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TEMPORAL DISTRIBUTION OF *ALEXANDRIUM* SPP. IN THE GULF OF TRIESTE (NORTHERN ADRIATIC)

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ABSTRACT

A monitoring program was carried out in the Gulf of Trieste in order to check the quality of shellfish and seawater in which blue mussels (Mytilus galloprovincialis) *are cultivated. The occurrence and temporal distribution of dinoflagellate* Alexandrium *spp., together with environmental conditions in inshore waters, were reported monthly to biweekly from 1994 to 1996. No blooms occurred, but in May 1994 4200 cells l^{-1} of* Alexandrium *species were found along the southeastern side of the Gulf, while on the northwestern side densities up to 4000 cells l^{-1} peaked in July 1995. An unknown species for the area, presumably related to* A. acatenella, *was observed for the first time with the scanning electron microscope.*

Key words: dinoflagellates, *Alexandrium* spp., environmental factors, PSP toxicity, Gulf of Trieste

INTRODUCTION

The regular occurrence of toxic dinoflagellates is well documented in the Northern Adriatic Sea (Boni, 1983; Honsell *et al.*, 1992; Mozetič & Obal, 1995). Scientific attention increased particularly since the first DSP (diarrhetic shellfish poisoning) intoxication in 1989 (Boni *et al.*, 1992; Sedmak & Fanuko, 1991) to which *Dinophysis* (Dinophyceae) species were connected as the potentially causative organisms. Since then, monitoring programs were set up to identify potentially toxic dinoflagellates and their temporal occurrence as well as toxicity of blue mussels on shellfish farms of the Gulf of Trieste (Northern Adriatic).

Up to now no cases of PSP (paralytic shellfish poisoning) human intoxication have been recorded from the Northern Adriatic, although PSP toxins have been detected in blue mussels from the Emilia Romagna (Italy) coast (Honsell *et al.*, 1996). Since the first observation of the genus *Alexandrium* on the western side of the Northern Adriatic in 1982 (Boni *et al.*, 1983), five species of the genus have been identified (Honsell *et al.*, 1992, 1996). However, there is still a lack of information regarding their temporal distribution and dynamic on a yearly basis.

The aim of this work was to study the seasonality of *Alexandrium* species in the Gulf of Trieste, together with the main environmental factors.

MATERIALS AND METHODS

The Gulf of Trieste (Fig. 1) is a shallow, semi-enclosed embayment with a maximum depth of ca. 25 m in its central part. It is characterized by large temperature variations (6-26°C in the surface layer and 6-20°C above the bottom) and, following the seasonal freshets, surface salinity oscillations (<30-38.5). Along the northwestern (Italian) coast there are large mussel farms, while on the southeastern (Slovenian) part there are three shellfish farms. Six stations near the shellfish farms were included in the monitoring program.

On the Italian side, the sampling for temperature, salinity and *Alexandrium* abundance was carried out monthly from July 1994 to December 1996 at stations

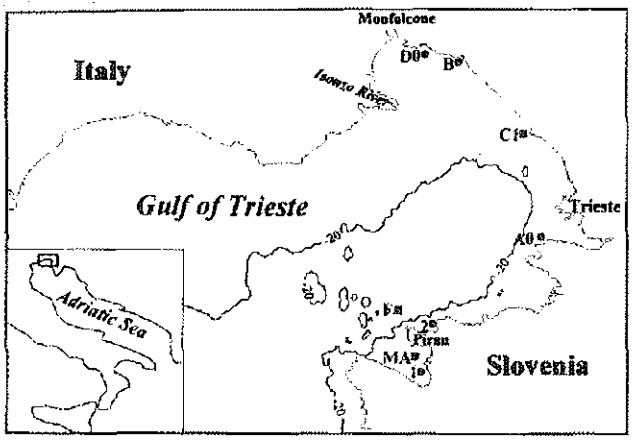

Fig. 1: Sampling locations in the Gulf of Trieste (Adriatic Sea): (●) stations close to the shellfish farms and (■) oceanographic stations.
Sl. 1: Vzorčevalna mesta v Tržaškem zalivu (Jadransko morje): (●) postaje v bližini školjčnih nasadov in (■) oceanografske postaje.

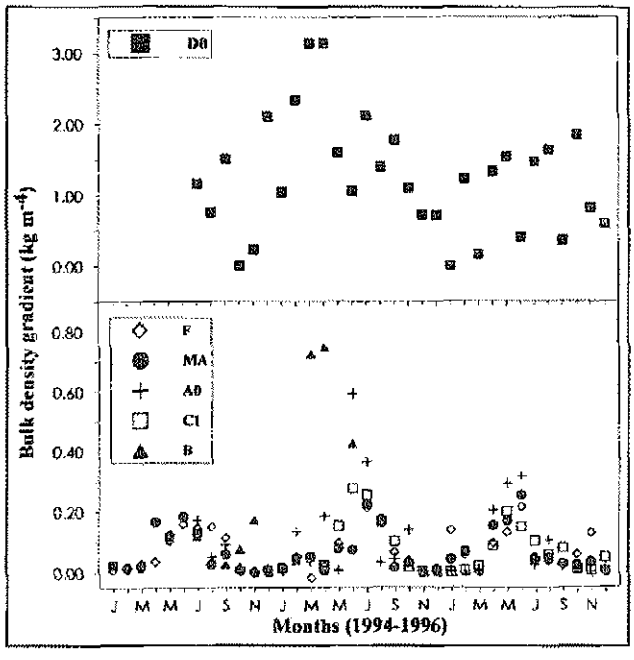

Fig. 2: "Bulk" density gradient at six stations in the period 1994-1996.
Sl. 2: Vertikalni gostotni gradient na šestih postajah v obdobju 1994-1996.

A0, D0 and B. Station B was from May 1995 to December 1996 replaced with station C1. Water samples (500 ml) for cell counts were collected at 0 m, 2 m, 5 m and above the bottom. Stations A0 and B are 10 m deep, while the depths of stations C1 and D0 are 17 and 5 m, respectively.

Samples for cell counts (800 ml) were collected biweekly at the subsurface (2.5 m) from April 1994 to November 1996 at stations 1 and 2 on the Slovenian side. From January 1994 to December 1996, temperature, salinity, phosphate and inorganic nitrogen (nitrate+nitrite+ammonium) concentrations were measured at two stations (F and MA) close to shellfish farms 1 and 2 (Fig. 1). Nutrients were measured at 0, 5, 10 m and above the bottom (21 and 16 m at stations F and MA, respectively), although, only mean concentrations from 0 and 5 m were used for this purpose.

Temperature and salinity profiles were recorded with a CTD probe, while nutrients were analyzed on unfiltered samples using standard colorimetric procedures (Grasshoff, 1976). Temperature and salinity data were used to calculate "bulk" density gradient (c) as follows

$$c = (\sigma_{Tb}-\sigma_{Ts})/H$$

where σ_{Ts} and σ_{Tb} are surface and bottom densities (kg m^{-3}), respectively, and H is water column depth (m).

Alexandrium species were identified in formaldehyde fixed subsamples and counted on the inverted microscope with a magnification of x200. Some samples with a high abundance of *Alexandrium* spp. were also prepared for scanning electron microscopy using a SEM Leica Cambridge Stereoscan 430i. Both microscopic methods, optical and electronic, are described in detail in Zingone *et al.* (1990).

RESULTS AND DISCUSSION

Hydrography and nutrients

During the investigated period the surface temperatures ranged from 7.3°C to 27.2°C. A decrease in surface salinity was observed in the periods of higher freshwater discharge, especially in late spring and autumn (from 27 to 34), while in other months higher salinity values were measured (>34 to 38). The exception was station D0, which is directly influenced by the Isonzo River, the largest freshwater source in the Gulf of Trieste. At this station extremely high salinity oscillations were observed in the surface layer (from 17 to 37). Stability of the water column is described with a "bulk" density gradient (Fig. 2). Generally, from April to October the water column was stratified ($c>0.05$), while during the other months it was mixed. Station D0 is shown separately on Figure 2, because the water column was most of the time density-stratified due to continuously diluted surface layer. In the period of thermohaline stratification, *Alexandrium* spp. was found in the seawater samples.

At the F and MA mean phosphate concentrations in the subsurface layer were most of the time below 0.10 or even 0.05 µmol l^{-1} (Fig. 3). The highest concentrations (from >0.20 up to 0.30 µmol l^{-1}) were measured in the summer periods of 1994, 1996, November 1994 and February 1996. Generally, mean phosphate concen-

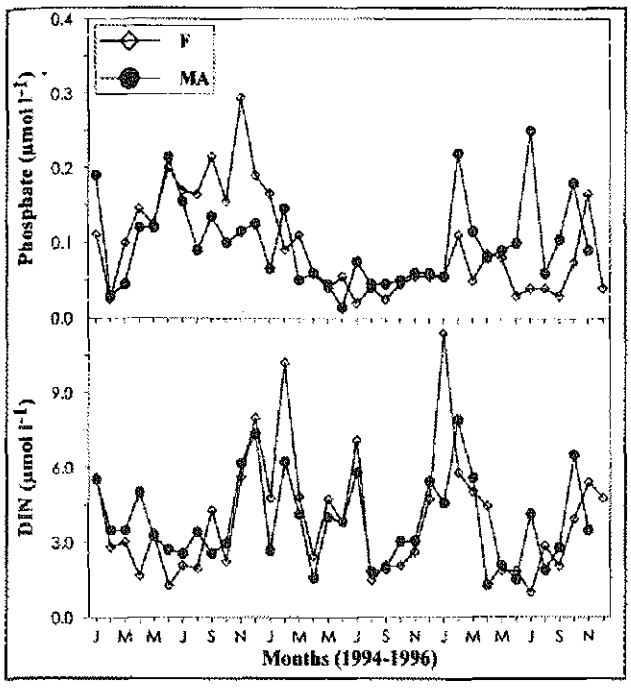

Fig. 3: Mean phosphate and DIN (dissolved inorganic nitrogen) concentrations at stations F and MA in the southeastern part of the Gulf (1994-1996).
Sl. 3: Povprečne koncentracije fosfata in raztopljenega anorganskega dušika (DIN) na postajah F in MA v jugovzhodnem delu zaliva (1994-1996).

trations increased from May to November in the years 1994 and 1996, whereas in 1995 the highest concentrations (>0.05 µmol l^{-1}) were measured in the first three months and July.

In the upper water column, nitrate was the predominant form of dissolved inorganic nitrogen. Highest mean concentrations (around 10 µmol l^{-1}) were found in the winter-spring and autumn periods (January-March, November), and only once in the summer period (July 1995) (Fig. 3). The peaks of inorganic nitrogen, especially nitrate, are connected to external nitrogen inputs from land during spring and autumn freshets of the Isonzo River, and occasionally with rain during the summer storms (Malej *et al.*, 1997). During late spring and summer, concentrations of dissolved nitrogen decreased and became the lowest in July 1996 (1.0 µmol l^{-1}). Besides low phosphate concentrations, calculated high N/P ratios (on average 58) reflect limited phosphate availability during almost the whole investigated period. Only during late spring-early summer in 1994 and 1996 the N/P was close to or below the Redfield ratio of 16.

Temporal and spatial dynamics of *Alexandrium* species

As the identification of *Alexandrium* species is very difficult with optical microscopy, we classified all the observed species as *Alexandrium* spp. *Alexandrium* species were present in the samples during almost each of the months of the 1994-1996 period, but their abundance increased from April to October each year (Fig. 4). In this period cell numbers ranged from undetectable to 4000 and 4200 cells l^{-1} in the northwestern and southeastern part, respectively. On both sides of the Gulf maximal densities were quite similar, but the months of peak abundances were different. The highest abundance was recorded in May-June in the southeastern part, while in the northwestern part abundance peaked later in the season (July-August). Following these peaks, abundance decreased significantly to increase slightly again in September and/or October at some stations. In the northwestern part cells counted at discrete depths were integrated in two layers: subsurface, low-salinity layer (from 0 to 5 m), and a deeper layer above the bottom. Generally, higher abundance was found in the subsurface layer, whereas at station B maximal densities were at 10 m.

Among *Alexandrium* species we identified *A. pseudogonyaulax* and *A. minutum*, two species already reported from the Gulf of Trieste (Honsell *et al.*, 1992). During the abundance maximum of genus *Alexandrium* in May 1994 new species for this area was found in the southeastern part. Based on scanning electronic observations it is presumably related to *A. acatenella* (Fig. 5; Fukuyo, *pers. comm.*). Interestingly, in 1996 a similar species appeared in the samples from the northwestern part and it was isolated. Although more detailed morphological study has to be done to ascertain the taxonomic position of *A.* cf. *acatenella*, it is likely that a new *Alexandrium* species did appear on both sides of the Gulf of Trieste. In our case the isolated species is now kept in cultures for morphological and possibly biochemical (HPLC analyses for PSP toxins) studies.

Environmental factors and *Alexandrium* abundance

The seasonality of *Alexandrium* spp. in the Gulf of Trieste is similar to those observed in other Mediterranean areas, with even higher maximal densities especially in the upper, less-saline layer and in connection with developed thermohaline stratification (Delgado *et al.*, 1990; Giacobbe *et al.*, 1996). In our study we observed that the increased cell numbers coincided with the decreased surface salinity in late spring-early summer at all stations. The exception was again station D0, where in a very diluted surface layer (salinity <20, April 1995) *Alexandrium* spp. was not found, but was present at 2 m (salinity >30). Although intense autumn freshets caused another surface-salinity decline, *Alexandrium* abundance did not increase. This is probably linked to the destratification of the water column and lower temperatures compared to the spring-summer period. Higher temperatures, a stratified water column and ab-

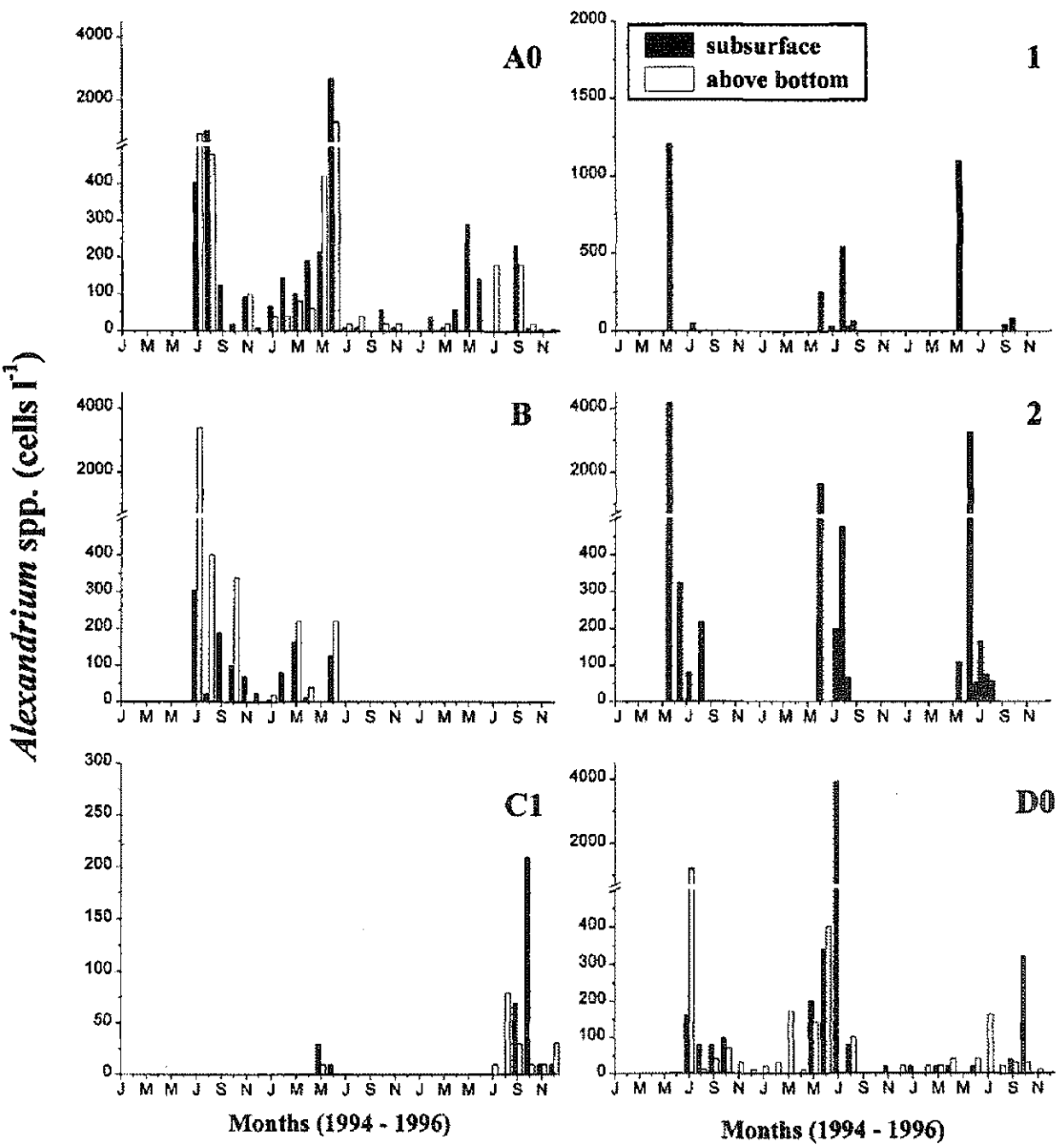

***Fig. 4:** Alexandrium* **spp. abundance at six stations in the period 1994-1996. (Note the different units on y axes.)**
***Sl. 4: Gostota vrst iz rodu** Alexandrium* **na šestih postajah v obdobju 1994-1996. (Upoštevaj različne enote na oseh y.)**

sence of turbulence seem to be favorable conditions not only for *Alexandrium* but for dinoflagellates as a group (Paerl, 1988). In mixed and nutrient enriched autumnal conditions diatoms prevail in the phytoplankton community of the Gulf of Trieste (Malej *et al.*, 1995; Cabrini *et al.*, 1994)

As mentioned above, mean phosphate concentrations increased from May to November in the years 1994 and 1996, and in July 1995. These periods coincide with the occurrence of *Alexandrium* species at the nearby stations 1 and 2. However, during the periods of favorable phosphate conditions *Alexandrium* abundance peaked (>1000 cells l^{-1}) only on few occasions (May 1994, 1995, 1996, June 1996), suggesting that

factors other than phosphate availability control the *Alexandrium* dynamics. Coincidence of high temperature, low salinity, stability of water column, high phosphate concentration and low N/P ratio, with high density of *Alexandrium* spp., was also observed in the Sicily lagoon, Mediterranean Sea (Giacobbe *et al.*, 1996). Several studies have also shown strong relationship between phosphate limitation and toxin production in *Alexandrium* species (Boyer *et al.*, 1987; Anderson *et al.*, 1990). However, in our case the results should be interpreted with care for at least two important reasons. *Alexandrium* abundance and nutrient concentrations were not measured in the same water sample, but from two, although close, different locations. It means that nutrient data allow to give an idea of the general situation in the area. Secondly, an important ecophysiological characteristic that should be taken into consideration is the ability of many free-living dinoflagellates to perform diel vertical migrations through the water column (Eppley & Harrison, 1975). For example, in our case inorganic nitrogen concentrations were low during the period of *Alexandrium* occurrence, which might suggest that *Alexandrium* cells could utilize deep nitrogen sources through vertical migrations. This behavioral adaptation enables a population of toxic dinoflagellates to persist in many nitrogen-depleted, summer-stratified surface coastal waters (Koizumi *et al.*, 1996; MacIntyre *et al.*, 1997).

In conclusion, our study confirmed the presence of *Alexandrium* spp. in the Gulf of Trieste and its increased abundance from April to October. Maximal cell numbers were recorded during thermohaline stratification of the water column, in a less-saline subsurface layer. A more detailed study on the influence of other environmental factors, mainly nutrients, on *Alexandrium* seasonality in the Gulf of Trieste is to be carried out in the future.

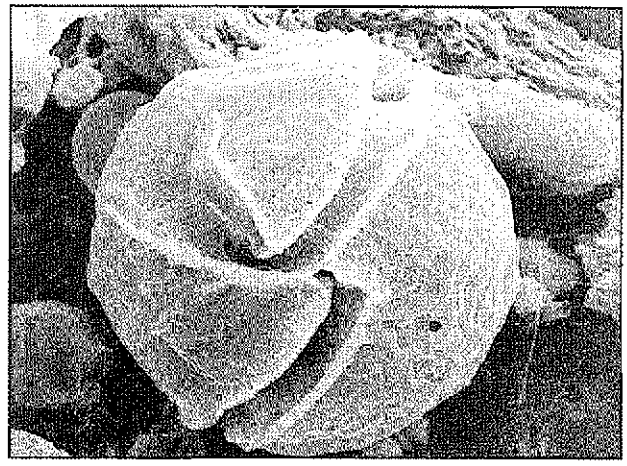

Fig. 5: Scanning electron micrograph of** Alexandrium **sp. (presumably related to** A. acatenella**) from the southeastern part of the Gulf of Trieste.
***Sl. 5: Posnetek (elektronski mikroskop) vrste** Alexandrium **sp. iz jugovzhodnega dela Tržaškega zaliva, ki je najverjetneje sorodna vrsti** A. acatenella.*

ČASOVNA PORAZDELITEV VRST IZ RODU *ALEXANDRIUM* V TRŽAŠKEM ZALIVU (SEVERNI JADRAN)

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POVZETEK

V prispevku avtorji podajajo rezultate triletnega spremljanja gostote toksičnih vrst dinoflagelatov na šestih postajah v bližini školjčnih nasadov užitne klapavice vzdolž italijanske in slovenske obale Tržaškega zaliva. Dinoflagelati iz rodu Alexandrium *so povzročitelji paralitične zastrupitve s školjkami (PSP) pri ljudeh, zato je že nekaj let na obeh straneh zaliva vpeljan program rednega spremljanja kakovosti morske vode in školjk. Poleg časovne in prostorske porazdelitve celic* Alexandrium *spp. v obdobju 1994-1996 so avtorji spremljali tudi nekatere fizikalno-kemijske parametre (temperatura, slanost, hranilne snovi).*

Vzorčevanje v mesečnih do dvotedenskih presledkih od leta 1994 do 1996 je pokazalo, da se Alexandrium *spp. pojavlja skoraj v vseh mesecih, vendar njegova gostota naraste v obdobju od aprila do oktobra vsako leto. V jugovzhodnem delu zaliva je bila največja gostota zabeležena v maju 1994 (4200 cel. l^{-1}), v severozahodnem delu pa julija 1995 (4000 cel. l^{-1}). Ti spomladansko-poletni viški so se ujemali z gostotno razslojenostjo vodnega stolpca,*

kot posledico povišanih temperatur in osiajevanja zgornjih slojev. V obdobju naraščajoče gostote vrst iz rodu Alexandrium *so bile v jugovzhodnem delu zaliva izmerjene povečane koncentracije fosfata, vendar so bili viški števila celic (>1000 cel. l^{-1}) izmerjeni le štirikrat v daljšem obdobju ugodnejših fosfatnih razmer.*

Avtorji opozarjajo na previdnost pri razlagi sezonske dinamike roda Alexandrium *zlasti zaradi dveh pomembnih dejstev: 1. število celic in koncentracija hranilnih snovi so bili merjeni na dveh različnih, četudi blizu ležečih postajah, zatorej izmerjene koncentracije hranilnih snovi odsevajo zgolj splošne hranilne razmere na tem območju; 2. za dinoflagelate je značilna dnevna vertikalna migracija po vodnem stolpcu, kar jim omogoča izkoriščanje globljih, bogatejših virov hranil zlasti v plitvih, temperaturno razslojenih priobalnih vodah.*

V vzorcih morske vode je bila z elektronskim mikroskopom odkrita do sedaj nepoznana vrsta Alexandrium *sp. iz Tržaškega zaliva, ki je najverjetneje sorodna vrsti* A. acatenella. *Vendar so za pravilno taksonomsko določitev in morebitno toksičnost potrebne nadaljnje morfološke in biokemijske raziskave na monokulturi izolirane vrste.*

Ključne besede: dinoflagelati, *Alexandrium* spp., dejavniki okolja, PSP zastrupitev, Tržaški zaliv

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