was also responsible for fish mortality of cultured yellowtail in Japan (Ono *et al.*, 1996). Its toxicity could be a function of nitrogen limitation (Benavides *et al.*, 1995). There is growing evidence for an increase in its abundance and geographical range around the Mediterranean, but it is rare in oceanic waters (Garcés *et al.*, 2000). The species is widespread in the Northern and Southern Hemisphere, known to produce a smooth-walled, cylindrical cyst with round end (Fukuyo, 1985; Hallegraeff *et al.*, 1988).

Alexandrium ostenfeldii (Paulsen) Balech, Tangen is a red tide forming species in the Mediterranean Sea (Fraga, Sanchez, 1985). Although no toxicity was reported when it was found alone (Moestrup, Hansen, 1988), the species would rank among the least toxic among the natural population of *Alexandrium* (Cembella *et al.*, 1988; Hansen *et al.*, 1992). Balech and Tangen (1985) found this species in Oslofjord in late Winter and early Spring, and occasionally in August.

Prorocentrum sigmoides Böhm is a known red tide species in Japanese coastal waters (Toriumi, 1980; Ueda *et al.*, 1998). The latter authors added that the species bloomed in a small semi-closed eutrophic inlet in November and December 1997. Very little is known about its distribution.

MATERIAL AND METHODS

The Eastern Harbour of Alexandria is a shallow, semi-closed basin located in the central part of Alexandria City. It has an area of about 2.53 km^2, average depth of 5 m and a total water volume of about 15.2x106 m^3. The harbour is directly affected by drainage water, and occasionally from Kayet Bey outlet (the main sewer of Alexandria) located nearby to the West and Mex Bay, about 10 km West.

Sampling was carried out twice a week, but daily during red tide outbreak periods, at a 4 m depth fixed station (figure 1) in the harbour from August 1998 to August 1999, and at intermittent periods from May to early November 2000.

The routine work included the measurement of water temperature, salinity (using a calibrated salinity refractometer, S/Mill) and surface dissolved inorganic nutrients (nitrate, silicate and phosphate, following Strickland, Parsons, 1972). Temperature and salinity were also measured over the bottom. The fresh phytoplankton samples were first examined for identification under a research microscope, then preserved by the addition of neutral formalin (4%) and a few drops of Lugol's acid solution and counted (Utermöhl, 1958).

RESULTS AND DISCUSSION

Fish killers

During the present study, *Chattonella antiqua* and *Gymnodinium mikimotoi* shared visible phytoplankton blooms with other causative species. Fishermen's reports and personal observation revealed discoloured water to have entered into the har-

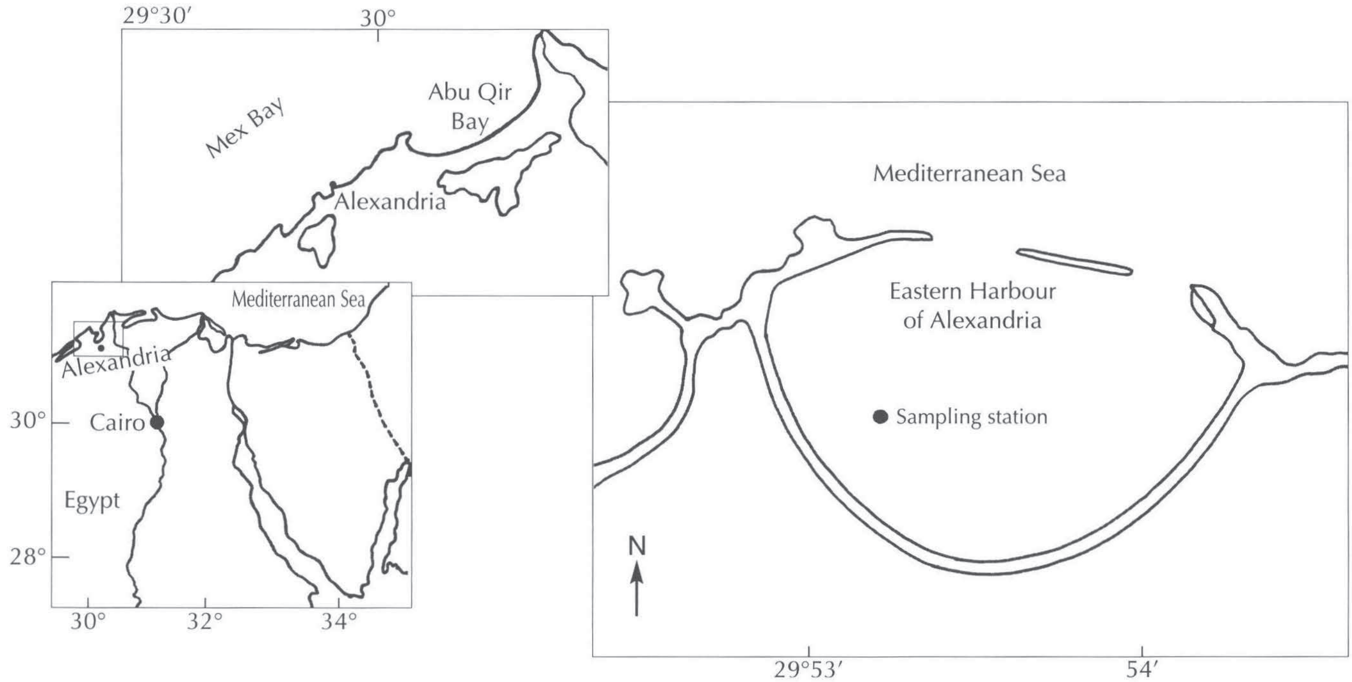

Figure 1 - The Eastern Harbour of Alexandria and location of the sampling station. / *Le Port Est d'Alexandrie et localisation de la station d'étude.*

bour from offshore. In all cases, the bloom was first observed west from Alexandria in Mex Bay about two days earlier, and probably transferred to the harbour by hydrographic processes (Abdallah, 1979). According to White (1987) blooms of *G. mikimotoi* usually develop offshore and are transported inshore thereby affecting fish farms.

Chattonella antiqua and *Gymnodinium mikimotoi* exhibited 5 peaks each, their frequency and density increasing during 2000, with longer duration periods. The major blooms are shown in figures 2 and 3 and table I.

Although the initiation of *C. antiqua* and *G. mikimotoi* blooms have been certainly triggered from another site, out of the harbour, the development and maintenance of their peaks in the harbour occurred under almost similar physico-chemical conditions within massive red tide blooms. *C. antiqua* maintained a bloom at a temperature range of 21-26°C, but more frequently between 24.5-26°C, a salinity range of 35-38.5 PSU, mostly 35-35.5 PSU. These levels were very close to those measured for *G. mikimotoi* (21.0-27.2°C, 34-36.5 PSU). In all cases, temperature above the bottom ranged between 19-25.5°C and salinity between 36-39 PSU. Yamaguchi *et al.* (1991) found that the suitable temperature for *C. antiqua* was 24.4-30.6°C. Kimura *et al.* (1999) reported that *G. mikimotoi* bloomed at a surface temperature of 22-26°C and preferred low salinity (28-32 PSU). These results together with those of Yamaguchi and Honjo (1989), Itoh *et al.* (1990) and Yamaguchi (1994) are in good agreement with the present study. Honjo (1987) stated that rainfall and salinity decrease account partly for the development of *G. mikimotoi* blooms but are by no means the only environmental factors responsible particularly in eutrophic semi-closed areas.

Generally, the nutrient concentrations during this study were relatively low, possibly affected by the presence of dense phytoplankton blooms and phosphate became reduced on peak days. Chang and Carpenter (1985) suggested that high nitrogenous nutrient availability is a prerequisite for blooms of *G. aureolum*, in accordance with other dinoflagellate species (Paerl *et al.*, 1990). This conclusion does not apply to the present observations.

The presence of *G. mikimotoi* in small numbers in mid-November and December 1998 (13.4×10^3 and 2.9×10^3 cell.L^{-1}, respectively) at low temperature of 18 and 15.4°C, under homothermal and homohaline conditions and at high nitrate and phosphate (ca, 3 µM for each, respectively on 12 December), appears to support the conclusion of Kimura *et al.* (1999), that populations of this species adapt well to turbulence where nutrient concentrations are relatively high. Several authors (e.g. Baba *et al.*, 1994) reported the overwintering motile cells of *G. mikimotoi*, which are likely to act as seeds for bloom formation in summer (Honjo *et al.*, 1991). This supposition is supported by the life cycle pattern of this species, since overwintering hypno-cysts of *G. mikimotoi* have not been found, although sexual reproduction has been demonstrated (Takayama, 1995).

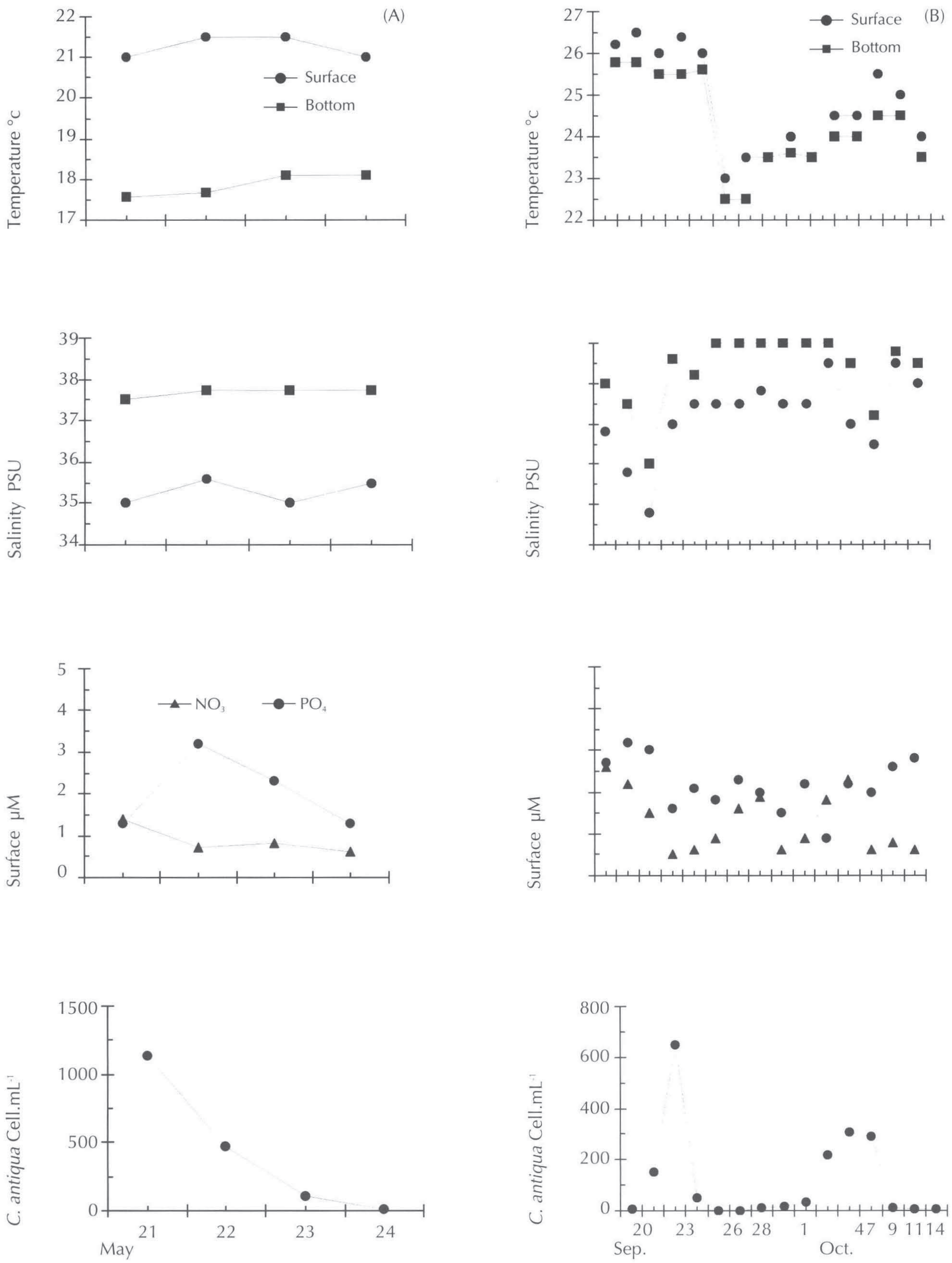

Figure 2 - Physicochemical parameters and density of *Chattonella antiqua* during May (A) and September-October 2000 (B). / *Paramètres physicochimiques et densité de* Chattonella antiqua *en mai (A) et septembre-octobre 2000 (B).*

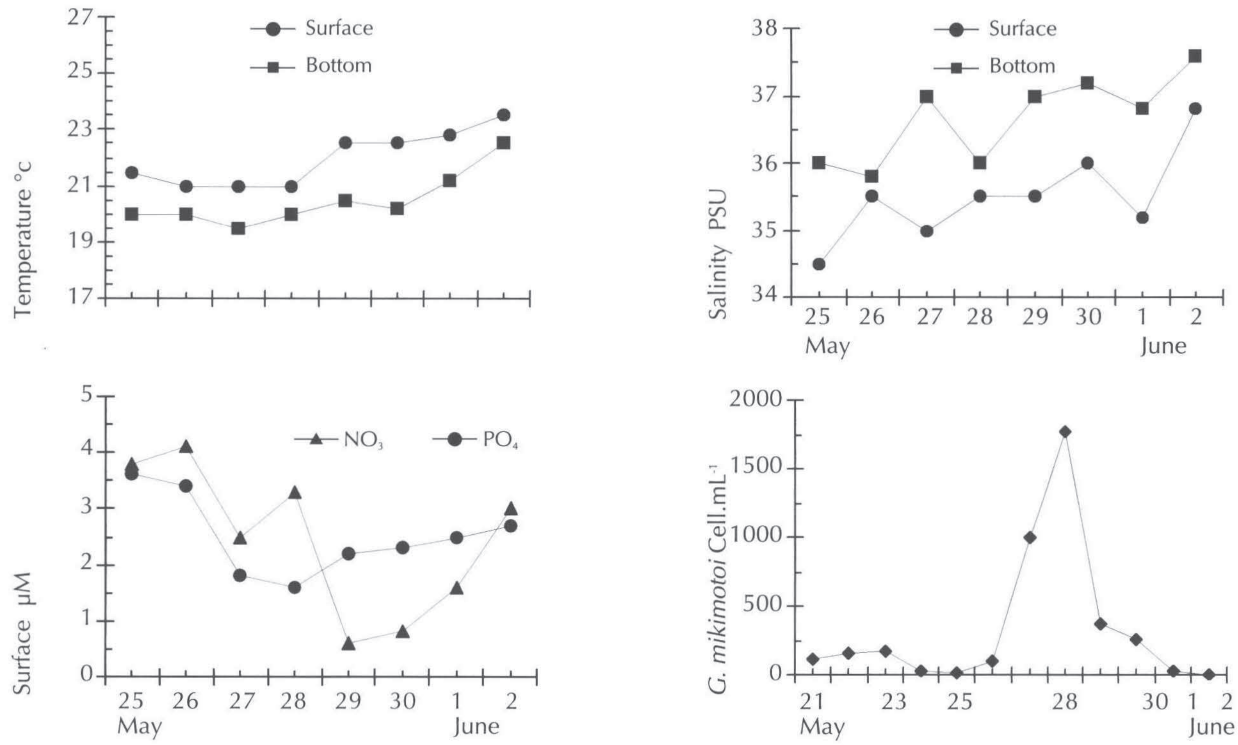

Figure 3 - Physicochemical parameters and density of *Gymnodinium mikimotoi* during May-June 2000. / *Paramètres physicochimiques et densité de* Gymnodinium mikimotoi *en mai-juin 2000.*

The peaks of *Chattonella antiqua* and *Gymnodinium mikimotoi* occurred together with diatom blooms, mainly of *Skeletonema costatum.* This later species is a common red tide form in the harbour during summer and early autumn, culminating at $27.3x10^6$ to $33x10^6$ $cell.L^{-1}$, respectively on 12 July and 17 October 1991 (Labib, 1994 a, b). It is reported in a culture experiment of species interaction that the luxuriant growth of *S. costatum* stimulated the growth of most tested red tide flagellates (Iwasaki, 1979) and this species may produce stimulating substances, that may help to explain the red tide occurrence.

Both *Chattonella antiqua* and *Gymnodinium mikimotoi* proved to be harmful on three occasions, 27 July 1999 and 21 May 2000 for the former and 30 May 2000 for the latter at maximum density given in Table I. The quick collapse of their peaks within a couple of days, despite the favourable physico-chemical conditions prevailing, caused limited fish and invertebrate mortality. Fish were stunned, swimming abnormally, crabs were seen migrating towards the beach at dawn or were found dead in fishing nets indicating signs of toxicity. These symptoms started to disappear when the cells began to change shape and lose motility. According to White (1987) a population of $0.1x10^6$ $cell.L^{-1}$ of *C. antiqua* can cause yellowtail death, which is known to be very sensitive to this species. Khan *et al.* (1996) stated toxicity of *C. antiqua* is hard to detect until the cell density reaches approximately $1.95x10^6$ $cell.L^{-1}$. The authors added that at low density fish did not die, but showed abnormal movements for 30-45 minutes, recovering gradually and swimming normally within a few hours. The same authors showed that morphology, motility and toxicity of cultured *C. antiqua* are influenced mainly by cell age: cells are motile and spindlelike, highly toxic during early to mid-logarithmic growth; oval-or spherical-shaped, non motile and weakly toxic during the stationary phase. Furuki, Kitamura (1981) pointed out that the cells became globular under phosphate deficiency and/or high ammonia concentration. The spindlelike, motile cells and the non-motile, oval to globular sometimes stretching to form pseudopodium like projections, were recognised during the present study. Imai and Itoh (1988) pointed out that *C. antiqua* overwinters in the bottom sediments as dormant cells, which vegetate and form active flagellate cells in early summer. *Gymnodinium mikimotoi* was reported to produce hemolysins, causing scallop mortality in French waters (Sournia *et al.*, 1990), as well as in Mediterranean lagoons (Romdhane *et al.*, 1998).

Potential PSP producers

Alexandrium catenella had irregular occurrence in the harbour (chains of 4-8 cells) with higher numbers on 26 October 1998, 10 August 1999 and 23 May 2000 (9.5, 9 and $10.7x10^3$ $cell.L^{-1}$, respecti-

Table I - *Chattonella antiqua* and *Gymnodinium mikimotoi* bloom characteristics from 1998 to 2000. / *Caractéristiques des efflorescences de* Chattonella antiqua *et* Gymnodinium mikimotoi *de 1998 à 2000.*

Species	Period	Peak day	Max.density cell.L^{-1} x10^6	Major species
Chattonella antiqua	29 Sept.-1st Oct. 1998	30 Sept.	0.54 (7.3%)	*Pyramimonas* sp. (5.35x10^6, 73.1%) *Cyclotella nana* (0.97x10^6, 13.3%)
	27 July, 1999	27 July	0.85 (28%)	*Skeletonema costatum* (1.5x10^6,49.5%) *Cyclotella nana* (0.39x10^6, 12.8%)
	21-24 May, 2000	21 May	1.14 (26.6%)	*S. costatum* (1.37x10^6, 32%)
	20-25 Sept.	24 Sept.	0.65 (5%)	*S. costatum* (8.43x10^6, 64.2%)
	1st-14 Oct.	7th Oct.	0.31 (5.5%)	*S. costatum* (2.94x10^6, 52.4%)
Gymnodinium mikimotoi	7-21 Sept. 1998	14 Sept.	0.5 (12.7%)	*Leptocylindrus minimum* (1.5x10^6, 38.4%) *C. nana* (0.79x10^6, 20.4%) *Nitzschia longissima* (0.5x10^6, 12.7%) *S. costatum* (0.46x10^6, 11.8%)
	10-17 Oct.	15 Oct.	0.13 (9%)	*C. nana* (0.49x10^6, 34.8%)
	10-12 Aug. 1999	11 Aug.	0.93 (14.7%)	*N. longissima* (3.7x10^6, 68%)
	21 May-11 June 2000	30 May	1.8 (29.6 %)	*Thalassionema nitzschioides* (3.87x10^6, 64.8%)
	26 Sept.-18 Oct.	9 Oct.	0.42 (10.8%)	*L. minimum* (2.24x10^6, 57.5%)

vely) at a temperature range of 19.3-28.5°C, a salinity of 35-37.8 PSU, and low nutrients, nitrate 0.5-1.2µM, and phosphate 1.8-2.5 µM. Its higher densities were associated with red tide blooms of different causative species, *Pyramimonas, Micromonas* spp., *Skeletonema costatum* and *Thalassionema nitzschioides*. Lechuga-Deveze and Morquecho-Escamilla (1998) showed maximum cell densities of *A. catenella* at 20-23°C in the Gulf of California, which seems coincidental with the present record. Takeuchi *et al.* (1990) pointed out that *A. catenella* forms red tides in Tanabe Bay, Japan, at surface temperature of 19.5-20°C, salinity of 33.9-34 PSU, nitrate and phosphate comparatively lower than the present records. Iwasaki (1979) found that motile cells of two strains of toxic *Gonyaulax catenella* (= *A. catenella*) appeared in winter at 12-14°C in Osaka Bay, which also confirms its existence in the Eastern Harbour at very low temperature of 13.5°C (30 cell.L^{-1} in late December). The present study has confirmed no cases of toxicity for *A. catenella* observed, probably due to its relative lower contribution.

Alexandrium ostenfeldii also represented a very minor component of the community in the present study, but its cell density was relatively higher than that of *A. catenella*. The species appeared in two periods, the first from16 September to 17 October 1998 (maximum density of 14.4-18.2x10^3 cell.L^{-1} between 27-29 September), the second from 5-17 September 2000 (14.3x10^3-36.5x10^3 cell.L^{-1} on 11-16 September). The highest density on 13 September occurred at 26.2°C, 36.5 PSU with a well stabilised water column. Nitrate and phosphate reached ca., 2.2µM for each. This peak accompanied a diatom red tide bloom period, *Rhizosolenia delicatula, Thalassiosira* sp. and *S. costatum* were the major species. Balech and Tangen (1985) who found this species in late winter and early spring and occasionally in August concluded that its occurrence is not restricted to low water temperature. Moestrup and Hansen (1988) linked its occurrence with low salinity. Although it produces toxins in some cases (Hansen *et al.*, 1992), no fish mortality accompanied its low occurrence in the Eastern Harbour. This species has been observed previously in Mex Bay by Mikhail (1997).

Red tide species

Prorocentrum sigmoides was irregularly recorded from October first 1998 till 30 May 1999, and from 18 September to 6 November 2000, with increased abundance on 10 and 15 October 1998 (12.2x10^3-13.4x10^3 cell.L^{-1}, respectively) and two relatively high numbers on 6 May 1999 (76.1x10^3 cell.L^{-1}) and 5 November 2000 (53.1x10^3 cell.L^{-1}). The accompanying conditions indicated a temperature range of 20-23.5°C, low salinity (34-37.2 PSU) and stratified water column, with relatively high nutrients. Ueda *et al.* (1998) pointed out that this species formed a red tide with maximum densities between 1.56x10^6 and 3.46x10^6 cell.L^{-1} at a temperature range of 15-30°C.

It is necessary to examine the ecology of these organisms, particularly in their original sites and in the initial phase of population growth and the relation with the physical mechanisms of dispersion and diffusion within and to these embayments. If the site where red tide initiation occurs can be determined, monitoring of phytoplankton species at this site might allow prediction of red tide outbreaks.

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