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Dynamics of potentially harmful microalgae in a confined Mediterranean Gulf—Assessing the risk of bloom formation

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A B S T R A C T

The population dynamics of potentially harmful microalgae was investigated in the semi-enclosed shallow Gulf of Kalloni, Greece (Aegean Sea, Eastern Mediterranean), during a 2-year period from August 2004 to March 2006. A total of 21 potentially harmful microalgae (bloom-forming and/or toxic) were identified including 3 diatoms and 18 dinoflagellates. The densities of each species were analyzed in time and space and in relation to environmental parameters. Some species such as *Alexandrium insuetum*, *Heterocapsa circularisquama*, *Karlodinium veneficum*, *Scrippsiella trochoidea*, and *Ceratium* spp. developed high cell concentrations, particularly during a *Pseudo-nitzschia calliantha* winter bloom. Other species such as *Dinophysis caudata*, *Ostreopsis ovata*, *Prorocentrum minimum*, and *Protoperidinium crassipes* were rare or appeared in small numbers. Densities of the most abundant species were closely associated with freshwater nutrient-rich inputs during winter, being negatively correlated with temperature and salinity and positively correlated with nitrogen. The spatial distribution of the abundant species exhibited a marked increase towards the inner part of the gulf, close to the main freshwater inputs, whereas some species were mainly concentrated in the dilute surface layer (1 m depth). Examination of the abundance–occupancy relationship revealed that the species more prone to bloom are those with wide spatial distribution and frequent presence throughout the year such as the diatom *P. calliantha*. Although blooms of cyst-forming species are rarer, an increased risk can be foreseen under favorable resource supply and environmental conditions during winter.

1. Introduction

Coastal ecosystems are increasingly becoming susceptible to nutrient enrichment mainly due to urbanization, tourism and agricultural activities (Justic et al., 1995). These enrichments result in the eutrophication of coastal waters (Nixon, 1995), accompanied by an increased occurrence of harmful microalgae and harmful algal blooms (HABs), especially in enclosed coastal embayments (Anderson et al., 2002; Hallegraeff, 1993). In the Mediterranean an increasing number of studies indicates harmful species proliferations, particularly along the northern coasts, possibly due to the variant morphology of its coastline and to nutrient-rich freshwater inputs from urban and agricultural activities (Collos et al., 2004; Spatharis et al., 2007a; Vila et al., 2005).

Harmful microalgal species tend to be bloom-forming, although the actual cell number characterizing a bloom cannot be strictly defined and varies intrinsically among species (Smayda, 1997a). Even at relatively low density ($\sim 10^4$ cells l^{-1}), harmful species may have detrimental effects on other organisms (Smayda, 1997b). These microalgal blooms may affect an ecosystem by disturbing the food chain (Graneli and Turner, 2006), being harmful to other organisms through the production of dangerous phycotoxins (Turner and Tester, 1997), and often resulting in anoxic or hypoxic conditions (Burkholder et al., 2008). Due to these adverse impacts on the ecosystem, as well as on the public health and certain economic sectors, harmful species have recently received great attention (Graneli and Turner, 2006).

Though the Eastern Mediterranean is oligotrophic (Krom et al., 1991), increased occurrence of HABs has been observed in coastal areas (Spatharis et al., 2007b) due to nutrient enrichment from human activities (urbanization, tourism, agriculture, industry) combined with low rates of water renewal. Since these coastal areas (gulfs, bays, lagoons, estuaries and deltas) are very important

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ecosystems from both the ecological and economic perspectives, specific actions have to be taken to mitigate the development of HABs. Therefore, it is essential to monitor the dynamics of species responsible for the development of HABs and find possible associations between their proliferation and environmental factors. Though monitoring is an important aspect of HAB research and produces data for modeling and decision-making, analysis and prediction of the population dynamics of HABs are still not well developed. Measures of species-specific bloom rates and their inter-annual variability, duration and occurrence are therefore important. In situ monitoring of microalgal blooms in small-scale coastal embayments is particularly useful for the investigation of their temporal and spatial dynamics. The simultaneous monitoring of the physicochemical parameters can further contribute to explain or predict HAB dynamics and the role of 'seed' populations (Figueiras et al., 2006).

The aim of the current study was the investigation of temporal and spatial dynamics of potentially harmful microalgae in the semi-enclosed ecosystem of Kalloni Gulf on the Island of Lesvos, Eastern Mediterranean. The investigation involved species identification, monitoring of species densities during the studied period, and the association of species density variability with physicochemical variables.

2. Methods

2.1. Study site and sampling

The Gulf of Kalloni is located in the central part of the Greek Island of Lesvos, Aegean Sea, Eastern Mediterranean (39°15′N 26°21′E). It is a semi-enclosed shallow gulf (mean depth of 10 m) surrounded by a watershed of about 400 km^2 where various human activities take place, mainly urbanization, tourism, and agriculture. Untreated by-products from these activities flow into the gulf and cause eutrophication phenomena, mainly during winter. According to the EU legislation, water and shellfish quality in Kalloni Gulf are monitored on a regular basis since 2001. Toxins related to paralytic and amnesic shellfish poisoning have been reported in low concentrations during the years preceding this study (Tsirtsis et al., 2004).

Samples were collected from 12 stations (Fig. 1). Six stations K1–K5 and K7, covering a wide area of the Gulf of Kalloni, were sampled on a monthly basis during a full annual cycle (August 2004–July 2005). Station K2 was located at the gulf's inlet and K1 in the open sea, serving as a control station. During the period from November 2004 to June 2005 additional sampling was carried out after main rainfall events at stations K5–K12 (Fig. 1), spaced along a gradient in the plume of River Tsiknias (the main freshwater input of the gulf). Additional information on species-abundance in station K8 (November 2005–March 2006) was available from a monitoring program related to the quality of commercial shellfish species (Tsirtsis et al., 2006).

Water samples were collected from 1 and 5 m depths, and vertical profiles of temperature and salinity (measured in psu) were recorded with a CTD profiler (Seabird SBE19). Samples from each depth were analyzed for nutrients and chl α, according to Parsons et al. (1984). Phytoplankton samples were preserved in a 2% Lugol's iodine solution and analyzed with a Zeiss inverted microscope following the Utermöhl method (Utermöhl, 1958). Cell counting was performed using 50 ml cylinders and 15% of the slide surface of each sample was examined at 250×. In some cases live material was maintained in Erlenmeyer flasks, for a few days after sampling, supplemented with L1 medium (Guillard, 1995). Samples from this material were prepared for bright field (BF) and transmission electron microscopy (TEM).

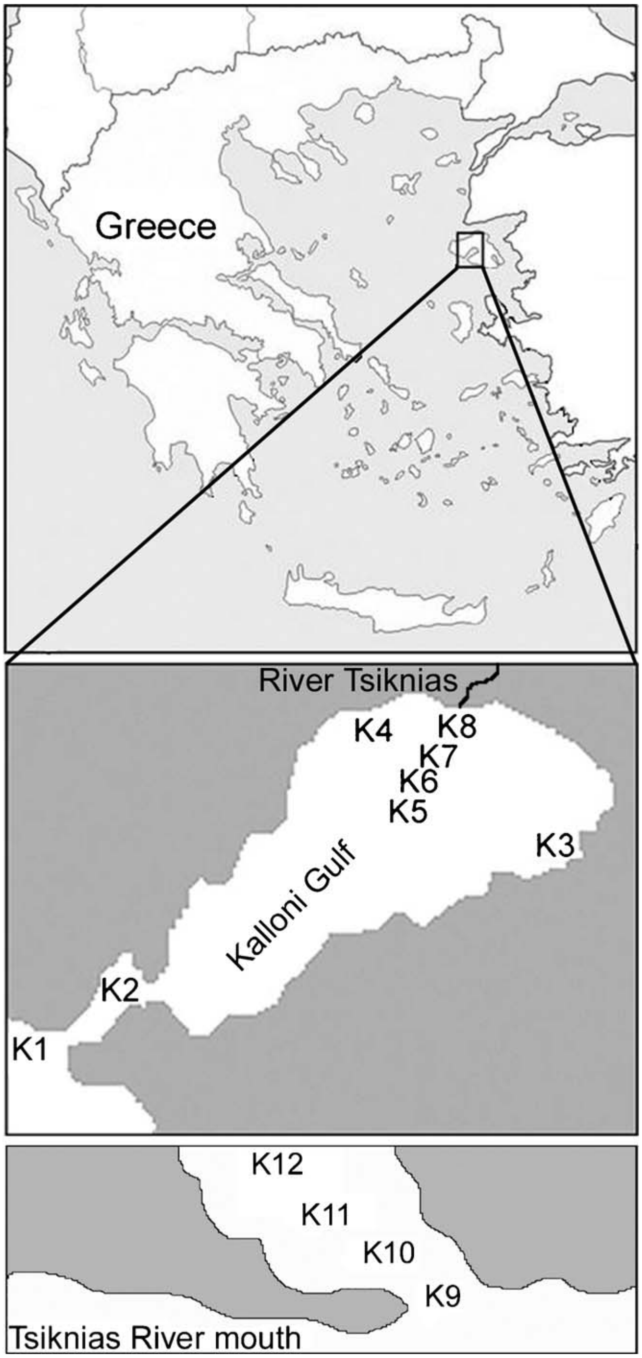

Fig. 1. Location of the eight sampling stations (K1–K8) in Kalloni Gulf, Lesvos Island, Greece.

2.2. Data analysis

Analysis of variance (ANOVA) was applied in order to test possible vertical differences of species (1 and 5 m depths) using abundance data from all stations in the inner part of the gulf. Potential relationships between species yielding substantial abundances and environmental parameters were tested by the Spearman rank correlation coefficient (Zar, 1984) using data from stations K1 to K8. Finally, aiming to examine whether the rare or common species are more likely to develop dense populations (i.e. blooms) in the study area, the average abundance of each species was plotted against the number of times that this species was present in the samples in an abundance–occupancy plot. This plot is commonly used in community ecology to determine the relationship between mean local abundance (at occupied sites) and site occupancy (number of sites occupied) (Gaston, 1996).

3. Results

3.1. Species dynamics

Overall 21 potentially bloom-forming and/or toxic taxa (Fig. 2), out of a total of 129 planktic species, were identified in the Gulf of Kalloni during the studied period (Table 1), most of them being dinoflagellates (18 species). A pattern of increased abundance during winter was observed for most species (Fig. 3), this being more pronounced in 2005 than in 2006. The diatom *Pseudo-nitzschia calliantha* was present in the water column throughout the year, with increased abundances during the winter period; massive blooms were developed in particular after intense nutrient enrichment from the watershed due to episodic rainfalls. On the other hand, *Pseudo-nitzschia pungens* was rare and recorded only during February and March 2005 at low cell densities.

Other abundant species such as *Alexandrium insuetum*, *Heterocapsa circularisquama*, *Ceratium tripos*, and *Scrippsiella trochoidea* proliferated during a narrow time period, mainly during February 2005 (Fig. 3), although their abundances were considerably lower in winter 2006. In particular, *A. insuetum* was almost totally absent from Kalloni Gulf throughout the year apart from February 2005 when it peaked along with the aforementioned diatom *P. calliantha*. The species *H. circularisquama* was present in relatively high densities in January, and also peaked during the *P. calliantha* bloom (max. 73.2×10^3 cells l^{-1}) in late February 2005. Other abundant species such as *Karlodinium veneficum*, and *Prorocentrum arcuatum* were present for a longer period from November 2004 to

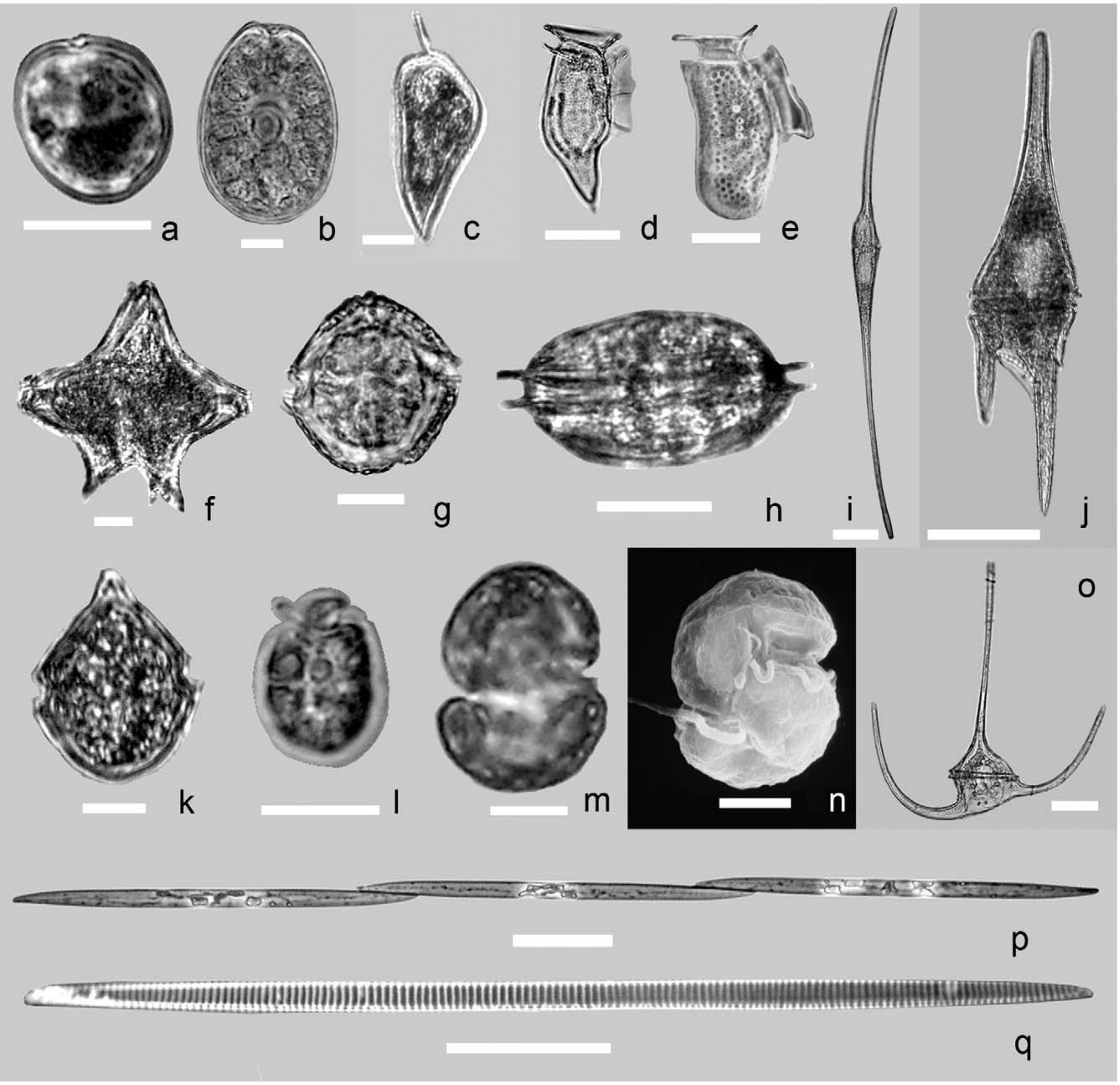

Fig. 2. Light and scanning electron micrographs of the potentially harmful species from Kalloni Gulf: (a) *Prorocentrum minimum*, (b) *Prorocentrum lima*, (c) *Prorocentrum arcuatum*, (d) *Dinophysis caudata*, (e) *Dinophysis sacculus*, (f) *Protoperidinium crassipes*, (g) *Alexandrium insuetum*, (h) *Diplopsalis lenticula*, (i) *Ceratium fusus*, (j) *Ceratium furca*, (k) *Scrippsiella trochoidea*, (l) *Amphidinium carterae*, (m) *Karlodinium veneficum*, (n) *K. veneficum*, (o) *Ceratium tripos*, (p) *Pseudo-nitzschia calliantha*, (q) *Pseudo-nitzschia pungens*. Scale bars: photos 'm' and 'n' = 5 μm, photos 'a'–'g' and 'k' = 10 μm, photos 'h', 'j', 'p', 'q' = 20 μm, photo 'i' = 25 μm, and photo 'o' = 40 μm.

Table 1
List of potentially toxic (reported at least once as toxic) and potentially harmful microalgae identified in Kalloni Gulf from August 2004 to July 2005, with information on their occurrence at the 168 samples, their maximum abundance in cells l^{-1}, and the month when the maximum abundance was observed.

Species	Impact	Times present	Max. abundance	Month of max. abundance
Potentially toxic				
Alexandrium insuetum Balech	PSP[a]	27	138.9×10^3	Feb.
Amphidinium carterae Hulburt	Ichthyotoxicity (Haemolycins)[b]	2	0.9×10^3	Feb.
Amphora coffaeformis (Agardh) Kützing	ASP (DA)[b]	74	10.4×10^3	Nov.
Dinophysis caudata Saville-Kent	DSP (OA, PTX)[b]	1	0.3×10^3	Sep.
Dinophysis sacculus Stein	DSP (OA)[b]	33	1.42×10^3	Dec., Jan., May, Aug.
Karlodinium veneficum (Ballantine) J. Larsen	Ichthyotoxicity (KmTx1, KmTx2)[b]	107	24.9×10^3	Dec.
Ostreopsis ovata Fukuyo	Toxic to marine fauna (PLTX)[b]	7	0.6×10^3	May
Prorocentrum lima (Ehrenberg) Dodge	DSP (OA, DTX-1, DTX-2)[b]	15	0.9×10^3	Apr., May, Jul.
Prorocentrum minimum (Pavillard) Schiller	Toxic to marine fauna (Haemolycins)[b]	2	0.9×10^3	May
Protoperidinium crassipes (Kofoid) Balech	DSP (AA)[b]	4	0.3×10^3	May
Pseudo-nitzschia calliantha Lundholm, Moestrup et Hasle	ASP (DA)[b]	145	$10{,}647 \times 10^3$	Feb.
Pseudo-nitzschia pungens (Grunow ex Cleve) Hasle	ASP (DA)[b]	5	1.4×10^3	May
Bloom-forming				
Diplopsalis lenticula Bergh	Red tides[c]	1	0.3×10^3	Nov.
Ceratium furca (Ehrenberg) Claparède et Lachmann	Red tides, anoxia, fish deaths[d]	21	2.84×10^3	Ap.
Ceratium fusus (Ehrenberg) Dujardin	Coastal blooms, fish deaths[e]	18	2.1×10^3	Aug.
Ceratium lineatum (Ehrenberg) Cleve	Coastal blooms[f]	37	3.6×10^3	Aug., Sep.
Ceratium tripos (Müller) Nitzsch	Coastal blooms, fish deaths[g]	43	5.7×10^3	Feb.
Heterocapsa circularisquama Horiguchi	Red tides, bivalve deaths[b]	40	73.2×10^3	Feb.
Peridinium quinquecorne Abé	Coastal blooms[h]	3	4.5×10^3	May
Prorocentrum arcuatum Issel	Coastal blooms[i]	110	35.5×10^3	May
Scrippsiella trochoidea (Stein) Loeblich III	Blooms, fish deaths[j]	29	2.4×10^3	Feb.

[a] Sako et al. (2004).
[b] Moestrup (2004).
[c] Feyzioğlu and Öğüt (2006).
[d] Glibert et al. (2002).
[e] Onoue (1990).
[f] Rost et al. (2006).
[g] Weaver (1979).
[h] Garate-Lizarraga and Muneton-Gomez (2008).
[i] Baric et al. (2003).
[j] Hallegraeff (1992).

April 2005, forming a peak during early winter (Fig. 3). The species *Ceratium lineatum* showed a different trend, with high abundances in early autumn for both the years studied. Other *Ceratium* species were less abundant, such as *Ceratium furca*, which showed maximum abundance in February 2005 (Fig. 3) and *C. fusus* which was more abundant during summer 2004 (Table 1).

Among the least abundant species, *Dinophysis sacculus* was frequently present in the water column particularly from December 2004 to March 2005. The species *Prorocentrum minimum*, *Dinophysis caudata* and *Amphidinium carterae* were rarely observed and in low cell numbers (Table 1). The species *Protoperidinium crassipes* was observed in May 2005 in a few stations in the interior of the gulf in low abundances. The brackish water species *Peridinium quinquecorne* was also recorded only in May 2005 at the four stations inside the River Tsiknias mouth, but at relatively high cell numbers (4.5×10^3 cells l^{-1}). The species *Diplopsalis lenticula* was recorded only once at low cell numbers (300 cells l^{-1}). Finally, three benthic, potentially toxic species were also recorded in samples from the water column in Kalloni Gulf. These species were the diatom *Amphora coffaeformis* which was found quite frequently in the water column in considerable numbers, the dinoflagellates *Ostreopsis ovata* observed at low numbers during May 2005, and *Prorocentrum lima* with low densities in April, May and July 2005.

3.2. Spatial distribution and relationship with environmental parameters

Almost all species examined showed extremely low abundances (<900 cells l^{-1}) or were completely absent in the open sea, whereas in most of the stations in the gulf the abundant species were present at high concentrations. The only exceptions were *K. veneficum* and *P. calliantha* which were present at the control station (K1) with densities $<2 \times 10^4$ cells l^{-1} during December and January. Considering vertical differences of species during the peak of the *P. calliantha* bloom in February 2005, dinoflagellate species as *H. circularisquama*, *K. veneficum*, *C. furca*, and *Prorocentrum arcuatum* were found with higher cell numbers at the depth of 1 m than at 5 m. This difference was even more pronounced and also statistically significant for *S. trochoidea*, *A. insuetum* and *C. tripos* (ANOVA, $p < 0.01$).

Temporal variation of various environmental parameters measured in the Tsiknias River mouth (stations K10–K12) is presented in Fig. 4. Temperature in the river mouth was lowest during the winter months, whereas the maximum flow rate was observed in February. In the beginning of the rainfall period (November 2004), dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) concentrations were extremely low, but both increased during winter and peaked between January and March 2005. This increase in nutrient concentrations, associated with an increase in river flow rate, resulted in a maximum nutrient loading of the receiving water body during February 2005. Very low DIN and DIP concentrations were measured in the river mouth in June 2005 while for the rest of the summer period the river was dry and the water in the river mouth was due to the intrusion of seawater. A similar trend of temporal variation was observed for nutrients in the inner part of Kalloni Gulf (Spatharis et al., 2007a), indicating the direct interaction between the receiving water body and the surrounding land through the network of rivers. Extreme conditions were also observed during February 2005, when the lowest annual values of seawater temperature and salinity were recorded in the inner part of the gulf (9.4 °C and 34.0 psu, respectively).

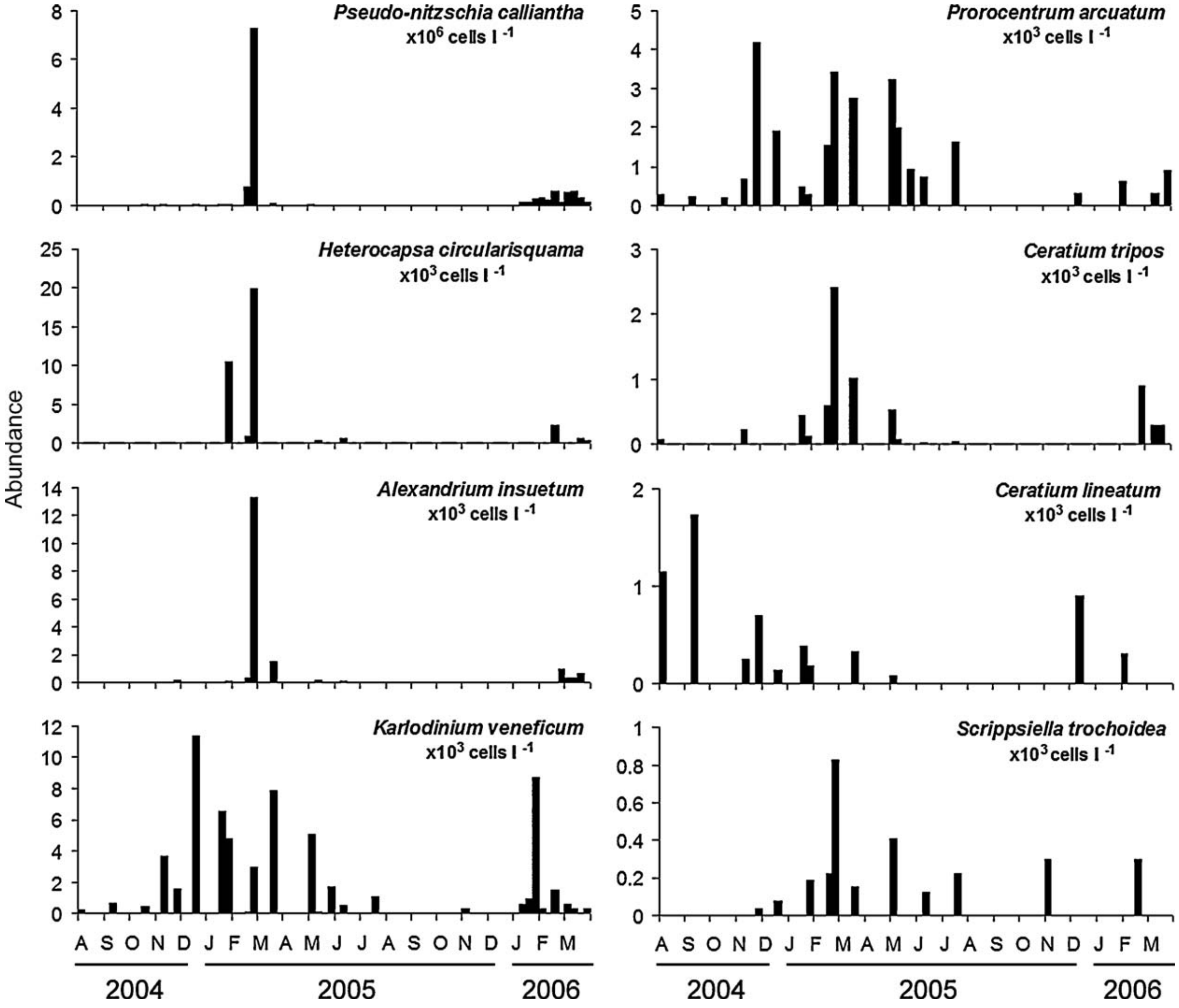

Fig. 3. Temporal variation of cell densities of the most abundant potentially harmful planktic species (August 2004–July 2005). Values represent the mean of stations K3–K8, whereas from November 2005 to March 2006 values represent data from station K8. Note that for *P. calliantha* abundance is $\times 10^6$ cells l^{-1} while for the rest of the species it is $\times 10^3$ cells l^{-1}.

Correlation analysis of species abundances and environmental parameters revealed that the increase in abundance of most of the potentially harmful species is associated with a rise in nutrient concentrations and a drop in seawater temperature and salinity in the gulf (Table 2). An exception is observed for *C. fusus* and *C. lineatum* which were usually more abundant during autumn and summer, when nutrients were low and temperature and salinity were high.

3.3. Abundance–occupancy relationship

The abundance–occupancy plot (Fig. 5) of each species (average density in relation to the number of times observed in the samples) showed a pattern according to which common species tended to appear in high densities (upper right part of the plot), whereas narrowly distributed species reached lower densities in the water column (lower left part of the plot). Species such as *P. calliantha* and *K. veneficum* that were present in most samples were often found in very high numbers or blooms.

4. Discussion

It has been widely recognized that accumulating data on the characteristics, causes, and consequences of HABs, contribute to the development of appropriate monitoring programs and preventative measures against the occurrence of such harmful events in coastal ecosystems (Cembella et al., 2005; Ranston et al., 2006; Riegman, 1991; Todd, 1993; Work et al., 1993). A prerequisite for this, is the detailed investigation of the spatial and temporal dynamics of the species involved across wide temporal scales, along with the corresponding physical and chemical information (ICES, 2005).

In the present study it was observed that the species *H. circularisquama*, *Ceratium tripos*, *S. trochoidea*, *P. calliantha* and *A. insuetum* co-occur and proliferate mainly during late winter as a result of the nutrient and freshwater runoff from the agricultural land. The species *P. calliantha* and *A. insuetum* bloomed in February 2005 soon after an episodic rainfall event, following the period of terrestrial fertilizer application (December–February) (see also Spatharis et al., 2007a). A similar bloom was also observed the following year, but it was less pronounced due to the fact that rainfalls were more evenly spaced in time and were of moderate intensity. Other abundant dinoflagellates such as *K. veneficum* and *Prorocentrum arcuatum* appear to peak in early winter after the first rainfalls when nutrient enrichment is still relatively low. Less abundant dinoflagellates, analyzed in detail in the present study, such as *Peridinium quinquecorne*, *P. arcuatum*, *D. sacculus*, *Prorocentrum minimum*, *C. furca*, and *Protoperidinium crassipes*

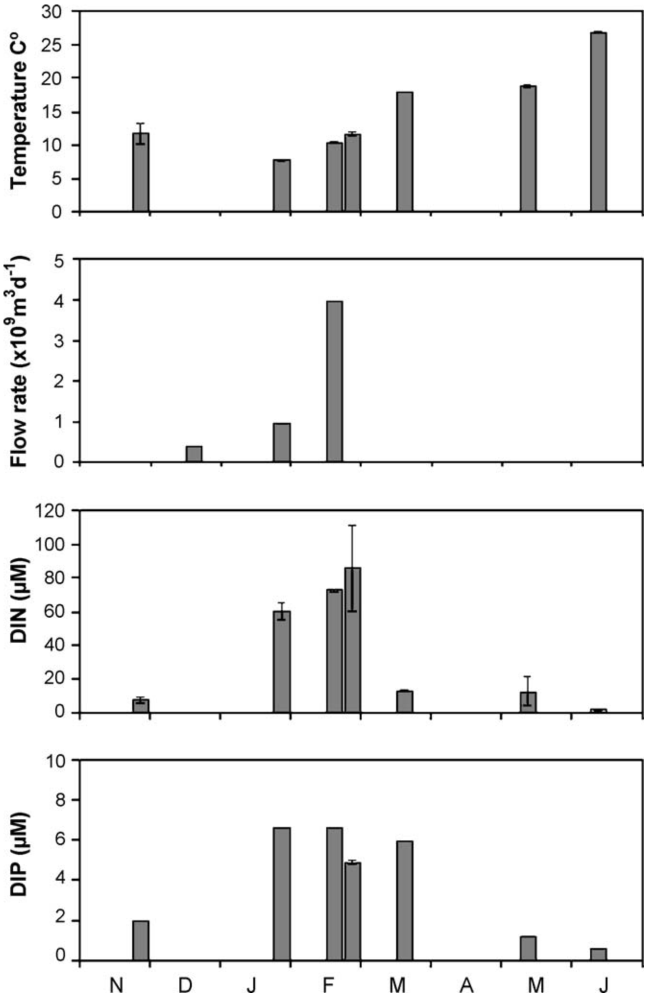

Fig. 4. Temporal variation of freshwater temperature, flow rate, dissolved inorganic nitrogen (DIN), and dissolved inorganic phosphorus (DIP) in the Tsiknias River mouth, averaged for stations K10–K12 from November 2004 to June 2005.

have been previously shown to co-occur with the spring diatom bloom (Spatharis et al., 2007b). Finally, the two benthic dinoflagellates *O. ovata* and *Prorocentrum lima* were rarely observed in the water column, and their presence may be attributed to the shallowness of the gulf and the continuous sediment re-suspension by the blowing winds.

Table 2
Spearman rank correlation coefficient for the most abundant potentially harmful species in the Gulf of Kalloni and environmental parameters in stations K1–K8 from August 2004 to July 2005 ($n = 188$).

Species	NO_3	NO_2	PO_4	SiO_2	Sal.	Temp.
A. insuetum	0.226**	–	–	–	−0.286**	−0.232**
D. sacculus	–	–	–	–	–	−0.242**
K. veneficum	–	0.229**	−0.319**	–	–	−0.374**
P. calliantha	0.271**	0.362**	–	0.406**	−0.243*	−0.665**
C. furca	0.161*	–	–	–	−0.212**	–
C. fusus	–	–	–	–	–	0.233**
C. lineatum	–	−0.153*	–	0.176*	0.417**	–
C. tripos	–	0.222**	–	0.233**	−0.388**	−0.334**
H. circularisquama	0.268**	0.210**	–	–	−0.331**	−0.327**
P. arcuatum	0.177**	–	−0.150**	–	−0.211**	−0.152*
S. trochoidea	–	–	−0.194*	−0.158*	−0.239**	–
N diatoms	–	0.151*	0.327**	–	–	–
N dinoflagellates	0.160*	0.224**	−0.275**	–	−0.256**	−0.471**

(–) not significant correlation.
* $p < 0.05$.
** $p < 0.001$.

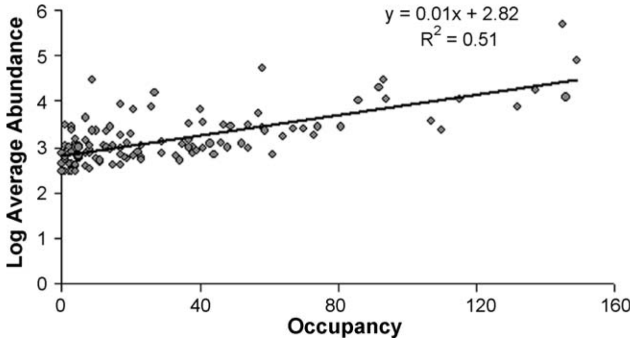

Fig. 5. Relationship between the abundance (in cells l^{-1}) of each of the 129 planktic species (averaged for the samples that the species was present), and the occupancy (number of samples that the species was present). Data were used from stations K1 to K8 in the inner part of Kalloni Gulf from August 2004 to July 2005.

The environmental conditions enhancing the growth and proliferation of the most abundant species in the gulf coincide with those observed in previous studies. More particularly, the species *K. veneficum* bloomed after the first freshwater inputs in the gulf, in agreement with previous records of this species blooming in estuaries ($>200 \times 10^6$ cells l^{-1}) when favored by high nutrient concentrations and reduced nutrient dispersal (Hall et al., 2008). In addition, the peak of *H. circularisquama* and *S. trochoidea* during the low salinity and nutrient-rich conditions of February 2005 is consistent with previous observations for these species from enclosed water bodies that justifies their characterization as estuarine (Matsuyama et al., 1996; Steidinger and Tangen, 1997). For *H. circularisquama* in particular experimental work has shown that the optimal salinity for growth was 30–34 psu (Leong et al., 2006) which was very similar to the field conditions in Kalloni during February. Finally, the species *C. furca* and *C. tripos* were found to bloom at much lower densities in the gulf than previously reported blooms in the Fire Island ($\sim 43 \times 10^3$ cells l^{-1}; Weaver, 1979) and in Chesapeake Bay (478×10^3 cells l^{-1}; Smalley and Coats, 2002). Although massive blooms of these species were not observed in Kalloni their widespread distribution and frequent occurrence in the gulf increase the risk of an episodic event.

The population sizes of potentially harmful species in the gulf are positively correlated with nutrient enrichments and negatively correlated with salinity and temperature. Furthermore, vertical differences of the most abundant dinoflagellates demonstrate the active migration of such populations to more nutrient-rich water layers, which is consistent with previous studies (Villarino et al., 1995). The fact that these potentially harmful species were almost totally absent from the oligotrophic station in the open sea indicates their preference to enclosed, nutrient-rich water bodies (Dolapsakis et al., 2008). Furthermore, during both the nutrient-rich conditions in February and the spring bloom of centric diatom species, diatoms were the dominant group over all dinoflagellates in terms of abundance, although the latter also seem to have increased diversity in the gulf (Spatharis et al., 2007b). The fact that this trend seems to be recurrent, indicates that environmental conditions in the gulf favor the proliferation of diatom species; however, the frequent presence of dinoflagellate species and their suppressed blooms suggest a potential threat to the ecosystem, demonstrated also by the abundance–occupancy relationship.

Most of the potentially bloom-forming and/or toxic species encountered in Kalloni Gulf appear to have established populations. Some of the dinoflagellates investigated in the present study such as *S. trochoidea*, *Prorocentrum lima*, *Diplopsalis lenticula* and *Amphidinium carterae* are known cyst-forming species (Matsuoka and Fukuyo, 2000). Dinoflagellate encystment and excystment are known to play a crucial role to the ecology and bloom potential of species (Peña-Manjarrez et al., 2005). Anderson and Keafer (1985) concluded that seeding by the gradual germination of cysts over many months, may provide multiple opportunities for bloom development. The ability of some of the identified species to form cysts, in addition to the increased tolerance to salinity and temperature variations (Graneli and Turner, 2006; Steidinger and Tangen, 1997) facilitates resistance to unfavorable environmental conditions and supports possible species adaptation at this given site. The dinoflagellate *A. insuetum* also belongs to a genus of cyst-forming species (Anderson, 1998). This species had no previous records in the gulf, apart from the sudden peak densities near the river plume in February, supporting the view that this bloom must have resulted from cyst germination under favorable resource supply and environmental conditions. This particular species appears as an outlier in the upper-left part of the abundance–occupancy plot, because it appeared in very high concentrations (high abundance) but in very few samples (reduced occupancy) in the gulf.

The assumption that the majority of the studied species has established populations in the gulf is also supported by their continuous presence in the water column throughout the year, also demonstrated by the abundance–occupancy relationship. The results from the population abundance–occupancy relationship in Kalloni Gulf are in agreement with previous ecological investigations showing an increase in population abundance as the occurrence of the species in samples increases (Gaston et al., 2000). One possible explanation for this trend is that species developing dense populations are more likely to have established local populations (possibly widespread) in a given area, thus enhancing their ability to bloom under favorable conditions. Furthermore, Kalloni Gulf is especially suitable for species establishment and blooming because it is a confined shallow gulf, strongly affected by nutrient-rich freshwater inputs (Spatharis et al., 2007a), offering a diverse aquatic breeding environment and seabed (Millet and Lamy, 2002). The probability of species establishment is further supported by the fact that species' densities used in the current study were averaged not only in space but also in time. Therefore, it seems that species which tend to be present throughout the year (i.e. established species) are likely to proliferate and form blooms. Consequently, apart from *P. calliantha*, blooms of which have already been observed in Kalloni Gulf, an increased risk of pronounced bloom formations can also be foreseen for the species *K. veneficum*, *H. circularisquama*, *C. tripos*, *C. lineatum*, *P. arcuatum*, and *D. sacculus*.

Therefore, it can be foreseen that nutrient loading during winter along with favorable environmental conditions (low temperature and salinity), increase the risk of HAB formation of species which have a continuous presence in the gulf throughout the year and of cyst-forming species occurring more rarely. This poses a particular threat for the ecological balance of the system, since the species described herein can be directly or indirectly harmful to other organisms (Turner and Tester, 1997). Subsequently, this may have adverse effects on human health and local economy imposing the need for monitoring at the community level especially during the winter period.

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