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Diel vertical migration and toxicity of *Alexandrium minutum* Halim red tide, in Alexandria, Egypt

Migration nycthémérale et toxicité d'une eau rouge à Alexandrium minutum *Halim, à Alexandrie, Égypte*

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Mots clés : Alexandrium minutum, *eau rouge, migration, mortalité, côte égyptienne*

ABSTRACT

Labib W., Y. Halim, 1995 - Diel vertical migration and toxicity of Alexandrium minutum *Halim red tide, in Alexandria, Egypt.* Mar. Life, **5** (1) : 11 - 17.

The dinoflagellate Alexandrium minutum *Halim was the causative organism of a heavy bloom, causing water discoloration in the neritic waters of Alexandria (Egypt) during October 1994. The bloom originated in the Eastern Harbour and then extended outside, along the coastline for about 20 km, in both east and west directions. The right combination of high nutrient load and density-stratified water column, induced by the input of drainage waters, triggered the bloom,* A. minutum *achieving a maximum population density of more than 24 x 10^6 cell l^{-1}. The species demonstrated a distinct diel vertical migration crossing the pycnocline with temperature and salinity gradients of 1.9°C and 1.3, respectively. The vertical migration was closely correlated to the light-dark cycle, but is probably less closely linked to the vertical gradient of nutrients. The species appears to be able to assimilate ammonia in addition to other nitrogen sources. The bloom was accompanied by massive mortality of different fish and invertebrate species in the coastal waters as well as in the aquaria of the National Institute of Oceanography and Fisheries, Alexandria (Egypt). The continous aeration of the aquaria excludes anoxicity as a possible cause of mortality.*

RÉSUMÉ

Labib W., Y. Halim, 1995 - [Migration nycthémérale et toxicité d'une eau rouge à Alexandrium minutum *Halim, à Alexandrie, Égypte].* Mar. Life, **5** (1) : 11 - 17.

En octobre 1994, le Dinoflagellé Alexandrium minutum *Halim a provoqué la décoloration des eaux côtières par sa prolifération anormale. Cette prolifération massive a commencé dans le port est, et s'est étendue en dehors, le long de la côte, sur environ 20 km à l'est, et à l'ouest. La charge élevée en sels nutritifs et la stabilité de la colonne d'eau, liées au déversement des eaux de drainage, semblent avoir déclenché la prolifération d'* A. minutum *atteignant un maximum de densité de plus de 24 x 10^6 cell l^{-1}. La migration nycthémérale d'* Alexandrium minutum *à travers la pycnocline et un gradient de température et de salinité de 1,9°C et 1,3 a été observée respectivement entre 0 m et 4,5 m. Contrairement à d'autres espèces de Dinoflagellés, l'accumulation en surface de la population d'* A. minutum *n'est pas affectée par l'intensité maximale de l'irradiation solaire à midi. La migration verticale était en corrélation très nette avec le cycle diurne, mais ne semble pas corrélée à la répartition verticale des sels nutritifs. L'espèce semble capable d'assimiler l'azote ammoniacal entre autres sources d'azote. Cette prolifération s'est accompagnée de la mortalité massive de plusieurs espèces de poissons et d'invertébrés dans les eaux côtières, aussi bien que dans les bassins d'élevage de l'Institut national d'océanographie et de pêches, à Alexandrie (Egypte). L'aération continue des bassins exclut l'anoxicité comme cause possible de la mortalité.*

INTRODUCTION

Alexandrium minutum was described as a new species and new genus from materials collected in the Eastern Harbour of Alexandria in 1958 (Halim 1960 a). Subsequent researchers placed the species under the genera *Pyrodinium, Gessnerium* or *Protogonyaulax* (Taylor 1979, Balech 1985, Sournia 1986). The identity of *Protogonyaulax* and *Alexandrium* was proved beyond doubt by Balech (1990), *Alexandrium* Halim having priority. According to Sournia (1990), Halim's description is satisfactory.

Alexandrium minutum Halim is a toxic dinoflagellate responsible for producing paralytic shellfish poisoning. Its occurrence and intensity, on a worldwide scale seem to be on the rise (Oshima *et al.*, 1989, Erard-Le Denn 1991). Blooms of *A. minutum* are reported from many localities to be associated with bivalve and fish mortality (Koray 1992), but this has not been documented yet in Alexandrian waters.

The coastal area of Alexandria is subjected to daily discharge of unprocessed sewage and waste wasters, which render the water highly eutrophic.

The present study is based on daily observations on the conditions accompanying the massive occurrence of *A. minutum* in a eutrophicated small bay. The role of its diel vertical migration in producing red tide was also monitored for the first time in Alexandrian waters.

MATERIAL AND METHODS

Daily sampling collections were conducted at a fixed station (5 m depth) in the middle of the bloom area, in the Eastern Harbour of Alexandria (Figure 1), a relatively shallow semi-closed basin, during the period from 30 September-12 October 1994. Temperature, salinity, dissolved oxygen and inorganic nutrient concentrations were measured in the surface water and at 4.5 m, over the bottom. Stability of the water column was calculated using temperature and salinity data (Williams 1962). Salinity was measured by salinity refractometer (S/Mill), oxygen by Winkler method and nutrient concentrations according to the method of Strickland and Parsons (1972).

Quantitative surface samples for the study of the standing crop were first examined under a research microscope, then preserved by addition of Lugol' acid solution and counted (Utermöhl 1958). Chlorophyll *a* was measured by spectrophotometer (Strickland, Parsons 1972). The diel vertical migration pattern of *Alexandrium minutum* was monitored at intervals during a 24 h cycle, at the same sampling station. Samples were collected at surface, 0.5, 1, 2, 3, 4 and 4.5 m. The vertical profiles of water temperature, salinity, nitrate, phosphate, ammonia, *A. minutum* density and chlorophyll *a* content were determined at the same depths.

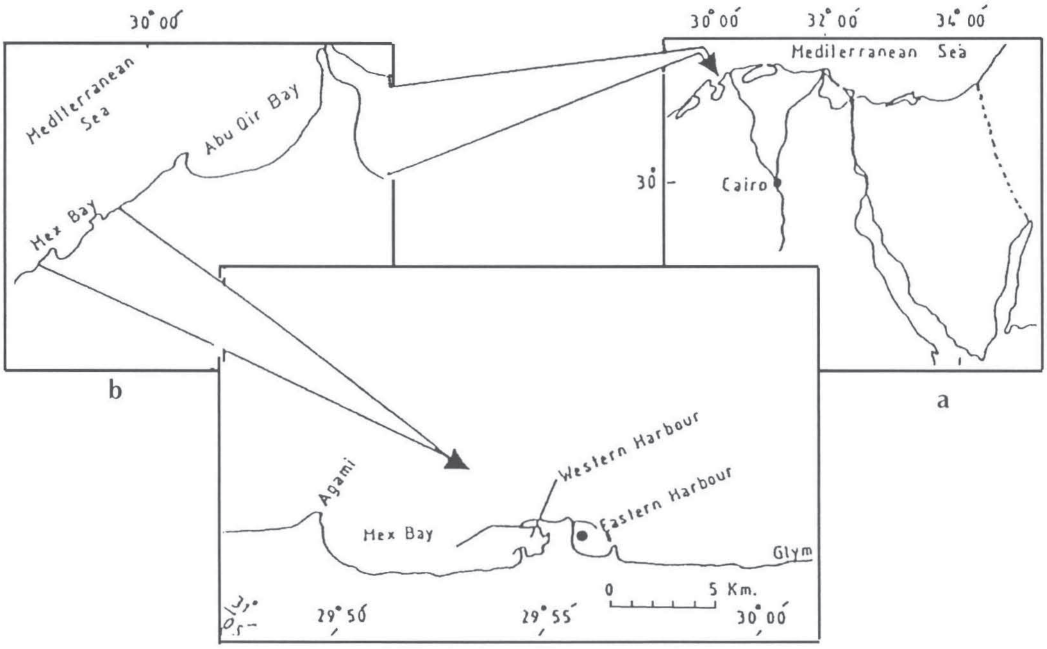

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Figure 1 - a) Egyptian Mediterranean coastline. b) Alexandria coastline. c) Area affected by the bloom. Location of the sampling station.
a) Côtes méditerranéennes d'Egypte. b) Côte d'Alexandrie. c) Zone affectée par la marée rouge. Position de la station étudiée.

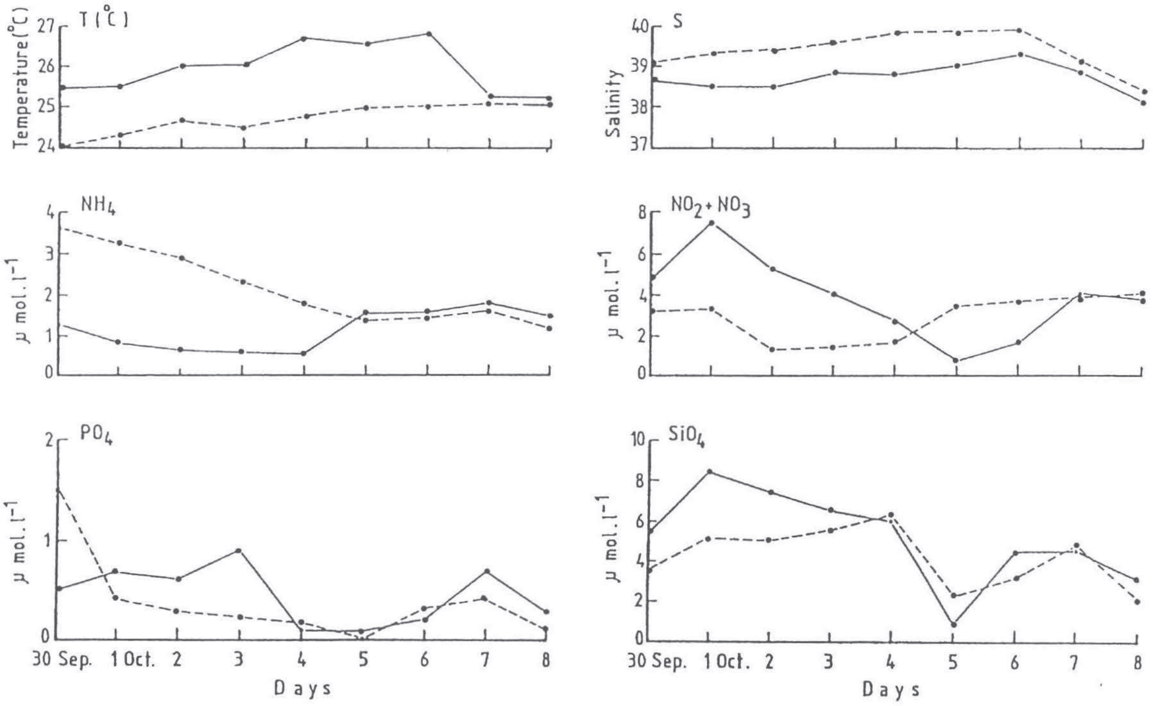

Figure 2 - Surface (.—.) and 4.5 m (.....) water température, salinity and nutrient concentrations from 30 September to 8 October 1994/ *Température en surface (.—.) à 4,5 m (.....), salinité, concentration des sels nutritifs, du 30 septembre au 8 octobre 1994.*

RESULTS

By mid-day 30 September 1994, yellow-brown water was observed to occupy most of the Eastern Harbour. The color became reddish by the next day and then extended outside, along the coastline for about 20 km, in both east and west directions. It persisted until 6 October. Water discoloration was found to be due to the massive outbreak of a heavy *Alexandria minutum* bloom (91.63 % to 98.98 % of the total phytoplankton community). At the start, on 30 September the density was 1.86 x 10^6 cell l^{-1}, chlorophyll *a* 10.8 µg l^{-1}, oxygen 7.6 ml l^{-1}. Surface temperature was at 25.5°C, salinity at 38.6 and the water column was highly stratified (Stability, 18.6). Temperature and salinity gradients between surface and 4.5 m depth were 1.5°C and 0.5, respectively, and density by 0.84 σ *t*. Surface nutrient levels were 1.3, 0.85, 4.1, 0.45 and 5.45 µmol.l^{-1} for ammonia, nitrite, nitrate, phosphate and silicate, respectively. The levels at 4.5 m were several times greater for ammonia (x 2.8), nitrite (x 1.4) and phosphate (x 3.3). Nitrate and silicate showed a reverse trend, decreasing with depth.

Alexandrium minutum exhibited its major peak on 2 October (24.4 x 10^6 cell l^{-1}, chlorophyll *a* 108.4 µg l^{-1}, oxygen 18.3 ml l^{-1}). This was followed by a decrease next day to 10.53 x 10^6 cell l^{-1} (chlorophyll *a* 48.9 µg l^{-1}, oxygen 15.8 ml l^{-1}). The density until 6 October fluctuated between 4.8 x 10^6 and 6.57 x 10^6 cell l^{-1}. The associated physicochemical condition during the bloom showed surface temperature to range between 25.5 - 26.8°C and salinity between 38.6 - 39.3. Water stability was maximal on 4 October (vertical density gradient 1.3 σ *t*, stability, 46.96). Surface nitrate, in spite of the progress of the bloom, increased considerably to 7.3 and 5.1 µmol. l^{-1} on 1 and 2 October, respectively. Phosphate showed the same trend (0.93 µmol. l^{-1} on 3 October). However, ammonia and nitrite decreased as days went by (Figures 2 and 3).

The phytoplankton community during the bloom period comprised a few numerically insignificant species. *Lithodesmium undulatum,* the most abundant contributed 0.112 x 10^6 to 0.223 x 10^6 cell l^{-1} between 1 and 4 October.

The dissipation of the bloom on 7 October, under strong wind stress, was dramatic, *A. minutum* falling to 1.1 x 10^6 cell l^{-1}, and two days later the discoloration was no longer visible (0.62 x 10^6 cell l^{-1}). This was followed immediately by flowering of the pennate diatom, *Nitzschia longissima,* becoming overwhelmingly dominant on 12 October (1.16 x 10^6 cell l^{-1}, 56.8 % of the crop), *Thalassiosira subtilis* (0.26 x 10^6 cell l^{-1}, 13.54 %) and *Euglena gracilis* (0.2 x 10^6 cell l^{-1}, 10.37 %) contributed in smaller numbers to the crop.

Diel vertical migration

Alexandrium minutum demonstrated a distinct diel migration pattern during the bloom. The results (Figure 4) showed : a- the species had the ability to cross a pycnocline with temperature and

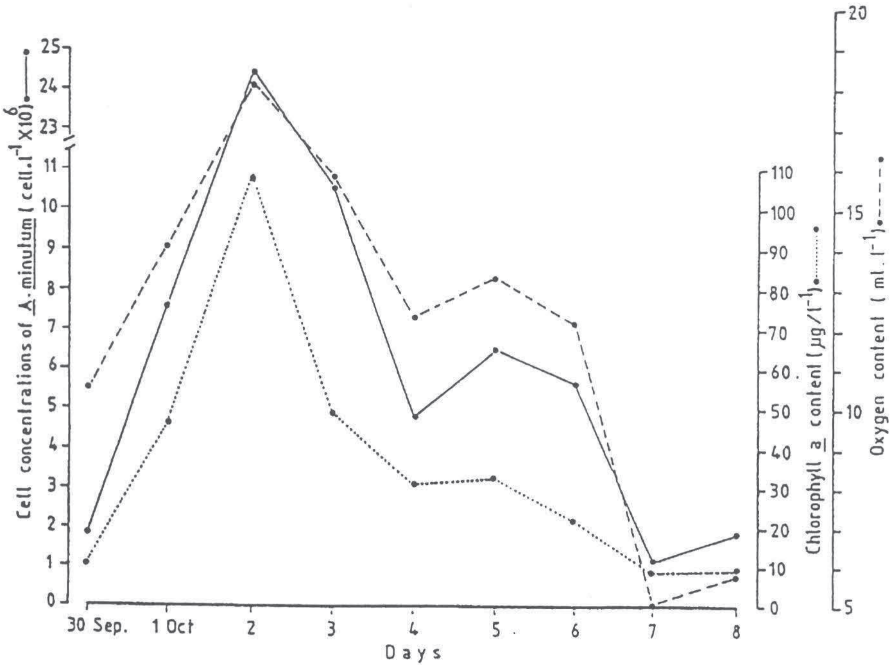

Figure 3 - *Alexandrium minutum* (.—.), chlorophyll *a* (.....) and dissolved oxygen (.----.), from 30 September to 8 October 1994/*Abondance d'*Alexandrium minutum (.—.) *chlorophylle* a (.....), *oxygène dissous* (.----.), *du 30 septembre au 8 octobre 1994.*

salinity gradients of 1.9°C and 1.3 %, respectively ; b- cells did not avoid the top meter at time of maximum irradiance (mid-day) ; c- massive downward shifting began prior to sunset, leading to homogeneous vertical distribution at night, within 1.4 x 10^6 to 2.6 x 10^6 cell l^{-1}, with a maximum density at 4 m ; d- cells started their ascent before sunrise, massive accumulation was observed at dawn between 0.5 m and 2 m ; e- the stable stratified water column did not always lead to nutrient surface depletion and higher concentrations at depth. The vertical distribution of nutrients was highly variable, bottom levels at night were significantly higher than the surface concentrations. Nitrate reached 3.32 µmol. l^{-1}(6 times greater), nitrite 2.5 µmol. l^{-1}(2.5 times) and ammonia 0.41 µmol. l^{-1}(5.8 times). Nitrate and ammonia were depleted at night at 4 m, coinciding with the maximum accumulation of *A. minutum.*

Chlorophyll *a,* data as expected, reflect almost exactly the diel trend of the vertical migration. The vertical movements of the population peak with time correspond to an approximate swimming speed of about 0.5 m h^{-1}.

DISCUSSION

Since the 1970s, eutrophication in the neritic waters of Alexandria has gradually intensified as a result of the increased input of waste waters. The progressive eutrophication was associated with annual red tide outbreak and other noxious blooms in the warm season. The most common species are *Alexandrium minutum, Prorocentrum triestinum, Prorocentrum minimum* and *skeletonema costatum* (Labib 1994). The major red tide organism, *A. minutum* has shown showed a regular occurrence during spring and summer of the last decades (Halim 1960 b, Sultan 1975, Zaghloul, Halim 1992, Labib 1994).

Alexandrium minutum blooms in temperate waters were reported to have taken place at 18-20°C (Koray 1992). In the present case, it achieved its bloom at a higher temperature (25.5-26.8°C).

The relatively high nutrient concentrations and the stratified water column, direct results of the input of waste waters, seem to be the right combination that allowed *A. minutum* to develop into a red tide. However, the stability of the water column appears to be more significant than the levels of nutrients. The dissipation of the bloom under severe reduction in stability forced by strong wind stress, confirms the more significant role played by stabilization rather than abundance of nutrients, supporting the results of Margalef *et al.,* (1979), Levasseur *et al.,* (1984), and the earlier studies carried out in the neritic waters of Alexandria (Labib 1992, 1994).

Diel vertical migration is a complicated phenomenon, and although both phototaxis (Forward 1976) and geotaxis (Yamochi, Abe 1984) have been proposed to explain the directionality of swimming behavior of dinoflagellate species, the different responses of different species to regulating parameters have hindered prediction and modeling efforts. Endogenous or circadian rhythm was also suggested by Kohata and Watanabe (1986) as a driving factor in vertical migration behavior of microorganisms.

The timing and rate of movement of *A. minutum* in the present study were similar to that reported for *Gonyaulax tamarensis* (Anderson and Stolzenbach 1985), and other dinoflagellate species (Heaney, Eppley 1981).

The population showed no avoidance of the maximum irradiance level (mid-day) when nutrients were in excess, similar to *Ceratium furca, Prorocentrum triestinum* and *Heterocapsa triquetra* (Cullen, Horrigan 1981, Anderson, Stolzenbach 1985, Labib 1990), but in contrast to *Gonyaulax tamarensis* (Anderson, Stolzenbach 1985) and *Ceratium hirundinella* (Heaney, Furnass 1980), which all avoided high irradiance under limiting nutrient conditions.

A large proportion of the *A. minutum* population had the ability to cross steep temperature and salinity gradients. It was reported that the flagellate *Heterosigma akashiwo* crosses a pycnocline with temperature and salinity gradients of 6.5°C and 5.7 respectively (Yamochi, Abe 1984) and the dinoflagellate *Cachonina niei* crosses a temperature gradient of 6°C (Kamykowski, Zentara 1977). Other

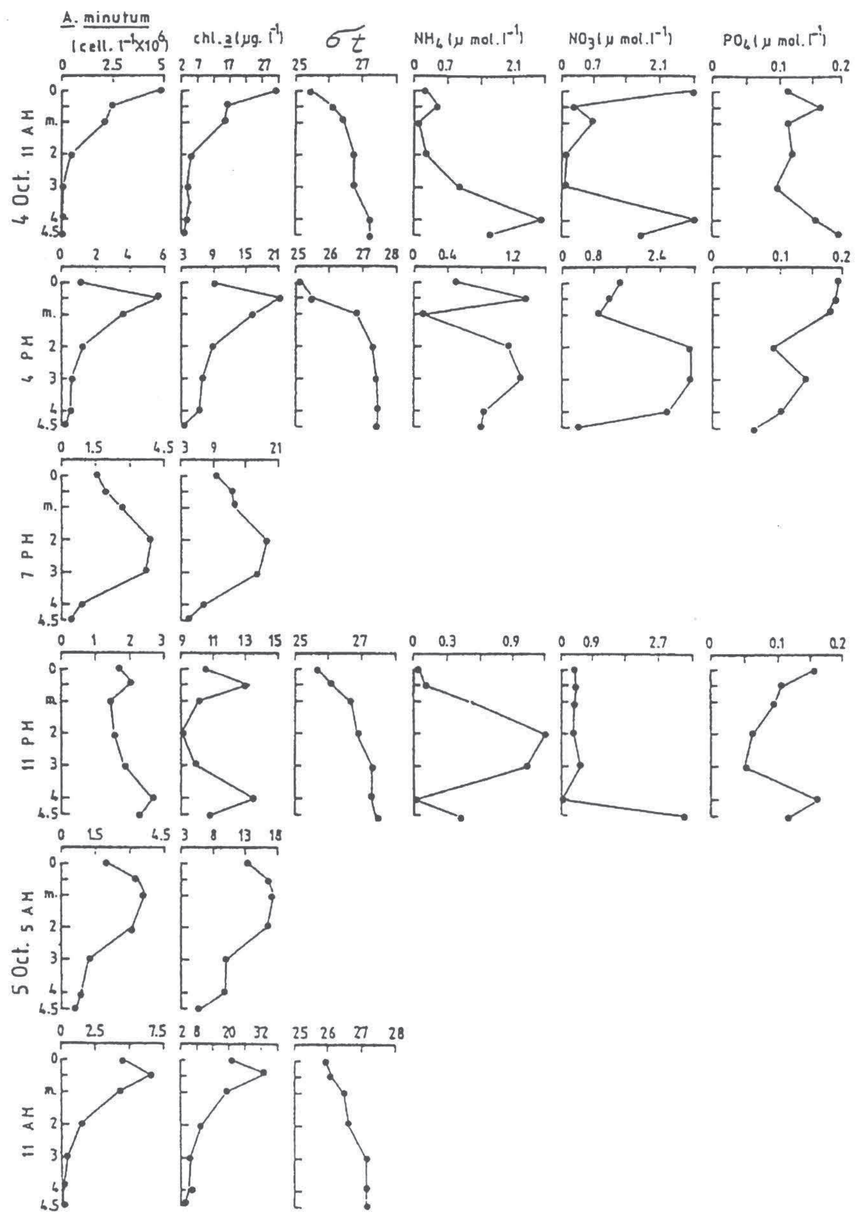

Figure 4 - Vertical profils of water temperature, salinity, σ t, nutrient concentrations, density of *Alexandrium minutum* and chlorophyll a content during a 24 h cycle on 4-5 October 1994.
*Profils verticaux : température, salinité, σ t, concentration en sels nutritifs, abondance d'*Alexandrium minutum *et chlorophylle* a *au cours d'un cycle de 24 heures (4 et 5 octobre 1994).*

researchers (George, Heaney 1978, Edler, Olsson 1985) have documented cessation of dinoflagellate vertical movements at steep temperature and salinity gradients.

Water column stratification did not always lead to nutrient depleted surface water and higher concentration at depth. The present migration therefore is probably not linked to nutrient uptake at depth since surface concentrations were not limiting. The complete consumption of nitrate and ammonia near the bottom at night might suggest the ability of *A. Minutum* to assimilate nutrients at night and migrate to surface at day-light to carry out photosynthesis. The results also showed the ability of *A. minutum* to utilize ammonia.

The vertical movement pattern of *A. minutum* was closely correlated with light-dark cycle, indicating that the species is phototactic.

The upward movement and surface accumulation were important factors for producing red tide.

In spite of the recurrence of *Alexandrium minutum* red tide, its toxicity in the waters of Alexandria has not been documented as yet, and apparently no cases of toxicity have accompanied its earlier bloom (Halim 1960 b), as also reported from elsewhere (Fraga 1988, Montresor *et al.*, 1990). However, occasional fish kills occurred in the Eastern Harbour during May 1987 when the species density exceeded 6 x 10^6 cell l^{-1} (Zaghloul, Halim 1992) and were attributed to localized oxygen stress and/or gill clogging. Unlike these earlier studies, our present observations give evidence of toxic events : a- massive fish and invertebrate mortality occurred throughout affected areas ; b- fish kills inside the aquaria of the National Institute of Oceanographies and Fisheries, receiving their water supply from the Eastern Harbour and where aerators are present ; c- dead fish exhibited symptoms of toxicity (yellowish coloration of the body and gills). At the same time symptoms of anoxia were also observed in the harbour : a- stunned demersal and pelagic fishes had lost their equilibrium, swimming either on their sides or upside down, gasping at the surface ; b- migration of hundreds of small crabs onto the sand beach of the Harbour at dawn, when oxygen deficiency occurred.

The regular occurrence of *A. minutum* in the Eastern Harbour during the last decades necessarily means the presence of its benthic cysts and a heavy growth might be stimulated by favorable hydrological conditions. The life cycle of *A. minutum* during subsequent blooms deserves more attention in future.

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