Potentially toxic dinoflagellates in Mediterranean waters (Sicily) and related hydrobiological conditions

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ABSTRACT: The seasonal occurrence of 3 potentially toxic dinoflagellates in different coastal environments of Sicily (Mediterranean Sea) and the associated hydrobiological conditions are reported. *Dinophysis sacculus* and *Alexandrium* sp. occurred, in 1993, in shallow inland waters (a brackish lagoon of the Tyrrhenian Sea), characterized by thermo-haline homogeneity. The densities of *Dinophysis* were maximal in April, when the waters were depleted in nutrients, the N:P ratio was 10:1 and the algal population, including synechoccoid cyanobacteria, bloomed. Afterwards, the cell concentrations decreased and in summer there was a total replacement of *Dinophysis* with *Alexandrium*. In late summer 1993, *Gymnodinium catenatum* was also recorded in offshore waters of the Malta Channel, during coastal upwelling associated with thermal stratification of the waters and the cells dispersed shorewards. DSP toxicity of blue mussels was detected in April, at a low level only, in the area affected by *D. sacculus*. No data is, however, available to date on PSP production by *Alexandrium* and *G. catenatum*, which are new records for these areas.

KEY WORDS: Dinoflagellates · Hydrobiological factors · Mediterranean Sea · Shellfish contamination

INTRODUCTION

In recent years, various species of both naked and armoured dinoflagellates which produce biotoxins have been recorded from diverse geographical areas (e.g. Anderson 1989, Maestrini 1991). Numerous studies have shown that blooms of harmful dinoflagellates are often closely related to changes in the structure of the water column, such as decreased turbulence and development of thermal or haline stratification (e.g. Delgado et al. 1990, Delmas et al. 1992), although no water column stratification has been observed in coincidence with blooms in Spanish Atlantic waters (Fraga et al. 1988).

Recently, the occurrence both of *Dinophysis* species and *Alexandrium minutum*, potential producers of DSP and PSP toxins, respectively (Diarrhetic and Paralysing Shellfish Poisons), has been reported from brackish environments of Sicily (Giacobbe et al. 1991, Giacobbe & Maimone 1994) where both exploitation of shellfish from natural banks and aquaculture are prac-

tised, as well as in other areas of the Tyrrhenian coastline, where artificial reefs and pilot plants for shellfish farming are located (Giacobbe et al. 1993). In 1993, *Dinophysis sacculus* Stein was again found in a confined Tyrrhenian site, where it co-existed with a species of *Alexandrium* Halim. *Gymnodinium catenatum* Graham was also recorded, for the first time, in an area of the southern coastline where upwelling of intermediate waters and their shoreward advection occur in summer (Magazzù 1988).

The present paper analyses the spring-summer changes in densities of these dinoflagellates, in relation to hydrobiological conditions.

MATERIALS AND METHODS

Two sites on the Sicilian coastline were investigated in 1993 (Fig. 1). The first was Green Lake (Oliveri-Tindari), a brackish Tyrrhenian lagoon not directly linked, by means of canals or otherwise, to the neighbouring
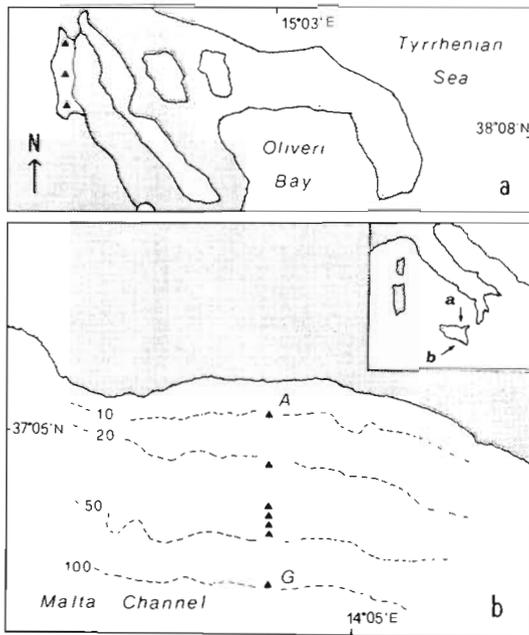
Fig. 1. Map of (a) the Oliveri-Tindari area and (b) the Malta
Channel with sampling stations

sea. The area is subject, however, to seawater inocula-
tion during storms, as well as to continental influence
— through water bearing inflows —, green discoloura-
tion of the waters and anoxic events (Giacobbe et al.
1992). Samplings were made monthly, from March to
September, at the subsurface (0.2 m) and near-bed
layer (2.0 to 3.0 m) at 3 stations. The second site was an
area of the southern coastline (Gulf of Gela, Malta
Channel). A transect from inshore to offshore waters (7
stations) was sampled in March and September, at the
subsurface layer (0.5 m) and at 3 or 4 points of the
water column (from 0.5 to 50 m).

Water samples were taken by Go-Flow Niskin bot-
tles (General Oceanics) and subsamples, preserved
with glutaraldehyde (2%), were settled and examined
by inverted microscope (Axiovert 35-Zeiss) for the
counts and identification of algal taxa. Direct cell
counts of picoalgae, ascribed to synechoccoid cyano-
bacteria, were made according to La Ferla & Crisafi
(1991).

Temperature and salinity measurements were made
by reversing thermometers, fitted to the sampling bot-
tles, and by a 8400A Autosal inductive salinometer,
Guildline Instr. Ltd (Millero & Poisson 1981), respec-
tively. Samples for macronutrients were analysed
according to Strickland & Parsons (1972) — except for
ammonia (Aminot & Chaussepied 1983) — and the
concentrations were measured using a Lambda 3
Perkin-Elmer spectrophotometer; the total concentra-
tion of nitrate, nitrite and ammonium is reported here
as DIN (dissolved inorganic nitrogen). N:P atomic

ratios were calculated as DIN:SRP (soluble reactive
phosphate). Chlorophyll a concentration was deter-
mined after overnight extraction with acetone (90%)
(Holm-Hansen et al. 1965) and fluorescence analysis
by a F-2000 Hitachi spectrofluorometer.

Shellfish Mytilus galloprovincialis, which are primary
transvectors of algal toxins, were introduced as bioas-
say organisms into Green Lake, where high concentra-
tions of Dinophysis sacculus have been recorded since
April 1988 (Giacobbe & Maimone 1991). A total of 200
individuals were placed at 1 m depth in a net hanging
from floats, at the central sampling point; the collection
of specimens (20 individuals randomly gathered) was
initiated 1 wk later (March 23) in coincidence with
water samplings, at monthly intervals. Following the
analytical procedure of UBE Ltd Research Laboratories
(Tokyo, Japan), 3 g of midguts was homogenized
(2 min) with 15 ml of 90% aqueous methanol solution
and centrifuged at 3000 rpm for 10 min. The super-
natant was diluted with deionized distilled water (1:1)
and 50 µl was placed in immunoplates for the competi-
tive inhibition ELISA assay, using monoclonal antibod-
ies with high specificity against okadaic acid (Usagawa
et al. 1989), a component of the DSP complex. Contents
of midguts were also examined microscopically for the
presence of Dinophysis cells.

RESULTS AND DISCUSSION

Dinophysis sacculus and Alexandrium sp.:
quantitative changes and associated hydrobiological
conditions (inland Tyrrhenian waters)

Fig. 2a shows monthly variations, from March to
September, of the densities of Dinophysis sacculus
Stein in the innermost area of Oliveri-Tindari. The cell
concentrations were maximal in April, in the upper
waters ($\bar{x}$ = 8000 l^{-1}) and declined in the subsequent
months, ending in total cell disappearance in July and
August; D. sacculus, which often dominated over a
small-sized Dinophysis sp., occurred again in Septem-
ber with average values of 1800 to 2350 cells l^{-1}

The experimental contamination of blue mussels
showed a weak toxicity of the shellfish in April only.
However, the presence in the mussel guts of Dino-
physis cells (D. sacculus only), or empty thecae, was
confirmed through the microscopical analysis of the
gut contents — except in July and August, when no
cells occurred in the waters at all. These findings sug-
gest a low production of okadaic acid by this strain. D.
sacculus has been previously associated with DSP
intoxications in the Adriatic Sea (Boni et al. 1992), but
a major mussel toxicity occurred when D. fortii domi-
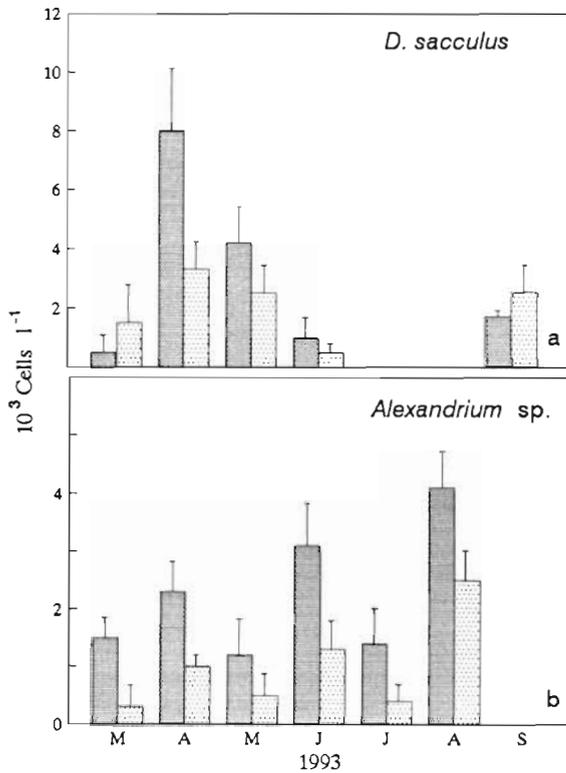nated over the other species.

Fig. 2. Spring-summer changes in abundance of *Dinophysis sacculus* and *Alexandrium* sp. in inland Tyrrhenian waters (Oliveri-Tindari). Bars represent averages of the 3 stations. Dark bars: sub-surface; dotted bars: near-bed layer; error bar = standard error of the mean (SEM)

tidal currents, the water column was well mixed (Fig. 3a), unlike other Tyrrhenian lagoons (Acosta Pomar et al. 1988). Temperatures ranged between a minimum of 15.4°C in March, and a maximum of 25.9°C in June, and the salinity values from 20.7 ppt in April, to 26.3 ppt in September.

As shown in Fig. 3b to f, in April — when the densities of *Dinophysis sacculus* were maximal — concentrations of inorganic nutrients were reduced, the N:P atomic ratios did not exceed 10:1 and algal blooms developed (total phytoplankton and chlorophyll *a* at the subsurface: 1.4×10^6 cells l^{-1} and 5.0 mg m^{-3}, respectively). During this period, the densities of synechoccoid cyanobacteria also increased, mostly in the subsurface layer, reaching a maximum in May (8×10^6 cells l^{-1}).

Experimental studies by Ishimaru (1988) showed that cells of *Synechococcus*-type cyanobacteria and cryptomonads are consumed by *Dinophysis fortii* and *D. acuminata*, resulting in an orange or orange-red fluorescence of *Dinophysis* cells, probably due to residual ingested chloroplasts. Although no data are available on the nutritional requirements of *D. sacculus*, our data show that high densities of this species corresponded to high availability of *Synechococcus*, whereas its disappearance (July-August) coincided with a decrease in synechoccoid cyanobacteria density. However, beyond this observation, no obvious relationship was found between the two. Neither were there significant correlations between *Dinophysis* density and inorganic

In spring and summer, an armoured dinoflagellate co-existed with *Dinophysis*; both the plate morphology and the tabulation were typical of the genus *Alexandrium* Halim (Balech 1985), which includes most of the saxitoxin-producing species (Taylor 1992). No data are, however, available to date on the PSP production by the strains occurring in the area. *Alexandrium* sp. was recorded for the first time and occurred in all the sampling periods, except for September; cell density was higher in the upper waters (Fig. 2b) and peaked in summer (August: $\bar{x} = 4080$ cells l^{-1} at the subsurface).

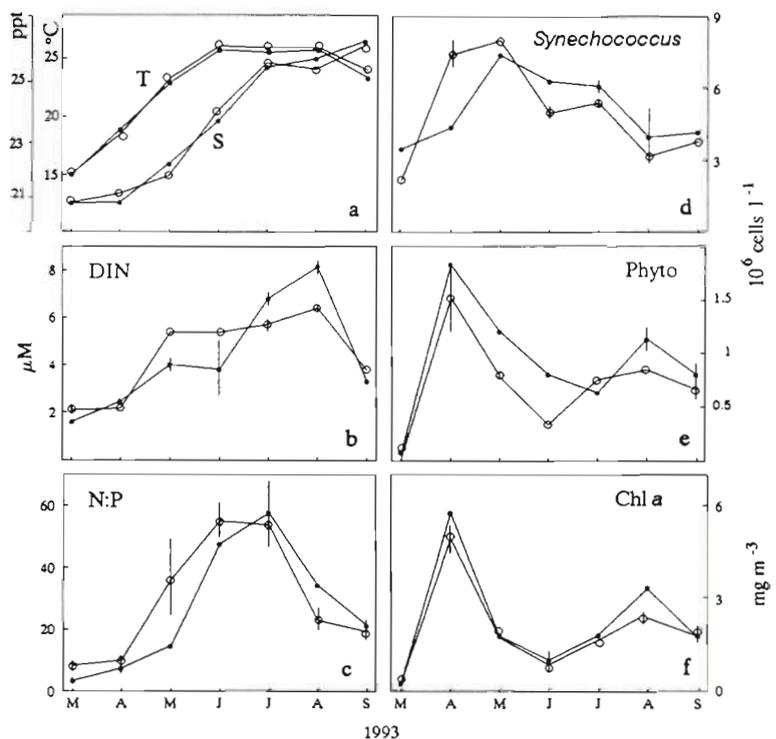Because of the shallowness of the waters (max. depth: 3 m) and the wind-driven and

Fig. 3. Temporal changes of hydrobiological parameters in shallow brackish waters (Tyrrhenian Sea). (a) Temperature and salinity; (b) DIN; (c) N:P ratio; (d) *Synechococcus*; (e) total phytoplankton; (f) chlorophyll *a*. Points represent averages of the stations. Error bars = SEM. (o) Surface; (•) near-bed layer
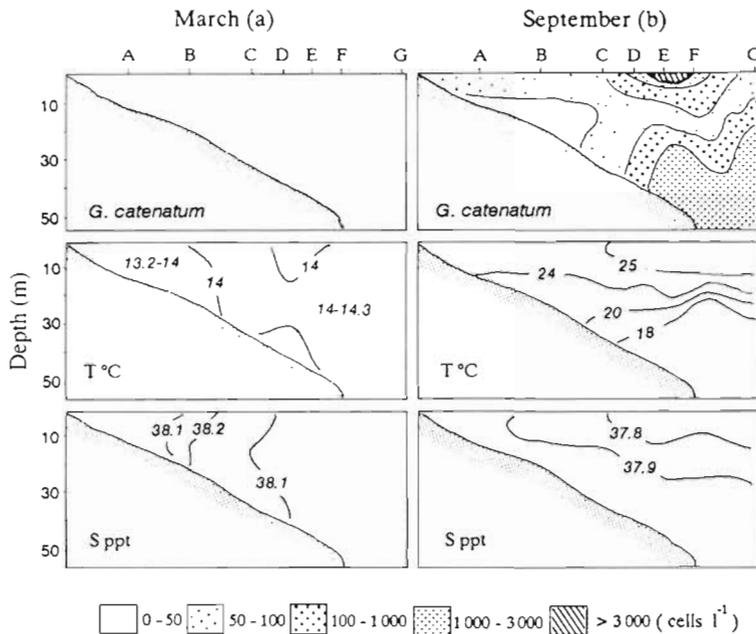
Fig. 4. Cell densities of *Gymnodinium catenatum* and thermo-haline structure of the waters in the Malta Channel in March and September

nutrient concentration. This is in agreement with studies from French Atlantic waters (e.g. Delmas et al. 1992) where, however, cell numbers increased during marked thermal stratification of the waters. In contrast, we observed, for the whole of the period of *Dinophysis* presence, a thermohaline homogeneity and, in April and May only, a slight stability of the water column in the most sheltered sector.

Finally, *Dinophysis* absence and *Alexandrium* maximal concentrations coincided, in August, with higher DIN amounts and N:P values (subsurface-nearbed layer: 6 to 8 µM and 23 to 34, respectively); phytoplankton and chlorophyll *a* peaked again (0.8 to 1.1 × 10^6 cells l^{-1} and 2.3 to 3.3 mg m^{-3}).

Gymnodinium catenatum distribution and related environmental parameters (Malta Channel)

No cells of *Gymnodinium catenatum* Graham occurred in the Malta Channel in March 1993, when the waters were both isothermal and isohaline (Fig. 4a); the amounts of inorganic nutrients were low (max: 1.36 µM DIN, Stn C; 0.16 µM SRP, Stn E) and the values of total chlorophyll *a* ranged between 0.01 and 0.3 mg m^{-3}

(Fig. 5a). Two-celled chains and single cells were recorded, for the first time, in September coinciding with thermal stratification. Abundance was highest offshore (max: 5400 cells l^{-1}, Stn E, 0.5 m; 1000 to 3000 cells l^{-1}, 25 to 50 m) and decreased shorewards (Fig. 4b). No data on the PSP production are available yet, but similar cell concentrations in waters of the Spanish Mediterranean coast corresponded to toxicity levels of 200 µg saxitoxin/100 g clams (Bravo et al. 1990).

In September, the thermal stratification of the water involved the whole area (Fig. 4b) and a thermocline was established offshore (Stns E and F). At these stations, the upper waters were warm and well mixed (T = 25°C) and separated from a homogeneously cool deep layer (T = 18°C) by a sharp thermocline. A slightly positive haline gradient was also observed with the increasing depth (from 37.80 to 37.92 ppt) (Fig. 4b). During September, there was a minor algal biomass and the highest DIN and SRP amounts were found shorewards and in the intermediate sector, respectively (max: 5.7 µM DIN and 0.82 µM SRP) (Fig. 5b); vice versa, offshore there were the lowest concentrations of inorganic nitrogen and a high abundance of *Gymnodinium catenatum*. A
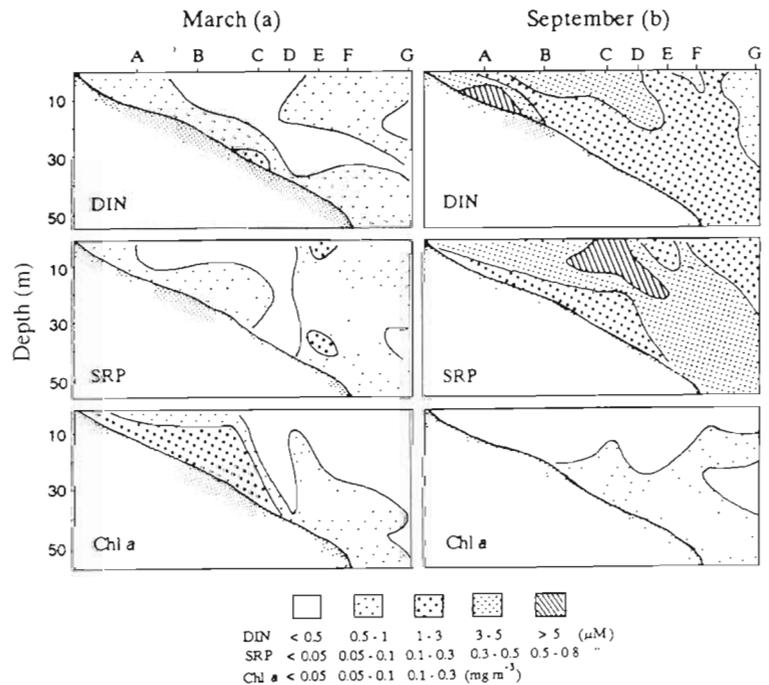

Fig. 5. Distribution of nutrients and total chlorophyll *a* in waters of the Malta Channel

significant inverse relationship was found, in fact, between cell densities and DIN values ($r = -0.72$; $n = 12$; $p < 0.01$); the cell numbers of *G. catenatum* inversely correlated with the ammonium concentrations ($r = -0.66$; $n = 12$; $p < 0.02$), whereas there was no correlation with nitrate or nitrite. Increases in cell numbers were also weakly associated to the decrease in the values of the N:P ratio ($r = -0.61$, $n = 12$; $p < 0.05$) which ranged, offshore, between 2 and 5 (data not shown).

CONCLUSIONS

The presence of both *Dinophysis sacculus* and *Alexandrium* sp. in the brackish Tyrrhenian area (Green Lake) may be partly attributed to an allochthonous inoculation of the cells either from the neighbouring bay, during sea storms, or from the nearby lagoons, during spring tides, when they are subject to water exchanges. The possibility of a locally initiated growth cannot, however, be excluded and further data are necessary from the adjacent waters and on the possible presence of *Alexandrium* hypnozygotes in the surface sediments.

In the Malta Channel, the late summer occurrence of *Gymnodinium catenatum* corresponded to the upwelling of cold intermediate waters and to thermal stratification. Blooms of *G. catenatum* have been recorded in the rias of NW Spain as well; their development strongly depended, in late summer, on the relaxation of upwelling and bloom prediction indices have been established (Fraga et al. 1990). This dinoflagellate, which may form long chains (e.g. Estrada et al. 1984), occurred in 2-celled chains and as a single form, suggested by other authors as indicative of suboptimal growth conditions (e.g. Blackburn et al. 1989). However, further studies in this area, as well as in mariculture sites of Sicily, are needed to clarify the allochthonous or autochthonous origin of these dinoflagellates, the mechanisms of bloom formation and factors regulating toxicity.

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