Climate influence on *Dinophysis* spp. spatial and temporal distributions in Greek coastal water

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Abstract: Presence of *Dinophysis* spp., the main phytoplankton genus responsible for the occurrence of lipophilic biotoxins of the okadaic acid group (Diarrhetic Shellfish Poisoning; DSP) in Greek mussels, was recorded in the period 2003–2008 in five major mussel culture areas of Greece. *Dinophysis* spp. abundances were simultaneously studied with climatological parameters (air temperature, rainfall, wind direction and velocity) and the occurrence of DSP toxic episodes in mussels. *Dinophysis* spp. blooms occurred with a seasonal periodicity. Highest cell abundances of the bloom appeared steadily between late winter and spring, whereas occurrence of DSP toxicity in mussels followed a similar time pattern, with mouse bioassay-positive samples ranging between 10.2–28.1% of the total samples analysed. Maximum mean *Dinophysis* spp. counts coincided with air temperatures in the range of 10–20°C; abundances higher than 1,000 cells L⁻¹ were more frequently recorded at air temperatures in the range of 4–20°C in combination with low rainfall and low velocity winds (<Beaufort scale 4), blowing from the north (NW, N and NE) and south (SE and S). To our knowledge, this is the first report to correlate the occurrences and abundances of potentially toxic *Dinophysis* spp. and climatological data for the majority of the shellfish production areas studied. Such observations may contribute to the clarification of the occurrence patterns of DSP toxic episodes, hence bettering the economical management of mussel production.

Key words: air temperature, Diarrhetic Shellfish Poisoning, *Dinophysis* spp., rainfall, wind

Introduction

Lipophilic biotoxins are toxic compounds mainly produced by species of the genera *Dinophysis*, *Prorocentrum*, *Protoceratium*, *Gonyaulax*, *Lingulodinium*, *Protoperidinium* and *Azadinium* (Daranas et al. 2001, Paz et al. 2004, Tillmann et al. 2009). The main toxin groups included are okadaic acid (OA) and dinophysistoxins (DTX), pectenotoxins (PTX), yessotoxins (YTX) and azaspiracids (AZA). Members of the OA group (OA, DTXs and PTXs), considered as one of the most important, are commonly referred to as Diarrhetic Shellfish Poisoning (DSP) toxins. The presence of DSP toxins is primarily connected to blooms of the toxic genus *Dinophysis* and to a lesser extent to the genus *Prorocentrum*. Species belonging to the genus *Dinophysis* have a wide distribution and are encountered in

tropical, subtropical and temperate seas (Hallegraeff & Lucas 1988, van den Hoek et al. 1995). Investigation of *Dinophysis* spp. biology has progressed considerably in recent decades, due to its increasingly recorded presence in numerous coastal areas worldwide and the accompanying shellfish toxicity (Hallegraeff 1993, Zingone & Enevoldsen 2000).

Geographical distribution and seasonal periodicity of the cosmopolitan genus *Dinophysis* varies even between adjacent areas. Potential factors contributing to these differences include, among others, environmental conditions, water movement and geomorphology of the area. Seasonal periodicity of *Dinophysis* spp. presence during the year is common in Thermaikos Gulf, Greece (Koukaras & Nikolaidis 2004, Nikolaidis et al. 2005), in other European countries (Bernardi Aubry et al. 2000, Vale & Sampayo 2003, France & Mozetič 2006, Ninčević-Gladan et al. 2008) and worldwide (Koike et al. 2000, Morton et al.

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2009, Swanson et al. 2010). These seasonal patterns seem to be partly related to regional parameters influencing *Dinophysis* blooms, such as the movement of water masses due to different densities or winds (Lindahl & Andersson 1996, Moita & da Silva 2001), temperature (Reguera et al. 1995, Hoshiai et al. 1996, Dahl & Johannessen 2001, Godhe et al. 2002) and rainfall (Vale & Sampayo 2003). Changes in these parameters may subsequently influence nutrient availability and/or water column stability, and can therefore greatly affect *Dinophysis* spp. growth patterns (Estrada & Berdalet 1997, Vale & Sampayo 2003). Moreover, other factors such as latitude, as well as water column and food web dynamics, have influences on geographical distributions (Reguera et al. 2012).

According to available data of the Greek National Reference Laboratory for Marine Biotoxins (NRLMB), marine biotoxins' episodes in Greece during the period 2000–2008 almost exclusively involved lipophilic toxins of the DSP group (Mouratidou et al. 2004, Prassopoulou et al. 2009, Louppis et al. 2010). These toxins occur in shellfish production areas almost steadily on a regular yearly basis; duration and time of toxicity occurrence, however, do not always coincide, even between neighboring areas. The possibility of reducing, or even avoiding, time coincidence between periods of lipophilic toxin-induced regulatory closures and shellfish commercialization in shellfish production areas, may be a significant contribution towards a more efficient time management of regional shellfish production. This could be partly facilitated by further clarification of *Dinophysis* spp. presence at seasonal and geographical levels in Greek coastal waters where mussel cultures are located, taking into account the potential influence of respective climatological parameters. The purpose of this study was therefore to investigate temporal and spatial distributions of potentially toxic *Dinophysis* spp. in Greek coastal waters, as well as the relationship between algal presence and climatological parameters (air temperature, rainfall, wind direction and velocity), using monitoring data from the five major Greek mussel culture areas, representing ca. 95% of Greek mussel production during the period 2003–2008. It should be pointed out that the actual target of the present study is the establishment of a practically applicable approach, and not the construction of a mathematical method. The approach, which would be easily perceivable and useful to relevant interested parties (e.g. shellfish farmers, official control personnel) is to be based on the clarification of the periodicity and effect of climatological data on the occurrence of DSP toxic episodes. In order to serve this purpose, the study design was based only on data freely accessible to the public, i.e. climatological data (air temperature, rainfall, wind direction and velocity) and official regulatory monitoring data on *Dinophysis* spp. counts and DSP toxins' presence.

Materials and Methods

Study area and sampling stations

All laboratory analysis data originated from the five major mussel culture coastal areas: Saronikos Gulf, Thermaikos Gulf, Maliakos Gulf, Gulf of Kavala and Amvrakikos Gulf (Fig. 1). Regulatory implementation of monitoring for these coastal areas falls within the jurisdiction of seven prefectural veterinary services: three for Thermaikos Gulf (Thessaloniki, Imathia and Pieria), one for Saronikos Gulf (Dytiki Attiki/Megara), one for Maliakos Gulf (Fthiotida), one for Gulf of Kavala (Kavala) and one for Amvrakikos Gulf (Preveza). The exact location of individual sampling stations for each prefecture, expressed by geographical coordinates (latitude and longitude), is provided in Table 1.

Sampling and laboratory analysis

Samples and respective data on *Dinophysis* spp. counts and presence of DSP toxins were derived from the Greek "National Program for Monitoring of Bivalve Molluscs' Production Areas for the presence of Marine Biotoxins" during the years 2003–2008; coordination was provided by the Ministry of Rural Development and Food (MRDF,

Fig. 1. Maps showing locations of sampling stations in (1) Saronikos Gulf, (2) Thermaikos Gulf, (3) Maliakos Gulf, (4) Gulf of Kavala and (5) Amvrakikos Gulf. Round marks (○) depict the location of individual sampling stations. Square marks (□) depict the location of the nearest meteorological station providing climatological data for the study. See Tables 1 and 2 for full geographic coordinates.

Table 1. Locations of sampling stations for each coastal area included in the study, expressed by geographical coordinates (longitude and latitude).

Coastal area	Prefecture	Declared annual cultured mussel production (tonnes)	Sampling station	Latitude (N)	Longitude (E)	Water column depth (m)	Mussel sampling depth (m)
Thermaikos Gulf	Thessaloniki	4,000	Kymina (HA)	40°30'22"	22°41'06"	max 15	4–8
			Peraia (TH11)	40°31'40"	22°56'33"	max 15	n/a ¹
			Aggelohori (TH14)	40°28'59"	22°48'20"	max 15	4–8
			Epanomi (TH15)	40°26'15"	22°51'33"	max 15	4–8
			Fanos Paliomanas (TH3)	40°35'01"	22°53'25"	max 20	n/a
			Paliomana (TH3-4)	40°33'59"	22°51'06"	max 20	n/a
			Naziki (TH4-5)	40°32'00"	22°47'48"	max 6 (pole cultivation) max 20 (long-line)	2–4 4–8
			Lefkoudi (TH5)	40°33'00"	22°46'24"	max 6 (pole cultivation) max 20 (long-line)	2–4 4–8
			Kavoura (TH6)	40°32'35"	22°46'02"	max 6 (pole cultivation) max 20 (long-line)	2–4 4–8
			Karabournaki (TH3A)	40°34'51"	22°6'06"	max 15	n/a
	Pieria	11,500	Alykes (P1)	40°22'30"	22°38'50"	6–15	3–4
			Makrygialos (P2A and P2B)	40°25'30"	22°37'30"	6–15	3–4
			Methoni (P3A and P3B)	40°27'37"	22°36'48"	6–15	3–4
			Variko	40°09'31"	22°33'54"	6–15	3–4
	Imathia	5,000	HA	40°30'22"	22°41'06"	max 20	2–5
			HB	40°29'15"	22°41'27"	max 20	2–5
			HPas	40°30'30"	22°40'40"	max 6	2–4
Saronikos Gulf ¹	Dytiki Attiki/Megara	300	Neraki	38°0'59.33"	23°27'55.16"	max 20	2–5
			Drepano	37°59'3.72"	23°24'44.55"	max 8	2–5
Maliakos Gulf	Fthiotida	700	Agios Ioannis Styliadas	38°53'26"	22°39'06"	6–10	2–4
			Agia Triada	38°50'49"	22°36'21"	6–10	2–4
			Molos	38°50'30"	22°39'05"	6–10	2–4
Gulf of Kavala ²	Kavala	3,000	Keramoti	40°51'42.11"	24°39'37.20"	max 15	2–5
			Nea Iraklitsa	40°51'42.80"	24°20'0.10"	max 25	2–5
			Vasova	40°52'01.15"	24°36'39.85"	max 17	2–5
			Agiasma	40°55'30.14"	24°33'23.38"	max 21	2–5
Amvrakikos Gulf ¹	Preveza	30	Koronisia	38°59'52.08"	20°56'33.29"	max 28	1–3
			Mazoma	39°1'5.51"	20°46'52.60"	max 20	1–3
			Sogono	38°59'34.93"	20°48'57.70"	max 20	1–3

¹ n/a: sampling point only for microalgal monitoring and for harvested (not cultured) shellfish species.² Sampling stations in these coastal areas are defined by four points; values provided correspond to the midpoint.

2002). The monitoring program requires weekly sampling during the whole year as the minimum frequency for regulatory controls, turning to twice-weekly whenever abundances of toxic and/or potentially toxic phytoplankton exceeded the nationally established surveillance limits. With regard to abundances of microalgal cells considered as po-

tentially toxic for the production of lipophilic toxins, including *Dinophysis* spp., the established national threshold limits designate the regulatory status of shellfish production areas as: open (1–199 cells L⁻¹), under surveillance (200–999 cells L⁻¹) and closed ($\geq 1,000$ cells L⁻¹). A closed regulatory status with regard to DSP toxicity in mussels

was designated in a production area by toxicity detection in mouse bioassays (MBA positive sample), whereas three consecutive negative samples in at least 8 days were required to re-open the production area. According to these requirements, each of the two parameters separately (increased cell abundances and MBA toxicity) or both in combination can be the cause of a regulatory closure.

All sampling was conducted by the relevant Prefectural Veterinary and/or Fisheries authorities. Cell counts of toxic and/or potentially toxic phytoplankton in seawater, specifically of the genus *Dinophysis*, were conducted by the Laboratory Unit of Toxic Marine Microalgae (LUTMM), Department of Biology, Aristotle University of Thessaloniki, and all data were forwarded to the NRLMB by the prefectural authorities, as indicated in the monitoring program. Analyses for presence or absence of DSP toxicity in mussel samples were conducted by the NRLMB, Institute of Food Hygiene of Thessaloniki, MRDF. At each station, integrated seawater samples (aliquots of 500 mL) for phytoplankton identification were collected on a weekly basis from the whole water column (as required by the EU Regulation 854/2004) using a dividable PVC tube-sampler equal to the depth of each station (see Table 1). Seawater samples were immediately preserved with Lugol's solution. At the same time shellfish (mussels: *Mytilus galloprovincialis* Lamarck, 1819) samples were also collected. Both seawater and shellfish samples were transferred to the relevant laboratories within 24 h.

Sea water samples were analyzed for the presence of toxic and/or potentially toxic phytoplankton by Utermöhl's sedimentation method (Utermöhl 1958), using inverted microscopy for identification and enumeration of the phytoplankton cells. Briefly, after homogenizing the samples, sub-samples were allowed to settle in 25 mL sedimentation chambers. *Dinophysis* spp. cells were counted under an inverted microscope at 100x and 400x magnifications so that the detection limit was 40 cell L⁻¹. The quantitative analysis for low abundances was performed by counting the detected cells on the entire surface of the settling chamber, whereas in the case of high level abundances a number of randomly selected fields were counted until reaching a count of at least 400 cells.

Mussel hepatopancreas (HP) samples were tested for the presence of lipophilic toxins by the mouse bioassay (MBA) protocol of Yasumoto et al. (1978). Briefly, a 20 g portion of HP was extracted thrice with 50 mL acetone each time and filtered through a cellulose filter. The combined toxin extract was evaporated to dryness and resuspended in 1% Tween-60 to a final volume of 4 mL (5 g HP/mL). Each one of three mice (Albino Swiss, 18–20 g body weight) was injected intraperitoneally with 1 mL of this solution. The criterion of toxicity established by the EU Regulation 2074/2005 (2005) was employed, which is the death of two out of three mice within 24 h of injection with an extract equivalent to 5 g of HP or 25 g of whole flesh tissue. This constitutes a positive result for the presence of the lipo-

philic toxins mentioned in the above Regulation, including OA, DTX-1, -2 and -3. The mice were allowed laboratory feed and water *ad libitum* throughout the observation period. All animal manipulations were performed in accordance with the EU Directive 86/609/EEC (1986) and the EU Recommendation 2007/526/EC (2007), under official license from the Prefectural Veterinary Service of Thessaloniki, Greece.

Climatological data

Climatological data (air temperature, wind direction and velocity, rainfall) were provided by the Hellenic National Meteorological Service (HNMS) from selected meteorological stations located as close as possible to the areas of interest (Table 2 and Fig. 1). The use of air temperature data in place of relevant seawater temperature measurements in the present study can be justified due to the rather limited maximum depth (up to 28 m) of the sampling sites (Table 1). According to Kara et al. (2009), the difference between sea surface temperature and water temperature at depths of at least 34 m in the Aegean Sea presents a maximum variation of 0.5°C, which indicates a well mixed water mass. On the other hand, Poulos et al. (1997) studied the relationship of air temperature versus sea surface temperature in the Aegean Sea and reported that the main factors affecting sea surface temperatures (SST) are the seasonal atmospheric conditions, which are reflected in the air temperature (AT). Theoharatos & Tselepidaki (1990) have established that the cross correlation coefficient between SST and AT, for 15 stations from the Aegean Sea, is $r^2 > 0.80$ (significant at the 95% level). Monthly variations in SST and AT showed that, on an annual basis, the sea temperature appeared to lag in relation to that of the overlying airmass by the order of a month. Finally, a principal component analysis carried out by Vlahakis & Pollatou (1993) showed that SST changes, related to AT fluctuations, occur almost simultaneously throughout the Aegean Sea.

Climatological data obtained by the HNMS usually required initial transformation in order to be in the appropriate form for a combined statistical analysis in conjunction

Table 2. Details and locations of the Hellenic National Meteorological Service meteorological stations included in the study.

HNMS Code	Station	Station location		
		Altitude (m)	Latitude (N)	Longitude (E)
619	Trikala Imathias	0.8	40°6'0.00"	22°33'0.00"
622	Mikra	4.8	40°31'0.00"	22°57'60.00"
624	Chrysoupoli	5.4	40°53'60.00"	24°36'0.00"
643	Aktio	4.0	38°58'0.00"	20°46'0.00"
675	Lamia	17.4	38°51'0.00"	22°23'60.00"
718	Elefsina	31.0	38°4'0.00"	23°33'0.00"

with the laboratory data. For air temperature and rainfall data, originally consisting of individual recordings at different times of day, daily averaging was considered necessary to obtain a single value for each day and meteorological station. Wind velocity data required transformation from knots to the Beaufort scale. For wind direction data, expressed initially in degrees in the range of 0–360° with no daily averaging possible, calculation of the prevailing wind direction on a daily basis was conducted according to the method described by the US Environmental Protection Agency (2000), taking into account both wind speed raw data and wind direction “azimuth”. Intensity of rainfall was classified according to the American Meteorological Society (2012) definitions as: a) “light” the rate of fall varying between a trace and 2.5 mm per hour; b) “moderate” from 2.6 to 7.6 mm per hour and c) “heavy” over 7.6 mm per hour.

Data processing and statistical analysis

Some initial data categorization and transformation were required in order to facilitate graphical representation and statistical analysis by the software package. Despite the fact that sampling intervals were weekly, sampling dates for each year were divided into 24 fortnights, encoded by an alphanumeric code indicating the specific month and fortnight, e.g.: JAN/1=January-first fortnight, JUN/2=June-second fortnight and DEC/2=December-second fortnight, in order to facilitate the statistical evaluation.

Statistical analysis and graphic representation was conducted using Minitab® version 15 (Minitab Inc., Coventry, UK). Normality of data was evaluated by the Kolmogorov-Smirnov test, and homogeneity of variance was examined using residual graphs and the Levene test. In cases where statistical normality and/or homogeneity was lacking, data transformation was applied, e.g. *Dinophysis* spp. counts were transformed onto a logarithmic scale [$\log(x)+1$] to obtain a continuous variable with no zero values. In all cases, statistical transformation produced normality and homogeneity of variance (Petridis 2000). Statistical methods employed included: (a) one-way Analysis of Variance (ANOVA), with the mean confidence interval set at 95% and calculated by the ANOVA error and significance of difference of means tested by the Fisher’s least significant difference (LSD) test and (b) Principal Components Analysis (PCA). PCA was conducted on the whole dataset to investigate the contribution of all studied parameters (fortnight of the year, wind direction, air temperature, rainfall, logarithmic *Dinophysis* spp. counts and wind velocity) to the occurrence of DSP toxic episodes in the total study area. Selection of principal components (PC) was based on a combination of criteria: (a) the Kaiser rule according to which selected PCs have to bear an Eigenvalue larger or equal/near to one (eigenvalue greater than one rule) and (b) explained variance of at least 70%, whereas loading factors for explaining contributions within a PC were taken into

account when their absolute value was greater than 0.5 (Jolliffe 2002). Finally, graphical representation of data involved the use of interval plots, dispersion charts, contour plots and a PCA loading plot, showing only the first two PCs due to restrictions of the Minitab software.

Results

Geographical distribution of *Dinophysis* spp.

Statistical evaluation of *Dinophysis* spp. abundances (total number of analyzed samples, $n=6,686$) over the whole study area across the total six-year monitoring period indicated that mean *Dinophysis* spp. abundances (cells L^{-1}) were comparable ($p>0.05$) for some areas, while statistically significant differences ($p\leq 0.05$, $F=75.72$, $F_{crit}=0.01$) were observed between certain others (Fig. 2). Based on statistical significance testing results, prefectures can be divided into three groups (Fig. 2; depicted within the same ellipse) with regard to their mean *Dinophysis* spp. counts. The first group contains only the area of Kavala (Gulf of Kavala), with very low *Dinophysis* spp. cell abundances, which could thus be characterized as the prefecture with the lowest incidence of DSP toxic episodes (Vlamiis et al. 2010). This is strongly supported by the fact that during the whole study period, with a total of 564 seawater samples analyzed, *Dinophysis* spp. abundances in Kavala reached the regulatory level of 1,000 cells L^{-1} only once, exceeded the surveillance level of 200 cells L^{-1} in only eight instances, and the presence of DSP toxins in mussels resulted in only three closures with a maximum duration of 17 days. The second group consists of the prefectures of Dytiki Attiki (Saronikos Gulf), Imathia and Pieria (Thermaikos Gulf) and Fthiotida (Maliakos Gulf), in which the mean *Dinophysis* spp. cell abundances ranged within similar ($p>0.05$) levels. Finally, the prefectures of Thessaloniki

Fig. 2. *Dinophysis* spp. abundance during the period 2003–2008 by Prefecture (⊕: mean value; ±: 95% confidence interval of the mean). Means within the same ellipse are not significantly different ($p>0.05$). Number of averaged samples (n) per area is presented above each mean value.

(Thermaikos Gulf) and Preveza (Amvrakikos Gulf) comprise the third group with the highest mean values of *Dinophysis* spp. abundances. It should be noted that the mean confidence interval range for Thessaloniki prefecture was more limited than that of Preveza, due to the fact that the mean value for the former was derived by the analysis of a significantly larger amount of raw data (see Fig. 2), as in the latter case samplings were conducted much less frequently or even sporadically in certain cases.

No statistically significant differences ($p>0.05$) were observed between *Dinophysis* spp. cell abundances at individual sampling stations located in the same area/prefecture in the cases of Saronikos Gulf (Dytiki Attiki: 2 sampling stations; $p>0.05$, $F=2.92$, $F_{crit}=3.87$), Gulf of Kavala (Kavala: 4 sampling stations; $p>0.05$, $F=0.57$, $F_{crit}=2.62$), Maliakos Gulf (Fthiotida: 3 sampling stations; $p>0.05$, $F=0.08$, $F_{crit}=3.02$) and/or Amvrakikos Gulf (Preveza, 3 sampling stations; $p>0.05$, $F=0.01$, $F_{crit}=3.06$) (data not shown). However, this was not the case with the results of the mean *Dinophysis* spp. abundances in Thermaikos Gulf. Significant differences ($p\leq 0.05$, $F=5.68$, $F_{crit}=1.88$) were noted between mean counts at certain sampling stations located in the northwestern Thermaikos Gulf and in Thessaloniki Bay (inner sea area of Thermaikos Gulf), within the jurisdiction of Thessaloniki prefecture (Fig. 3). Specifically, sampling stations located outside the boundaries of Thessaloniki Bay, in central Thermaikos Gulf, had significantly lower *Dinophysis* spp. cell abundances compared to all other stations, except for the sampling station TH14, located at the boundaries of Thessaloniki Bay. Counts at the latter station significantly differed only from those of stations located in the inner part of Thessaloniki Bay, whereas no significant differences ($p>0.05$) were found between *Dinophysis* spp. abundances at any of the sampling stations located inside Thessaloniki Bay.

During the whole study period the maximum *Dinophy-*

Fig. 3. *Dinophysis* spp. abundance at the sampling stations in Thessaloniki prefecture during the period 2003–2008 (⊕: mean value; ±: 95% confidence interval of the mean). Mean values with the same letter (a, b, c) on the right of the bars are not significantly different from each other.

sis spp. cell abundances were recorded in the mussel production areas of western Thermaikos Gulf (Thessaloniki, sampling stations TH4-5: 87,280 cells L⁻¹ and TH6: 86,680 cells L⁻¹, both in February 2008) and Saronikos Gulf (Dytiki Attiki, sampling station Neraki: 76,280 cells L⁻¹ in December 2006), in full agreement with the longest regulatory closure periods recorded for the same sampling stations due to presence of DSP toxins in mussels, i.e. 153 days (December 2007–May 2008), 160 days (December 2007–June 2008) and 139 days (December 2006–April 2007), respectively. The dominant *Dinophysis* species in these cases was *Dinophysis* cf. *acuminata* (LUTMM, personal communication), later redefined by Papaefthimiou et al. (2010) as *Dinophysis* cf. *ovum*.

Temporal variation of *Dinophysis* spp. abundances

Available data during the monitoring period 2003–2008 demonstrate an evident increase of *Dinophysis* spp. mean abundances during the year 2004 followed by 2006, with these maximum values being statistically significantly different from those of the remaining years, as well as between each of those years ($p\leq 0.05$, $F=25.78$, $F_{crit}=2.22$). On the other hand, mean *Dinophysis* spp. cell abundances for the year 2003 were considerably lower ($p\leq 0.05$) compared to the other five years included in the study (Fig. 4a). These findings are mostly in disagreement with the intensity of the relevant DSP toxic episodes in mussels, as represented by the number and percentage rate of DSP positive samples per year (Fig. 4b). Except for the fact that both maximum values occurred in 2004, all other studied years fail to show a clear correlation between cell abundances and frequency of DSP positive samples, indicating that there are other factors determining the intensity of the episodes.

Yearly trends for individual areas followed more or less the patterns outlined above for the mussel production areas of Saronikos, Thermaikos and Maliakos Gulfs. Slight differences were observed in the Gulf of Kavala, where the highest *Dinophysis* spp. abundances were recorded in the year 2006, followed by 2005, although with generally very low values as indicated in the previous section ("Geographical distribution of *Dinophysis* spp."). Moreover, in the case of Amvrakikos Gulf, the year 2004 also had the highest *Dinophysis* spp. abundances but this was followed by the year 2005, while no data exist for the year 2003 for this area (Fig. 4c).

The lowest mean *Dinophysis* spp. counts within the calendar year (Fig. 5) were recorded in late August (fortnight AUG/2). From this point on, a slow but rather steady increase was observed, while the highest values were noted between late March (fortnight MAR/2) and early June (fortnight JUN/1), with an evident peak in early May (fortnight MAY/1). Minor differences in the exact starting time of *Dinophysis* spp. blooms were observed between the individual years included in the study, but in all cases the highest counts appeared from late winter to spring and the

Fig. 4. (a) *Dinophysis* spp. abundance in the total study area, by year ($\oplus$: mean value; $\pm$: 95% confidence interval of the mean). Number of averaged samples (n) per year is presented above each mean value; (b) total counts of DSP positive (filled bars) and negative (unfilled bars) mussel samples and percent rates of DSP positive over total samples during the period 2003–2008, by year; (c) *Dinophysis* spp. abundance in Amvrakikos Gulf (Prefecture of Preveza), by year ($\oplus$: mean value; $\pm$: 95% confidence interval of the mean).

seasonal periodicity of the phenomenon was evident (Fig. 6). The distribution of the mean number and relevant percent rate of DSP- positive mussel samples during the calendar year indicates that the highest numbers occurred in late winter–spring time (Fig. 7), coinciding with the

increase in *Dinophysis* spp. abundances (Fig. 5). The increase observed in the number of DSP negative and total samples at the time when mussel toxicity tends to decline (MAR/2 to JUN/1; Fig. 7) is a result of the national monitoring programme requirements of three consecutive negative samples at an interval of at least 8 days for the re-opening of a closed production area. A second, although minor, increase of DSP positive samples occurred during late summer–autumn, due to the simultaneous increased presence of benthic dinoflagellates, such as *Prorocentrum* spp. (NRLMB, unpublished data).

Correlation of individual climatological parameters with *Dinophysis* spp. cell counts

The whole dataset (individual counts) of *Dinophysis* spp. abundances exceeding levels leading to closures ($\geq 1,000$ cells L⁻¹) were plotted by use of dispersion charts against the climatological parameters studied. Results indicate the following trends for individual parameters:

a) *Air temperature*: Abundance levels higher than 1,000 cells L⁻¹ were more frequently recorded at air temperatures within the range of 4–20°C, with the extremes being –2°C and 31°C, whereas the maximum abundance of *Dinophysis* spp. (87,280 cells L⁻¹) was recorded at an ambient air temperature of 10°C (Fig. 8a). A minor deviation was noted in the mussel production zones of Maliakos Gulf (Fthiotida prefecture), where such abundances were encountered more frequently at a somewhat higher air temperature range, between 15–21°C, and occurred towards the end of spring/beginning of summer (data not shown). When changes of mean cell abundances of *Dinophysis* spp. were compared with those of environmental air temperature within the calendar year in an interval plot (Fig. 5), the highest increasing trends were observed in the air temperature range between ca. 10–20°C, which coincided with two time periods: one between early March and early May (see also the previous section “Temporal variation of *Dinophysis* spp.”), and another between early October and December.

b) *Rainfall*: *Dinophysis* spp. cell counts exceeding 1,000 cells L⁻¹ coincided more frequently with drought or low rainfall levels and much less frequently with more intense rainfall (Fig. 8b). Despite the fact that all *Dinophysis* spp. abundances exceeding 10,000 cells L⁻¹ coincided with rainfall of moderate intensity, it is evident that the majority of closure *Dinophysis* spp. levels were recorded during periods of light rain or drought.

c) *Wind direction*: Increased *Dinophysis* spp. counts leading to regulatory closures were recorded with most wind directions. All three highest cell densities observed coincided with NW winds, followed by the next highest values being encountered with prevailing winds of SE, NE and N directions (Fig. 8c). Where the yearly pattern of wind directions is observed in relation to *Dinophysis* spp. abundances (Fig. 6), it is noteworthy that a prevailing SW wind (225°) seems to be present quite regularly around the

Fig. 5. *Dinophysis* spp. abundance and ambient air temperature (°C) based on the total dataset of sampled Prefectures during the period 2003–2008 (⊕: mean value; ⊥: 95% confidence interval of the mean). Vertical dotted lines depict the most important timepoints regarding increase of *Dinophysis* cell abundances versus air temperature.

Fig. 6. *Dinophysis* spp. abundance on each sampling date across the whole study area of Greece during the period 2003–2008 and prevailing wind direction (●: *Dinophysis* spp. mean daily count; ◆: prevailing daily wind direction expressed in degrees).

time that *Dinophysis* spp. abundances start to decline in the majority of the yearly blooms.

d) *Wind velocity*: *Dinophysis* spp. counts higher than 1,000 cells L^{-1} coincided exclusively with weak (Beaufort 1–2) to moderate (Beaufort 3–4) winds (Fig. 8d). All three highest recorded *Dinophysis* spp. values (87,280 cells L^{-1} , 86,680 cells L^{-1} and 76,280 cells L^{-1}) were observed in conjunction with wind velocities of Beaufort 1–2.

Combined effects of climatological parameters on *Dinophysis* spp. cell counts

The four climatological parameters studied were contour-plotted in pairs together with *Dinophysis* spp. cell counts [total dataset (individual counts) of the study], in order to investigate the possible existence of combined climatological effects. Contour levels for *Dinophysis* spp. abundances were selected in order to coincide with the

Fig. 7. Distribution of total counts of DSP positive (filled bars) and negative (unfilled bars) mussel samples and percent rates of DSP positive over total samples per fortnight within the calendar year during the period 2003–2008.

nationally established regulatory levels leading to surveillance and/or closure of mussel production areas. All possible combinations of parameters were studied; however only results showing specific trends are presented and further discussed.

a) *Wind direction versus time period of the calendar year*: *Dinophysis* spp. cell counts exceeding regulatory closure levels (1,000 cells L^{-1}) tended to appear in combination with specific wind directions (N, NW, NE and SE) only during winter months (December to February). No other evident tendencies were observed for the remaining months of the year (Fig. 9a).

b) *Air temperature versus wind direction*: abundances of *Dinophysis* spp. above the regulatory level of 1,000 cells L^{-1} were recorded in two separate combinations. The first case was low air temperatures (below 5°C)

Fig. 8. *Dinophysis* spp. abundances (dots represent individual counts) at levels exceeding the national regulatory closure limits ($\geq 1,000$ cells L^{-1}) versus: (a) air temperature; (b) rainfall; (c) wind direction and (d) wind velocity.

combined with SE prevailing wind directions, while in the second case air temperature ranged between 8–15°C and was exclusively associated with NW, N and NE winds (Fig. 9b).

c) *Air temperature versus time period of the calendar year*: regulatory levels of *Dinophysis* spp. were exceeded towards the end of the year in combination with air temperature ranges of either -2 – $2^{\circ}C$ or 11 – $17^{\circ}C$, whereas in the beginning of the year they coincided with air temperatures between 9 – $13^{\circ}C$ (Fig. 9c).

d) *Air temperature versus rainfall*: increased *Dinophysis* spp. counts leading to regulatory closures ($\geq 1,000$ cells L^{-1}) were recorded in drought periods when air temperature ranged between -2 and $2^{\circ}C$ or between 18 and $20^{\circ}C$. When low rainfall occurred, such increased counts occurred in combination with an air temperature of ca. $14^{\circ}C$, whereas with more intense rainfall similar *Dinophysis* spp. abundances were recorded at an air temperature range of 9 – $12^{\circ}C$ (Fig. 9d).

e) *Wind direction versus wind velocity*: counts of *Dinophysis* spp. exceeding $1,000$ cells L^{-1} , tended to occur with a combination of low velocity wind (Beaufort 1) blowing from the NE and NW directions (Fig. 9e).

Correlation of parameters influencing the occurrence of DSP toxic episodes

Results of the principal components' analysis (PCA) are

summarized in Table 3 and Figure 10. Analysis of the correlation matrix indicated that the first four PCs (PC1, PC2, PC3 and PC4) complied with the set criteria, accounting in total for a proportion of 78.6% of the data variance (Table 3). Proportions of accounted variance, together with the loading factors of the parameters studied for each of the PCs, indicate that PC1, accounting for 25.2% of the variance is most probably related to the factor of time, expressed either as individual points of time or as seasonality/periodicity. This hypothesis is supported by the fact that the highest loading factor values in this PC belong to the parameters of air temperature and fortnight of the year, which are tightly connected with time and seasonality. The same observation applies for *Dinophysis* spp. counts which, as shown in previous sections, are also highly influenced by the time of the year with apparent periodicity. As expected, these parameters were strongly correlated (Fig. 10); air temperature and fortnight of the year were strongly positively correlated, and both were negatively correlated with *Dinophysis* spp. counts. PC2, accounting for 21.8% of the variance, seems to be related to the interactive influence of wind, with high loading factor values for both wind velocity and direction (Table 3). These two parameters were strongly positively correlated (Fig. 10), whereas there is no apparent connection with either fortnight of the year or *Dinophysis* spp. counts. PC3 accounted for 16.8% of the data variance and is evidently strongly related to the

Fig. 9. *Dinophysis* spp. abundances in relation to: (a) wind direction and time period of the year; (b) air temperature and wind direction; (c) air temperature and time period of the year-graph areas indicated by “x” represent impossible combinations; (d) air temperature and rainfall; (e) wind direction and velocity.

influence of rainfall on DSP toxic episode occurrence, whereas PC4, accounting for a further 14.8% of the data variance, was connected with the individual influence of wind direction (Table 3).

Discussion

Geographical distribution of *Dinophysis* spp.

Highest mean counts of potentially toxic *Dinophysis* spp. during the years 2003–2008 were recorded in the mussel production areas of Thermaikos (Thessaloniki prefecture) and Amvrakikos Gulfs. Nikolaidis et al. (2005),

investigating the presence of harmful microalgal episodes in Greek coastal waters in the period 2000–2004, also noted that most blooms occurred in the Thermaikos and Amvrakikos Gulfs. This could be due to intense eutrophication in both areas resulting from increased nutrient inputs from river discharges and/or anthropogenic factors, such as intensive aquaculture, industrial, sewage and land-farming runoff (Balopoulos & Friligos 1993, Kormas et al. 2001, Sylaios & Theocharis 2002, Lekka et al. 2004, Dolapsakis et al. 2008). On the other hand, the significantly lower *Dinophysis* spp. abundances in the mussel production zones of Saronikos (Prefecture of Dytiki Attiki), Western Thermaikos (Prefectures of Imathia and

Table 3. Principal Component Analysis (PCA): Eigenanalysis of the correlation matrix to investigate contribution of the studied parameters (fortnight of the year, wind direction, air temperature, rainfall, logarithmized *Dinophysis* spp. counts and wind velocity) and loading factors for the parameters studied (variables) for each of the principal components selected for further analysis by PCA. Bold characters indicate statistically significant ($P \leq 0.05$) contributions within a principal component.

	Principal Component (PC)					
	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	1.5109	1.3084	1.0085	0.8901	0.7220	0.5601
Proportion of variance accounted for	0.252	0.218	0.168	0.148	0.120	0.093
Cumulative proportion of variance accounted for	0.252	0.470	0.638	0.786	0.907	1.000
Variable						
Fortnight of year	0.490	0.424	-0.147	-0.133		
Wind direction	0.138	-0.521	-0.206	-0.769		
Air temperature	0.617	-0.150	0.145	0.384		
Rainfall	-0.020	-0.122	-0.936	0.325		
LOG- <i>Dinophysis</i> spp.	-0.504	-0.324	0.117	0.277		
Wind velocity	0.327	-0.638	0.156	0.246

Fig. 10. Correlation matrix presenting the loading factors of the parameters influencing DSP toxic episode occurrence tested by the Principal Components' Analysis (PCA). Only the first (PC1) and second (PC2) principal components are depicted. Proportions of variance accounted for: PC1=25.2%, PC2=21.8%, PC3=16.8%, PC4=14.8%.

Pieria) and Maliakos Gulfs (Prefecture of Fthiotida) may be attributed to the more open character of Saronikos and Maliakos Gulfs, whereas the mussel production zones of western Thermaikos are located in the central and external parts of the Gulf where hydrographic conditions differ to those of the inner part of the Gulf (Balopoulos & James 1984). The latter observation is also confirmed by the study of Koukaras & Nikolaidis (2004) reporting lower abundance levels of *D. cf. acuminata* at the sampling stations of Pieria and Imathia (Thermaikos Gulf), possibly as a result of dilution of coastal waters with offshore waters from the North Aegean Sea. This is further supported by the significantly lower *Dinophysis* spp. abundances in the Gulf of Kavala (present study), a particularly open gulf with reduced input of nutrients from freshwater discharges.

The significant differences in *Dinophysis* spp. cell counts leading to the observed sampling station "grouping" in the mussel production areas of Thermaikos Gulf, of the Thessaloniki prefecture jurisdiction, are also in agreement with the findings of Koukaras & Nikolaidis (2004), who reported the outer parts of Thermaikos Gulf (middle and southern parts) always have significantly lower numbers of *Dinophysis* cells than in the inner gulf. In the same context, Balopoulos & Friligos (1993) recorded significant differences in inorganic nutrient concentrations between the Gulf of Thessaloniki and the central and external parts of Thermaikos Gulf, with the highest values obtained in the Gulf of Thessaloniki, followed by northwestern Thermaikos and southeastern Thermaikos Gulf. The highest values, measured in the Gulf of Thessaloniki, were mainly attributed to the discharge of unprocessed urban waste from the city of Thessaloniki in the inner part of the Gulf (Nikolaidis et al. 2006) and could also point to a connection with the long durations of regulatory closures observed in the mussel production areas of the western Thermaikos Gulf.

On the other hand, cell count differences among sampling areas could be attributed to a number of factors influencing water column stability (stratification), such as water circulation, bottom morphology/topography and depth, which lead to different patterns of vertical mixing/turbulence, as for instance it is known that *Dinophysis* spp. counts start to increase when there is water column stability (Maestrini 1998). Furthermore, thin layer formation during marked stratification or intense physical accumulation forced by upwelling relaxation, which are commonly reported for *Dinophysis* spp., should also be taken into account. Since in the present study the entire water column was sampled with a tube, the factor of individual depths could also play a role in the significantly different *Dinophysis* counts observed between areas (Reguera et al. 2012).

Temporal distribution of *Dinophysis* spp.

Significant differences between mean counts of potentially toxic *Dinophysis* spp. during the years 2003–2008 might be attributed to yearly differences in climatological and hydrological parameters, which could influence nutrient availability in the respective areas and/or change the water column conditions, as disruption of the latter seems to have an important impact on growth of *Dinophysis* spp. (Estrada & Berdalet 1997, Vale & Sampayo 2003). Moreover, such parameters could also affect growth and availability of species used as prey by *Dinophysis*, such as the ciliate *Mesodinium rubrum* (Lohmann, 1908) [synonym *Myrionecta rubra* (Jankowski, 1976)], as well as competition between species (Crawford & Lindholm 1997, Park et al. 2006). For example, four *Dinophysis* species [*D. acuminata* (Claparède & Lachmann, 1859), *D. caudata* (Saville-Kent, 1881), *D. fortii* (Pavillard, 1923) and *D. infundibulus* (Schiller, 1928)] feed on the marine ciliate *M. rubra* when grown with the cryptophyte *Teleaulax* (Park et al. 2006, Nishitani et al. 2008, Nagai et al. 2008). Relevant experimental data clearly showed that these species could not grow by ingestion of only the *Teleaulax* sp. but instead required *M. rubra* as prey for enabling their vegetative growth and sequestering the ciliate plastids in order to utilize them as kleptoplastids (Nagai et al. 2008). Kamiyama et al. (2010) and Nagai et al. (2011) clarified that toxin production by *Dinophysis* species was confirmed only when they fed on *M. rubra* as the food source, indicating that abundances of the latter species are also very important for *Dinophysis* in order to grow and produce toxins in natural environments.

On the other hand, the lack of agreement between yearly *Dinophysis* spp. counts and the intensity of the relevant DSP toxic episodes in mussels, indicated by the number of DSP positive samples per year, may be attributable to a number of reasons, such as variation in toxin production capacity of implicated *Dinophysis* species, the possible existence of different strains of a single species with variable dominance between different years, and fluctuating abundance of other phytoplankton species which could also be ingested by the mussels. Furthermore, there were fluctuations in the numbers of mussel samples received for testing, as well as fluctuations in the relative contribution of DSP-positive samples originating from benthic dinoflagellates (e.g. *Prorocentrum* spp.), which is a quite common phenomenon in late summer–autumn periods in Greek coastal areas (Vlamiš et al. 2010, NRLMB, unpublished data). It is noted that both the sampling method (entire water column) as well as the cell counting method employed (as defined by the relevant legislation) result in certain limitations which could influence both apparent spatial variation in cell counts and the lack of a relationship between cell density and the occurrence of DSP-positive mussel samples. Natural populations of *Dinophysis* cells are known to aggregate within thin layers; there-

fore by sampling the entire water column, the number of cells in contact with the mussel stock may be under or overestimated. In addition, the use of a pipe or hose to sample the entire water column, could cause a “dilution effect”, which could be enhanced at sampling sites with greater depth. Furthermore, the counting method involves *Dinophysis* determination at the genus level rather than recognition of individual species, thus ignoring differences in toxin profiles and concentrations between cells of different species. These two limitations need to be taken into account when discussing issues like spatial variation in cell counts and the lack of an apparent of relationship between cell density and the occurrence of DSP-positive mussels samples (Reguera et al. 2012).

During the whole study period (2003–2008), *Dinophysis* spp. presence followed an evident periodicity within the calendar year in most of the areas studied, with maximum abundances recorded in the winter–spring months and negligible abundances in summer and autumn. Seasonal periodicity has also been reported in other studies from the Thermaikos Gulf, Greece (Koukaras & Nikolaidis 2004, Nikolaidis et al. 2005), several European countries (Bernardi Aubry et al. 2000, Vale & Sampayo 2003, France & Mozetič 2006, Ninčević-Gladan et al. 2008), and worldwide (Koike et al. 2000, Morton et al. 2009, Swanson et al. 2010). The only deviation between studies from different countries concerns the exact time period in which maximum abundances occur within the calendar year, which is a result of differences in the timing of seasonal climate periodicity and hydro-chemical parameters in different geographic regions.

Effect of climatological parameters on *Dinophysis* spp. counts

Air temperature

The trend of *Dinophysis* spp. presence increasing within the air temperature range of 10–20°C, as well as the more frequent occurrence of abundances exceeding 1,000 cells L⁻¹ when air temperatures ranged between 4°C and 20°C, at least in the mussel production zones of Thermaikos and Saronikos Gulfs, is connected to the fact that *D. cf. acuminata* is the dominant *Dinophysis* species in these areas (Koukaras & Nikolaidis 2004, Nikolaidis et al. 2005, LUTMM, personal communication). This a species has a preference for seawater temperatures common during the winter and spring months (Table 4) in Greece (Ninčević-Gladan et al. 2008). In a recent study, Papaefthimiou et al. (2010), using ribosomal and mitochondrial genetic sequence data, re-identified the *D. cf. acuminata* described from Greek coastal waters as *D. cf. ovum* (Schütt, 1895), a species belonging to the same complex. In agreement with our findings, maximum abundances of *D. cf. acuminata* have been recorded at the seawater temperature range of 12–16°C in Thermaikos Gulf in the years 2000–2002 (Koukaras & Nikolaidis 2004), and other researchers have also reported similar seawater temperature ranges for

Table 4. Winter and spring seawater temperatures of the Greek Gulfs included in the study, according to the available bibliography.

Coastal area	Season	Water temperature range (°C)	Reference
Thermaikos Gulf	Winter	11.5–12.5	Koukaras & Nikolaidis (2004)
	Spring	13–16	
Saronikos Gulf	Winter	12.5–15	Siokou-Frangou et al. (1998)
	Spring	14–16	
Amvrakikos Gulf	Winter	12–13	Vareli et al. (2012)
	Spring	18–20	
Gulf of Kavala	Winter	11–12.5	Sylaios et al. (2005)
	Spring	12–20	
Maliakos Gulf	Winter	11.8–14.1	Kormas et al. (2002)
	Spring	16.9–23.9	

maximum counts of *Dinophysis* spp. when *D. acuta* (Ehrenberg, 1840), *D. acuminata* or *D. norvegica* (Claparède & Lachmann, 1859) were the species responsible for the blooms (Reguera et al. 1995, Godhe et al. 2002, Díaz et al. 2011).

In Maliakos Gulf, abundances of *Dinophysis* spp. higher than 1,000 cells L⁻¹ were more frequently recorded when the air temperature ranged between 15–21°C, probably due to the fact that *D. sacculus* (Stein 1883) and *D. caudata*, the growth of which is favored at slightly higher seawater temperatures, constitute the dominant *Dinophysis* species in this area (Bernardi Aubry et al. 2000, Koukaras & Nikolaidis 2004, Nikolaidis et al. 2005, France & Mozetič 2006). Similarly, maximum abundances of *D. sacculus* and *D. caudata* have been systematically recorded in the warmer seasons of the year by other researchers (Bernardi Aubry et al. 2000, Caroppo 2001, France & Mozetič 2006, Ngyuen et al. 2006).

Temperature is the most important factor affecting growth of *Dinophysis* spp. (Reguera et al. 1995). Consequently, the observed differences in seasonality with regard to development of the same or closely related *Dinophysis* species between different areas and/or countries are actually related to the exact time periods when the optimum temperature range is reached due to variation in local climatological conditions. Nevertheless, the influence of other environmental conditions such as water stability due to thermal stratification and/or prevalence of low winds and low rainfall, which occur during periods of the year when the air temperature is indicative of periods when mussel stocks are more susceptible to the occurrence of DST contamination), should not be neglected, as it is possible that there is no direct cause-effect relationship between air temperature and shellfish toxicity.

Wind direction and velocity

Maximum *Dinophysis* spp. abundances in the present study occurred during a variety of prevailing wind directions, but were mostly correlated with northern (NW, N and NE) and southern (SE and S) directions and were generally favored by low velocity winds up to Beaufort 4. Prevailing wind direction is closely related to the phenomena of upwelling and downwelling, as well as with changes in water circulation (Escalera et al. 2010). Reports in the literature are often controversial with regard to specific wind directions favoring the development of *Dinophysis* spp., as well as of other dinoflagellates, but there is a rather general consensus that the main controllers of *Dinophysis* spp. growth, in agreement with our findings, are connected to winds blowing from northern and southern directions and affecting the phenomena of water upwelling and downwelling (Reguera et al. 1995, Blanco et al. 1998, Tilstone et al. 2000, Reguera 2003, Velo-Suárez et al. 2008). Reguera (2003) specifically indicated that high concentrations of *Dinophysis* spp. can develop after wind reversal (from northerlies promoting upwelling, to southerlies favouring downwelling events); in the latter case, high abundances are the result of physical accumulation (interacting with behaviour) rather than of active vegetative growth. It is highly possible that such influences are regulated by the particular climatological conditions and geomorphology of each of the different areas under investigation. In agreement with the results of the present study where increased abundances of *Dinophysis* spp. coincided with winds of low velocity, Estrada & Berdalet (1997) have pointed out that dinoflagellates generally have higher mean abundances during periods of calmness, in contrast to diatoms, which are favored by the presence of intense winds.

Rainfall

Results of the present study indicated that *Dinophysis* spp. abundances exceeding the established closure limit of 1,000 cells L⁻¹ were more frequently recorded in conjunction with drought or low rainfalls. Vale & Sampayo (2003), who studied the effects of rainfall on the seasonality of DSP in Aveiro lagoon, Portugal, concluded that low rainfall volume, preceded by heavy rainfall that would lead to a higher nutrient input into the basin, was associated with higher *D. acuminata* abundances and the occurrence of more intense DSP toxic episodes. Rainfall was identified as an extremely important factor controlling *in situ* blooms of toxic *Dinophysis* spp. and consequently its toxicity implications in shellfish in Aveiro lagoon. Lowering of rainfall resulted in lower river drainage, which translated into higher salinity and increased stability of the water column inside the lagoon. Growth of *Dinophysis* spp. and dinoflagellates in general, is reported to be closely connected to water column stability. Consequently, factors that contribute to limiting water column disruption, such as low rainfall, can be favorable for dinoflagellate development (Estrada & Berdalet 1997). The findings of Vale & Sampayo (2003) are in partial agreement with those of the present study, excepting that no cases of heavy rainfall preceding the low rainfall periods when *Dinophysis* spp. reached their maxima were observed in the present study (data not shown). This difference is attributed to both the specific conditions of the study area, as well as to the fact that Greece is a country with generally low rainfall. Aveiro lagoon is characterized as a complex network structure with an intertidal morphology and, furthermore, lagoons are considered to be productive ecosystems where the hydrological conditions and water salinity can change rapidly due to geomorphology (Lopes & Dias 2007). In contrast, open systems such as the gulfs in our study, have geomorphological, hydrographic and dynamic differences that do not always allow for easy intercomparisons (Álvarez-Salgado et al. 2011). In this context, the model proposed by Vale (2012) for predicting DSP contamination according to rainfall accumulation during the months preceding the relevant toxic episodes would be of limited use in the case of Greek coastal areas.

Combined effects of climatological parameters

Development of *Dinophysis* spp. blooms and highest abundances were mainly favored by two combinations of climatological parameters: a) northerly winds at air temperatures between 10–15°C during the initial months of the calendar year and b) southerly or south-easterly winds with lower air temperatures within the range of -2°C and 2°C towards the end of the calendar year. Both combinations also involved winds of low velocity and rainfall ranging from drought to low levels. These findings obviously do not constitute the exclusive conditions that may favour the development of *Dinophysis* spp. blooms in Greek coastal waters, but might be taken as a rough indicator in order to

anticipate increases in *Dinophysis* spp. abundances.

The principles upon which both the above combinations can be explained are the least possible disruption of water column conditions in conjunction with the presence of a favorable temperature range. The various algal taxonomic groups demonstrate a different level of susceptibility to agitation and therefore changes in water column stability, with an ascending order of sensitivity as follows: chlorophytes, Cyanobacteria, diatoms and dinoflagellates (White 1976, Thomas & Gibson 1990, Petersen et al. 1998). This is further confirmed by observations in the natural environment where the presence of dinoflagellates is connected with areas exhibiting minimal disturbance (Margalef 1978, Estrada & Berdalet 1997). In agreement, therefore, to the findings of our study, climatological conditions disrupting water column stability, such as strong winds and heavy rainfall, are not considered in the literature as a favorable environment for dinoflagellates, and thus for species belonging to the genus *Dinophysis*.

Conclusion

Harmful blooms of *Dinophysis* spp. in Greek coastal waters during the period 2003–2008, showed a clearly evident periodicity, peaking in the winter and spring months, with highest counts recorded regularly in winter and spring months and a negligible presence in the summer and autumn months of the year. The highest *Dinophysis* spp. abundances were recorded in Amvrakikos and Thermaikos Gulfs, and specifically in the Gulf of Thessaloniki, while the lowest values occurred in the Gulf of Kavala. Air temperature seemed to be highly correlated with *Dinophysis* spp. presence; maximum counts coincided with the air temperature range of 10–20°C, while abundances exceeding the nationally established limits for closure of mussel production areas ($\geq 1,000$ cells L⁻¹) were more frequently recorded at air temperatures between 4–20°C. Northerly (NW, N and NE) and southerly (SE and S) wind directions and low wind velocities generally favored the presence of *Dinophysis* spp., whereas maximum counts occurred mostly coinciding with drought or low rainfall. Such observations, derived from historical monitoring data, could be of significant value with regard to clarification of DSP toxic episode occurrence patterns and might subsequently lead to better economical management of mussel production.

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