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Harmful microalgal episodes in Greek coastal waters

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In the frame of the harmful algae monitoring program conducted in the main fishing and shellfish growing areas of Greece during the period 2000-2004, two types of harmful algae were recorded: 1) microalgae that produce water discoloration because of their massive growth and 2) microalgae related to toxin production. The majority of algal blooms associated with water discoloration were observed in Thermaikos Bay, where species of the genus *Prorocentrum* (*P. redfeldii*, *P. micans*, *P. triestinum* and *P. obtusidens*), the heterotrophic *Noctiluca scintillans*, the raphidiophyte *Chattonella* cf. *globoda* and a naked dinoflagellate *Gymnodinium* sp. reached high cell densities. Algal blooms causing water discoloration were also observed in Amvrakikos and Elefsis Bays, where the causative organisms were *Alexandrium insuetum* and *Prorocentrum minimum*. The main harmful dinoflagellates, which so far cause the greatest economic losses in Greece, due to the shellfish intoxication, belong to the genus *Dinophysis*. *Dinophysis* species were mainly observed during late winter and spring, causing recurrent diarrhetic shellfish intoxication outbreaks in the last five years in Thermaikos Bay. Amvrakikos Bay is also affected by toxic microalgae, which cause diarrhetic and paralytic shellfish intoxication. *Pseudo-nitzschia* species a further group of harmful algae related with amnesic shellfish intoxication episodes were found also in all monitored areas.

Key words: Harmful algae, Greek coastal waters, monitoring, biotoxins, shellfish intoxication.

INTRODUCTION

Amongst the living microscopic algae in the oceans, a few thousands cells can at times cause harm to humans and to marine ecosystems. The negative effects of harmful algae are many and diverse, ranging from economical losses in aquaculture, fisheries and tourism industry to major environmental and human impacts.

Attending the problems that they cause, harmful algae can be categorized into three major groups. The first group is formed by "harmless" organisms that discolor the water due to their massive occurrence. However, under certain conditions, oxygen depletion caused by bacterial respiration during the decay of the algal biomass, results in killing of both fish and invertebrates. Examples of such algae are some dinoflagellates included in the genera *Gonyaulax*, *Scrippsiella*, *Prorocentrum* and *Noctiluca* and some diatoms of the genera *Skeletonema*, *Rhizosolenia* and *Leptocylindrus* (Hallegraeff *et al.,* 2003). The second group, recently recognized, because of the increased interest in intensive aquaculture systems for finfish, includes algae that can damage fish gills either mechanically or chemically through the release of exogenous substances with hemolytic action (Yasumoto, 1989). Examples of algae belonging to this group are the diatoms *Chaetoceros convolutus* and *C. concavicornis*; the dinoflagellates *Karenia mikimotoi*, *K. brevisulcata* and *Karlodinium micrum*; the prymnesiophytes *Prymnesium* spp. and *Chrysochromulina polylepis* and some raphidophytes species such as *Heterosigma akashiwo* and *Chattonella* spp. The third and most important algal group includes microalgal species that produce potent toxins which can cause serious gastrointestinal and neurological illnesses

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and death to humans, after contaminated seafood consumption and deaths to other organisms (fish, marine mammals, birds) (Scholin *et al.,* 2000). The occurrence of toxic microalgae represents a significant threat to human health, aquaculture, fishery resources and marine ecosystems throughout the world (Hallegraeff *et al.,* 2003). Based on the symptoms observed in humans, having consumed contaminated shellfish, five types of poisoning due to marine biotoxins are distinguished; Paralytic Shellfish Poisoning (PSP), Diarrhetic Shellfish Poisoning (DSP), Amnesic Shellfish Poisoning (ASP), Neurotoxic Shellfish Poisoning (NSP), Azaspiracid Poisoning (AZP) and Ciquatera Fish/Shellfish Poisoning (CFP/CSP). The latter is transferred to humans also by consuming contaminated coral reef fish.

The first publication related to harmful algae impacts in Greece goes back to the sixties. Anagnostidis (1968) has reported a "red tide" phenomenon along the coastline of Thessaloniki city resulting in mass fish mortalities. Recently, mass finfish mortality (Veterinarian Authorities, personal communication) was recorded in Amvrakikos Bay (December 1998), probably due to the massive presence of the raphidophyte *Chattonella veruculosa*.

Water discoloration due to massive growth of microalgae (algal blooms), with no fish mortality, has been reported for the inner part of Thermaikos Bay by Nikolaidis (1994), Nikolaidis & Evangelopoulos (1997) and Nikolaidis *et al*. (1997). Causative organisms for these events were mainly species of the genus *Prorocentrum* (*P. micans*, *P. triestinum*, *P. obtusidens* and *P. rostratum*), the photosynthetic ciliate *Mesodinium rubrum*, the heterotrophic unarmored dinoflagellate *Erythropsidinium* sp. and the phagotrophic dinoflagellate *Noctiluca scintillans*. The occurrence of several potentially harmful phytoplanktonic species was reported, apart from Thermaikos Bay, also in other Greek coastal waters (Ignatiades, 1976; Pagou & Ignatiades, 1990; Ignatiades *et al.*, 1995).

The massive growth of the toxins producer (DSP), dinoflagellate *Dinophysis* cf. *acuminata*, that was observed on January 2000 in Thermaikos Bay (Koukaras & Nikolaidis, 2004), was the first documented toxic outbreak in Greece (Mouratidou *et al*., 2004), which resulted in economical losses of about 5 million Euros in the shellfish industry. This toxic episode was the reason for establishing a monitoring program for marine biotoxins. This program is focused on the occurrence of toxic and potentially toxic microalgae in the shellfish production areas of Greece. In the frame of this phytoplankton monitoring program a number of harmful algal episodes have been recorded. The aim of this paper is to present a survey of harmful algae associated with these episodes in Greek coastal waters during the last five years (2000-2004).

MATERIALS AND METHODS

Study area and sampling stations

Water samples for identification and enumeration of harmful, toxic and/or potentially toxic microalgae were collected from a wide network of fixed points within the separated geographical areas as they are shown in Fig. 1. The number of sampling stations of each area is presented in Table 1.

Sampling and analyses

Water samples from the whole water column (integrated water samples) were taken with a PVC tube equal in length to the depth of each station. Subsamples of 500 ml were placed in plastic bottles and fixed immediately with Lugol's solution. Species were identified according to the official guidelines of UNESCO (Hallegraeff *et al.,* 2003) and additional specific monographs such as those by Schiller (1933), Hustedt (1962), Sournia (1986) and Tomas (1993,

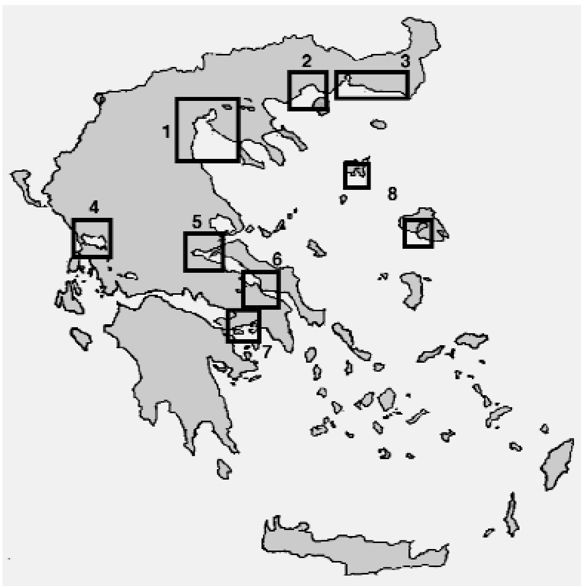

FIG. 1. Map of Greece showing the study areas. 1: Thermaikos Bay, 2: Kavala coasts, 3: Thrace coasts, 4: Amvrakikos Bay, 5: Maliakos Bay, 6: Euvoikos Bay, 7: Elefsis Bay, 8: Limnos and Lesbos Islands.

TABLE 1. Spatial distribution of the main harmful algae recorded in the Greek coasts and the number of sampling stations in each area during the present study

Location (sampling stations)	**Species**									
	D. cf. *acuminata*	*D. sacculus*	*D. caudata*	*A. insuetum*	*A. minutum*	*P. minimum*	*Gymnodinium* sp.	*N. scintillans*	*Chattonella* spp.	*Pseudo-nitzschia* spp.
Thermaikos Bay (15)	++	+	+	-	-	-	++	+++	++	+++
Kavala coasts (3)	-	+–	–	–	–	–	–	+–	–	++
Thrace coasts (5)	–	–	–	–	–	++	–	–	–	+
Amvrakikos Bay (3)	+–	+	+	+++	+	++	+–	–	++	+
Maliakos Bay (3)	+–	+	+	–	+	++	+–	–	–	+
Euvoikos Bay (2)	–	+–	+	–	–	–	–	–	–	+
Elefsis Bay (2)	–	+–	–	–	–	++	–	–	–	+
Limnos & Lesbos Islands (4)	–	+–	–	–	–	–	–	–	–	+

+++: $>10^6$ cells l^{-1}; ++: $10^4 - 10^6$ cells l^{-1}; +: 10^3 cells l^{-1}; +–: 10^2 cells l^{-1}; –: abundances below detection level

1996). Cells were counted with an inverted microscope, by applying the Utermöhl's sedimentation method (Utermöhl, 1958). The potentially toxic species were counted using 25 ml (blooming period) and 50 ml (non-blooming period) sedimentation chambers by scanning the whole bottom of the chamber (magnifications ×63, ×100). Scanning electron microscope (SEM, ZEISS DSM 940A) was additionally used for species identification.

RESULTS

During the last five years (2000-2004), water discoloration due to massive occurrence of microalgae has been observed mostly in the inner part of Thermaikos Bay (Fig. 2a). The most common organism was *Noctiluca scintillans* (Fig. 2b), a heterotrophic dinoflagellate mainly observed in late winter (February) and at the beginning of spring (March-April) reaching high abundances, between 2.0×10^6 and 5.4×10^6 cells l^{-1}. Another organism, the massive growth of which in the inner part of Thermaikos Bay caused water discoloration during spring (April-May) in the years 2001-2003, was the raphidophyte *Chattonella* cf. *globosa* (Fig. 2c). Its abundance ranged between 2.0×10^4 and 4.0×10^4 cells l^{-1}. *Chattonella* cf. *globosa* cells are yellowish brown, spherical in shape with two unequal flagella; one long and the other extremely short, almost invisible. *Gymnodinium* sp. (Fig. 2d), an unarmored dinoflagellate, also occurred in the inner part of Thermaikos Bay (mainly in September 2002) reaching abundances up to 65.4×10^3 cells l^{-1}. Its massive occurrence caused yellow-greenish water discoloration.

Discoloration of water was also caused by species of the genus *Prorocentrum* in different regions of Greece. *Prorocentrum* cf. *obtusidens* (Fig. 2e) occurred in the inner part of Thermaikos Bay during the winter 2000-2001 at abundances of about 1.2×10^6 cells l^{-1} and was responsible for water discoloration in January 2001. Water discoloration in the same region was similarly caused by another species of this genus (*P. redfeldii*, Fig. 2f) with an abundance ranging between 1.2×10^6 and 6.0×10^6 cells l^{-1}. *Prorocentrum minimum* (Fig. 2g) mass occurrence (up to 11.8 $\times 10^4$ cells l^{-1}) was observed in April 2003 in Elefsis Bay and also along the coasts of Thrace (Porto-Lagos and Alexandroupolis). In Amvrakikos Bay, *P. minimum* reached high abundances every year during autumn.

Another species in Amvrakikos Bay that caused water discoloration mainly during spring was *Alexandrium insuetum* (Fig. 2h). In April 2003 (Fig. 3), mass occurrence (2.54×10^6 cells l^{-1}) of this species caused brownish water discoloration without toxic episodes.

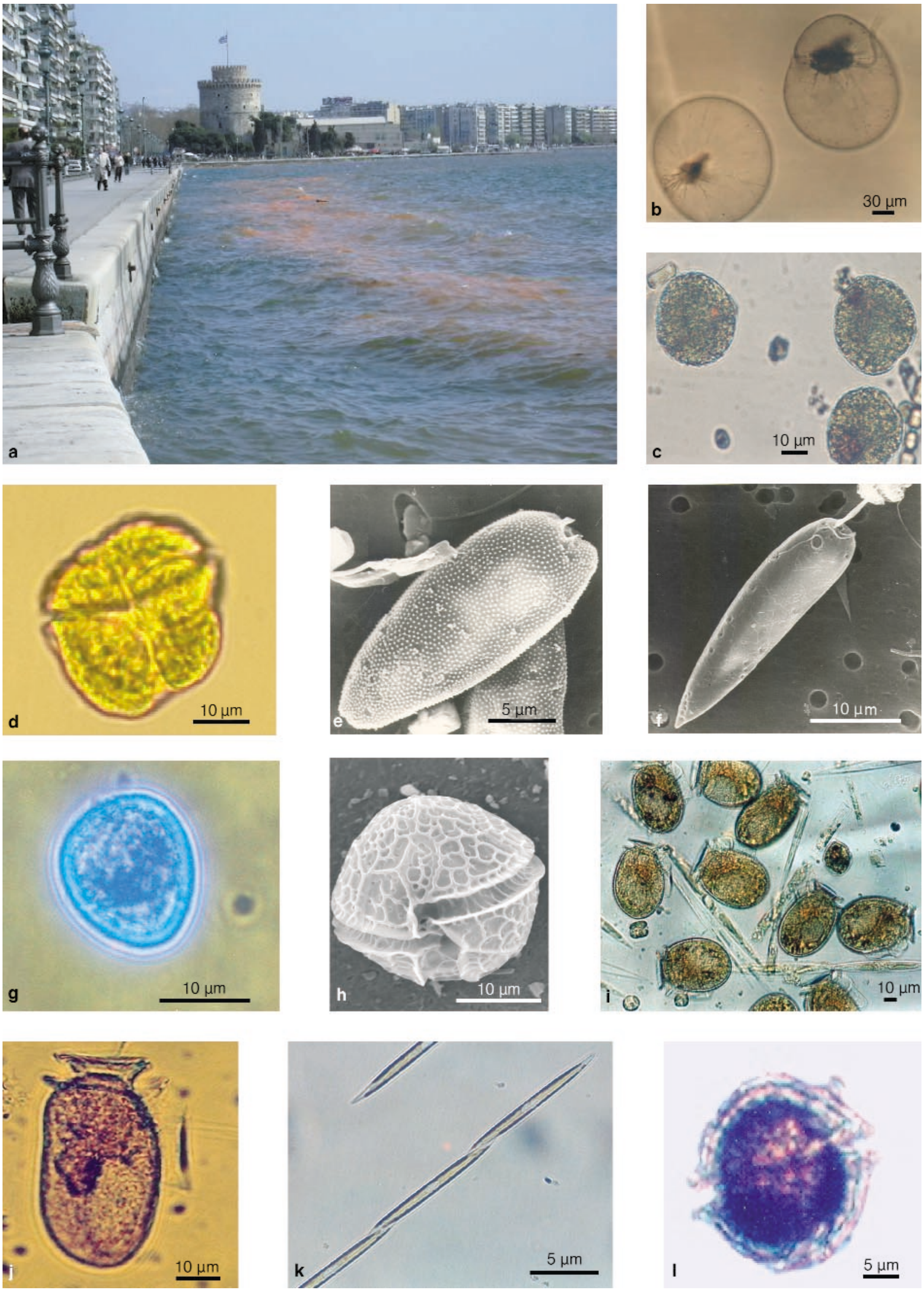

FIG. 2. **a**: Red tide phenomenon along the coastline of Thessaloniki city (Thermaikos Bay, April 2003) caused by *Noctiluca scintillans*. **b**: *N. scintillans* cells from Thermaikos Bay in light microscope (LM). **c**: *Chattonella* cf. *globosa* cells from Thermaikos Bay (LM). **d**: *Gymnodinium* sp. cell from Thermaikos Bay (LM). **e**: *Prorocentrum* cf. *obtusidens* cell from Thermaikos Bay in scanning electron microscope (SEM). **f**: *P. redfeldii* cell from Thermaikos Bay (SEM). **g**: *P. minimum* cell from Amvrakikos Bay (LM). **h**: *Alexandrium insuetum* cell from Amvrakikos Bay (SEM). **i**: *Dinophysis* cf. *acuminata* cells from Thermaikos Bay (LM). **j**: *D. sacculus* cell from Amvrakikos Bay (LM). **k**: *Pseudo-nitzschia* cf. *pungens* cells from Thermaikos Bay (LM). **l**: *A.* cf. *minutum* cell from Amvrakikos Bay (LM).

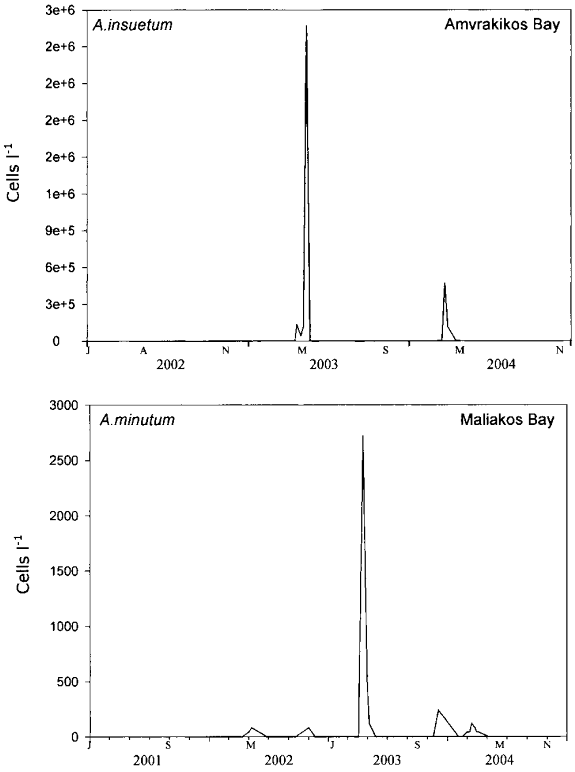

FIG. 3. Temporal distribution of *Alexandrium insuetum* in Amvrakikos Bay (2002-2004) and *A. minutum* in Maliakos Bay (2001-2004) at selected stations. J: January, A: April, M: May, S: September.

A. insuetum was observed also in May 2004 at high cell densities (4.71×10^5 cells l^{-1}) in Amvrakikos Bay and its bloom lasted approximately three weeks (Fig. 3).

Microalgae related to toxic episodes have been reported only in Thermaikos and Amvrakikos Bays. These belonged to the genera *Dinophysis* and *Alexandrium* which caused DSP and PSP intoxication. High cell densities (up to 85.4×10^3 cells l^{-1}, middle depth water sample, February 2000) of *Dinophysis* species were recurrently recorded in the inner part of Thermaikos Bay between February and April during the study period (Fig. 4). 98% of the *Dinophysis* population was occupied by the species *D.* cf. *acuminata* (Fig. 2i). The bloom always initiated in the inner part of the Bay and expanded after 3-4 weeks to the southern part along the coast of Pieria in relation to the water masses circulation in Thermaikos Bay. *Dinophysis* cf. *acuminata* cells were also observed in Maliakos and Amvrakikos Bays in low abundances. The dominant species within the *Dinophysis* population, either in Maliakos or in Amvrakikos Bays, were *D. sacculus* (Fig. 2j) and *D. caudata*

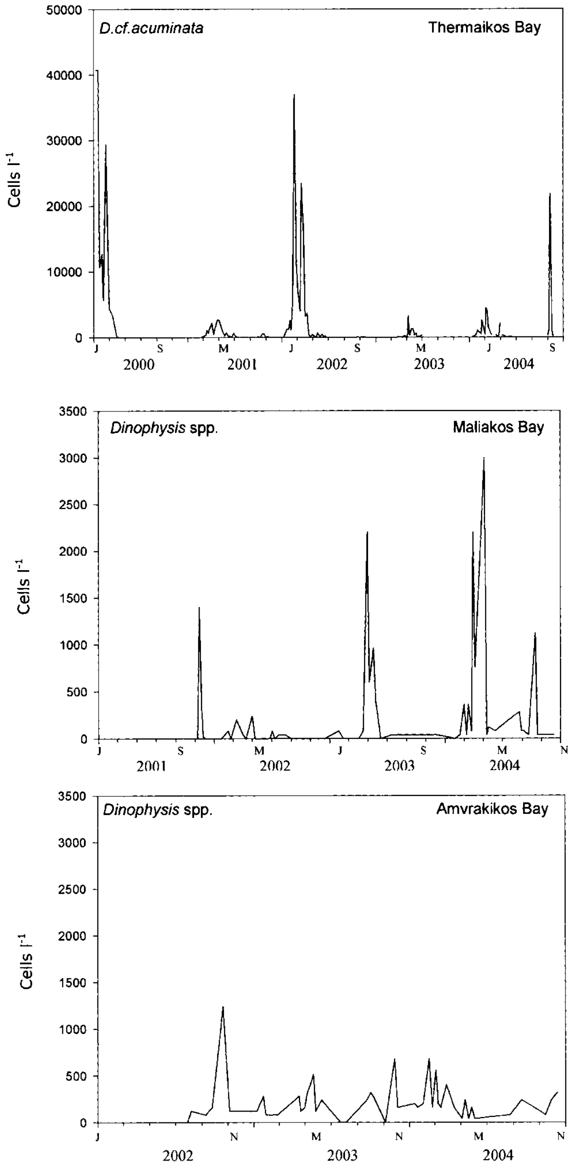

FIG. 4. Temporal distribution of *Dinophysis* species at selected stations of the Bays of Thermaikos (2000-2004), Maliakos (2001-2004) and Amvrakikos (2002-2004). J: January, S: September, M: May, N: November.

which reached abundances up to 3.0×10^3 cells l^{-1} (Fig. 4). *Dinophysis* species in lower cell densities (<200 cells l^{-1}) were further observed in Lesbos and Limnos islands without DSP episodes. In coastal waters of Thrace, Kavala, Euvoikos and Elefsis Bays, *Dinophysis* species were sporadically observed in low abundances (<80 cells l^{-1}).

Pseudo-nitzschia species (*P.* cf. *pungens* [Fig. 2k], *P. pseudodelicatissima*) were recorded throughout the year in all monitored areas. Blooms of the above species were mainly observed during the period March to May (Fig. 5) reaching a maximum abun-

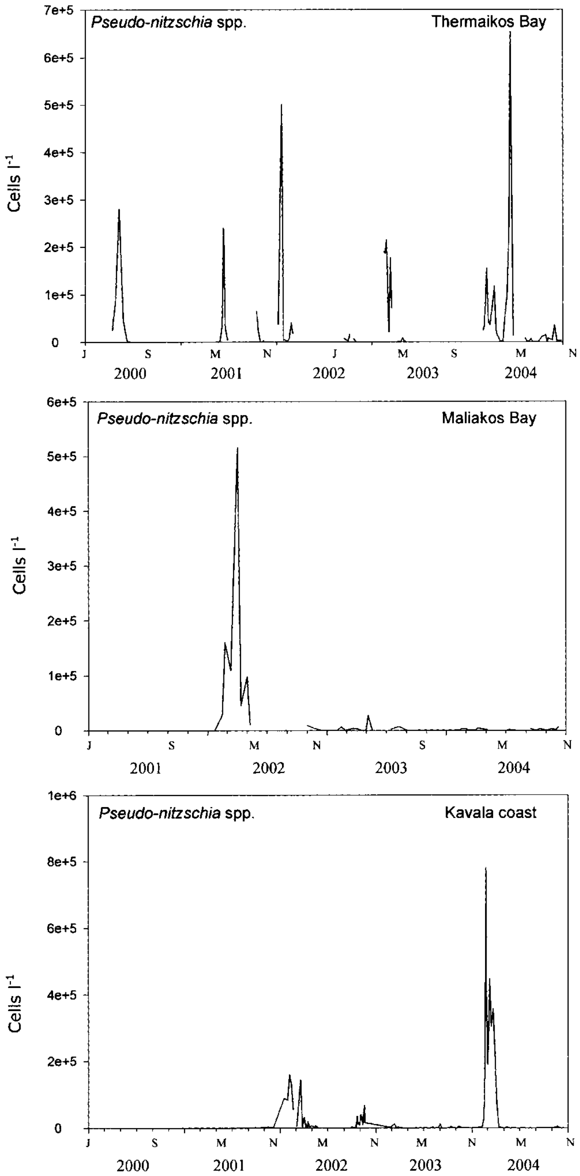

FIG. 5. Temporal distribution of *Pseudo-nitzschia* species at selected stations of the Thermaikos (2000-2004) and Maliakos (2001-2004) Bays and Kavala coast (2000-2004). J: January, S: September, M: May, N: November.

dance up to 1.76×10^6 cells l^{-1}.

Another potentially toxic, small-sized species of the genus *Alexandrium* (Fig. 2l), probably *A. minutum,* was recorded particularly during spring and early summer in Maliakos and Amvrakikos Bays. Specifically, in Maliakos Bay, this species reached abundance up to 2.7×10^3 cells l^{-1} (Fig. 3).

The spatial distribution of the main harmful phytoplankton species detected in the Greek coast during the present study is presented in Table 1.

DISCUSSION

Greece is one of many countries affected by harmful algae. During the present study, the majority of algal blooms were observed in Thermaikos and Amvrakikos Bays. It is generally accepted that one of the most important factors promoting the development of algal blooms in coastal waters is the nutrient supply from terrestrial sources (Cadée, 1986; Radach *et al.*, 1990; Smayda, 1990). Thermaikos and Amvrakikos Bays are strongly influenced by different human activities and the development of recurrent algal blooms could be explained by the continuous nutrient discharging from rivers and urban sewage. High levels of nutrients could promote algal blooms, but this is not the only factor for bloom development. Nutrient imbalance could also be a parameter that favors certain species *versus* others (Hodgkiss & Ho, 1997; Bulgakov & Levich, 1999). Because of the increase of nitrogen and phosphorous inputs due to eutrophication, the ratio of these nutrients to silicate becomes very high. This could favor non diatom species including several harmful and toxic species (Smayda, 1989).

During the last five years, silicate concentration in Thermaikos Bay seems to decrease (Koukaras & Nikolaidis, 2004) compared to previous measurements (Nikolaidis & Moustaka-Gouni, 1992; Nikolaidis *et al.*, 1995). In Thermaikos Bay, the majority of algal blooms are formed by flagellates, mostly dinoflagellates, that dominate over diatoms as they have specialized behaviors, like mixotrophy (Granéli & Carlsson, 1998; Stoecker, 1999) and capability for vertical migration (Hasle, 1950; Villarino *et al.*, 1995).

According to our results, a temporal pattern for algal blooms does not seem to exist, since bloom development was observed throughout the year. However, the majority of blooms were recorded during spring when thermal stratified conditions in the water column begin to establish. Massive growth of dinoflagellates associated with water discoloration was observed in Amvrakikos Bay, where *A. insuetum* population reached high cell densities during spring 2003 and 2004, respectively. *Alexandrium insuetum*, which has been first described by Balech (1985) in Korean Pacific waters, is not a common species of the genus. This species was found in Japanese waters (Yuki & Yoshimatsu, 1990) and recently in the Mediterranean Sea (Daly Yahia-Kefi *et al.*, 2001), but bloom of this species has never been reported. According to our findings, the first bloom of *A. in-*

suetum was observed in Amvrakikos Bay during the present study.

Water discoloration resulted from massive growth of several microalgae was, until the beginning of 2000, the only documented impact of the occurrence of harmful algae in Greek coastal waters (Nikolaidis *et al*., 1997). Presence of toxic or/and potentially toxic algae in Greek coastal waters without toxic events has been reported by Nikolaidis & Moustaka-Gouni (1990), Nikolaidis *et al.* (1995) and Nikolaidis & Evangelopoulos (1997). Since 2000, when the first toxic outbreak was recorded, attention was focused on toxic and potentially toxic microalgae from Greek coastal waters. The first documented toxic outbreak (Koukaras & Nikolaidis, 2004; Mouratidou *et al*., 2004) that caused humans DSP intoxication was recorded on January 2000 in Thermaikos Bay. The causative organism was confirmed to be the dinoflagellate *D.* cf. *acuminata* the population of which reached unusually high cell densities during the winter. Blooms of *D. acuminata* associated with toxic events are known mainly from waters of NW Europe (Maestrini, 1998; Godhe *et al.*, 2002), New Zealand (Chang, 1996) and the Mediterranean Sea (Lassus *et al.*, 1991; Giacobbe, 1995). In Maliakos and Amvrakikos Bays the most abundant species of the genus *Dinophysis* were *D. sacculus* and *D. caudata*. DSP episodes associated with the presence of *Dinophysis* species were recorded only in Amvrakikos Bay (Kaniou-Grigoriadou *et al*., 2005), whereas cell densities of *Dinophysis* species ranged at similar levels with those of Maliakos Bay. These results are probably due to the variability of toxin production among *Dinophysis* species concerning regional and seasonal morphotypes of one species (Johansson *et al.*, 1996; Sato *et al.*, 1996; Giacobbe *et al.*, 2000).

Another harmful algal species which has been world widely related to PSP events is *Alexandrium minutum* (Delgado *et al.*, 1990; Honsell, 1993; Vila *et al.*, 2005). This species has been well documented, mainly in confined areas of the Mediterranean Sea and it was also observed during the present study in Maliakos and Amvrakikos Bays. PSP toxicity (mouse bioassay) in one mussels-sample has been detected only in Amvrakikos Bay (March 2004) (Kaniou-Grigoriadou *et al.*, 2004) during the occurrence of *A. minutum* (<500 cells l^{-1}) along with high abundances of marine cyanobacteria.

The genus *Pseudo-nitzschia* is a further group of harmful algae with worldwide distribution (Fryxell *et al.*, 1997). The ubiquitous distribution of the genus and its association with domoic acid (DA) production has brought serious attention to this phytoplankton group (Bates *et al.*, 1998). Nine species of *Pseudo-nitzschia* have been documented to produce DA (*P. multiseries, P. australis, P. seriata, P. pseudodelicatissima, P. delicatissima, P. multistriata, P. turgidula, P. fraudulenta* and *P. pungens*) (Hallegraeff *et al.,* 2003). In Greek coastal waters, *Pseudo-nitzschia* species are important component of the phytoplankton community and only two of the previously mentioned DA producers were recorded (*P. pseudodelicatissima* and *P. pungens*). However, ASP toxicity in mussels (above the regulatory level of DA: 20 μg g^{-1}) has not been detected (Kaniou-Grigoriadou *et al*., 2005) in areas where *Pseudo-nitzschia* species were observed in high abundance. These findings are supported by the results of other researches who have found non-toxic clones of *P. pseudodelicatissima* from around the world and a low amount of DA in *P. pungens* (Bates *et al.*, 1998; Rhodes, 1998).

The potential hazard of future toxic blooms requires further investigation for harmful algae distribution in Greek coastal waters in order to provide additional information and improve our understanding of these phenomena.

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