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Management of the production and harvesting in Greek mussel culture areas, according to the occurrence of lipophilic toxic episodes

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Abstract

Toxic episodes due to lipophilic biotoxins, mainly Diarrhetic Shellfish Poisoning (DSP) toxins, constitute one of the major problems in Greek mussel culture, leading to significant economic losses per year. The present study aimed at establishing occurrence trends of lipophilic toxic episodes throughout the year, in order to serve as a tool for better scheduling and management of production and harvesting, and involved five major mussel culture areas of Greece, representing ca. 95% of Greek production (Saronikos, Thermaikos, Maliakos, Gulf of Kavala and Amvrakikos Gulf). Data were obtained during the years 2003-2008 and included closure time periods due to both presence of lipophilic biotoxins in shellfish and increased abundance of *Dinophysis* spp. populations, the main phytoplankton genus responsible for the occurrence of DSP lipophilic biotoxins in mussels, as well as respective climatological parameters (wind direction and intensity, rain precipitation and temperature). Statistical analysis of the data confirmed the empirically evident yearly periodicity of DSP toxic episodes occurrence, presenting significant differences between specific production areas. For instance, closures in production areas of Saronikos and Thermaikos Gulfs occurred mostly during winter/spring, whereas spring/autumn was the common occurrence time for areas in Maliakos and Kavala Gulfs. The suggested approach of evaluating past data regarding time period, duration and factors affecting the occurrence of DSP toxic episodes, can significantly contribute towards a more efficient financial management of mussel cultures.

Keywords: Diarrhetic shellfish poisoning, *Dinophysis* spp., Management, Mussel culture, Lipophilic toxins, Production areas

Introduction

Occurrence of biotoxins and possibility of human poisoning is an international multidisciplinary phenomenon, with serious implications on both public health as well as in the economy of the shellfish culture industry. It can unexpectedly arise at any time, regardless of region and climatological conditions (Hallegraeff 2003), depending on the presence of the relevant harmful algal bloom (HAB). The current situation in Greece, according to data of the

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National Reference Laboratory on Marine Biotoxins (NRLMB) of the Ministry of Rural Development and Food (MRDF), is not different from the international trends (Katikou 2005).

The major reason for the importance of biotoxins control is the fact that marine biotoxins are chemical compounds with increased heat tolerance and therefore heat processing of bivalve molluscs prior to their consumption can not reduce their toxicity. Depending on the poisoning symptomatology, which is connected to biotoxins' structure and mode of action, five types of syndromes are recognized (UNESCO 1995; Daranas et al. 2001):

- Diarrhetic Shellfish Poisoning (DSP)
- Paralytic Shellfish Poisoning (PSP)
- Amnesic Shellfish Poisoning (ASP)
- Neurotoxic Shellfish Poisoning (NSP)
- Ciguatera fish Poisoning (CFP)

Currently, the only approach towards removal of toxins from shellfish is natural detoxification, whereby shellfish are maintained in the sea for several weeks once the toxic episode has disappeared (Gestal-Otero 2000). The problem is that natural detoxification is a slow process and depends on the metabolic activity of the molluscs, which is inhibited by several environmental conditions such as low temperature (Gonzalez et al. 2002). In this context, the best and until now the only available means of confronting this problem is the adoption of preventive measures, which require implementation of an intensive system for the monitoring of both marine environment and shellfish.

Since 1999, the MRDF, as the competent authority of Greece for the implementation of European Union (EU) legislative requirements regarding marine biotoxins (initially EU Directive 91/492/EEC and currently EU Regulations 853/2004 and 2074/2005), has set up and conducts the "National Program for Monitoring of Bivalve Molluscs Production Areas for the Presence of Marine Biotoxins". This program imposes monitoring at a weekly basis for the presence of the three marine biotoxins groups included in the EU legislation (DSP – Diarrhetic Shellfish Poisoning or now termed as lipophilic toxins group, PSP – Paralytic Shellfish Poisoning and ASP – Amnesic Shellfish Poisoning) in all Greek areas where authorized production (aquaculture or harvesting) of bivalve molluscs takes place.

According to the available data of the NRLMB, in the time period of 2000-2008, marine biotoxins toxic episodes in Greece are almost exclusively caused by the DSP/lipophilic toxins group, and specifically toxins of the okadaic acid (OA) group. Presence of the OA group toxins is mainly connected to blooms of the toxic dinoflagellate genus *Dinophysis* spp. (Mouratidou et al. 2004; Prassopoulou et al. 2009; Louppis et al. 2010), and to a lesser extent of the genus *Prorocentrum* spp. Lipophilic (DSP) toxins occur almost steadily at a yearly basis in different sampling areas; however time occurrence and duration of toxicity does not always coincide between different, even adjacent, sampling areas. In this context, investigation and clarification of lipophilic toxins temporal and spatial distribution in the whole of Greece, which constitutes the purpose of the present study, could result in a more effective time management of shellfish production in different areas, in order to avoid as much as possible coincidence of regulatory closure periods due to lipophilic toxins' episodes with periods that shellfish attain their marketable size.

Materials and methods

Data analyzed in the present study refer to the time period of the years 2003-2008. Data regarding cell counts of toxic and/or potentially toxic phytoplankton, and specifically of the genus *Dinophysis*, were provided by the Laboratory of Marine Toxic Microalgae, Department of Biology, Aristotle University of Thessaloniki, whereas data concerning toxic episodes and specifically presence or absence of DSP toxicity in mussels come from the NRLMB, Institute of Food Hygiene of Thessaloniki, MRDF.

According to the requirements of the "National Program for Monitoring of Bivalve Molluscs Production Areas for the presence of Marine Biotoxins", during the whole year the minimum sampling frequency for the conduction of regulatory controls was weekly, or even more frequent whenever abundances of toxic and/or potentially toxic phytoplankton exceeded the established surveillance limits.

Production areas and sampling points

Data originate from implementation of the aforementioned monitoring program in the five most important Greek gulfs where mussel culture activity is in place, namely: Saronikos, Thermaikos, Gulf of Kavala, Amvrakikos and

Maliakos Gulfs (Fig. 1). Regulatory controls implementation competence for the above gulfs is distributed among seven prefectures in total and specifically in the respective prefectural Veterinary and Fisheries Services.

Graphical representation – Statistical analysis

Graphical representation and statistical analysis of the data included in the study was conducted using the logistical software Microsoft Excel® (Microsoft Corporation) and the statistical package Minitab® version 15 (Minitab Incorporation). Statistical analysis involved the methods of single factor analysis of variance (One-way ANOVA) with confidence interval of the mean set at 95% and calculated from the ANOVA error, as well as Multiple Binary Logistic Regression with goodness of fit checked using the Pearson chi-square (χ^2) test.

Fig. 1. Satellite view of Greece indicating the gulfs where mussel production and sampling points of the present research are localized. 1: Saronikos Gulf, 2: Thermaikos Gulf, 3: Gulf of Kavala, 4: Amvrakikos Gulf, 5: Maliakos Gulf.

Results

Overall presence of DSP toxicity in mussels – Intensity of toxic episodes

During the time period of 2003-2008, a total of 5,137 mussel samples, derived from the areas included in the present study, were tested for DSP toxicity. Of these, 432 samples (8.4%) were found to be DSP positive by mouse bioassay, whereas majority of both tested as well as positive samples originated from the production areas of Thermaikos Gulf.

Single factor ANOVA for investigation of the relationship between *Dinophysis* spp. cell abundances and mussel DSP toxicity revealed a statistically significant connection (F=209.90, P < 0.01). Toxic episodes intensity (occurrence of mussel toxicity) was further studied by means of the Pearson chi-square (χ^2) test, taking into account the respective mean abundances of *Dinophysis* spp., for each different year and prefecture. Within the time period of 2003-2008 (Table 1a), mussel toxicity was found to be very intense compared to mean *Dinophysis* spp. cell concentrations in the years 2004 and 2007, whereas in the case of years 2005 and 2006 occurrence of the phenomenon was extremely weak.

With regard to different prefectures of the study, the same analysis method revealed that intensity of mussel toxicity occurrence was significantly larger in production areas of Thessaloniki and West Attiki compared to relevant mean *Dinophysis* spp. cell concentrations, while the phenomenon occurred in production areas of Fthiotida and Imathia at a significantly weaker level (Table 1b).

Table 1. Intensity of mussel DSP toxicity in relation to mean abundances of *Dinophysis* spp cells: (a) by year and (b) by prefecture with Pearson's chi-square (χ^2) test. Probability values related to intensity of the phenomenon and falling within the range of -2.0 to +2.0 are considered as statistically significant

(a)		(b)	
Year	Probability of toxicity occurrence	Prefecture	Probability of toxicity occurrence
2003	0.930	West Attiki	2.8344
2004	7.742	Imathia	-2.2992
2005	-7.524	Thessaloniki	3.4154
2006	-5.380	Kavala	-0.2974
2007	3.191	Pieria	-0.3688
2008	0.889	Preveza	0.2093
		Fthiotida	-4.4157

DSP toxicity in mussels – Evident particularities of production areas

Distribution of DSP positive samples within the year in individual production areas (Fig. 2) shows two distinct time periods for the presence of DSP toxicity in mussels. The first one which occurs in winter – spring months of the year, concerns production areas of Saronikos, Thermaikos and Amvrakikos Gulfs (Fig. 2a,g) and seems to be directly related to cell concentration levels of *Dinophysis* spp. The second period of DSP toxicity occurs in summer – autumn months of the year and does not seem to be whatsoever related to abundance levels of *Dinophysis* spp. This toxicity is obviously derived from the presence, in high concentrations, of benthic microalgal species also capable of producing DSP toxins, such as Prorocentrum spp. which commonly occurs in Greek waters mostly in summer months. Presence of DSP toxicity in production areas of the Gulf of Kavala and Maliakos Gulf occurs almost exclusively in summer – autumn months (Fig. 2h-i). In certain production areas both types of DSP toxic episodes can be found; such areas are located in Saronikos Gulf and the East coasts of Thermaikos Gulf (Fig. 2a,d).

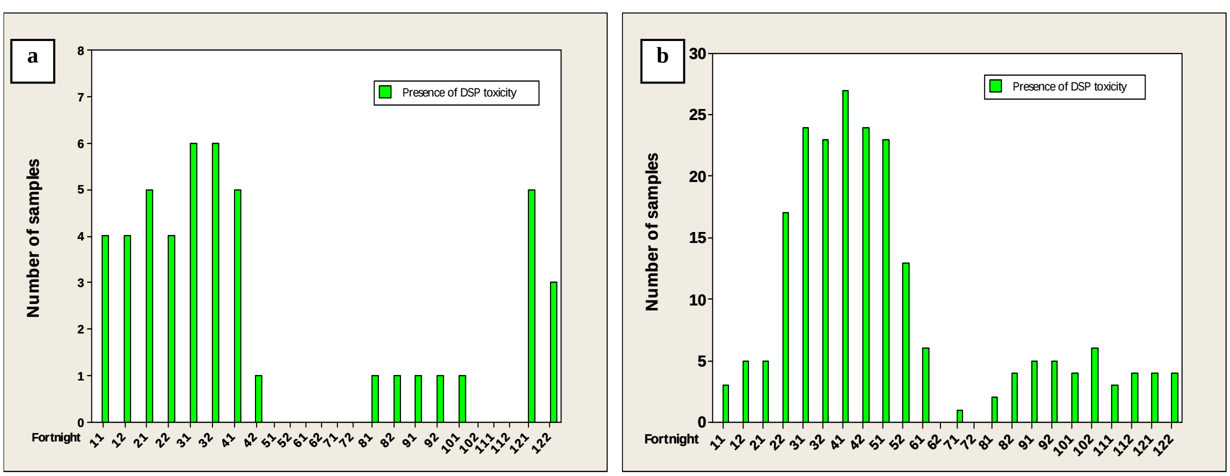


Fig. 2. Distribution of DSP positive samples during the year, categorized at fortnights, in the production zones of the prefectures: a) West Attiki, b) Thessaloniki (all areas), during the years 2003-2008.

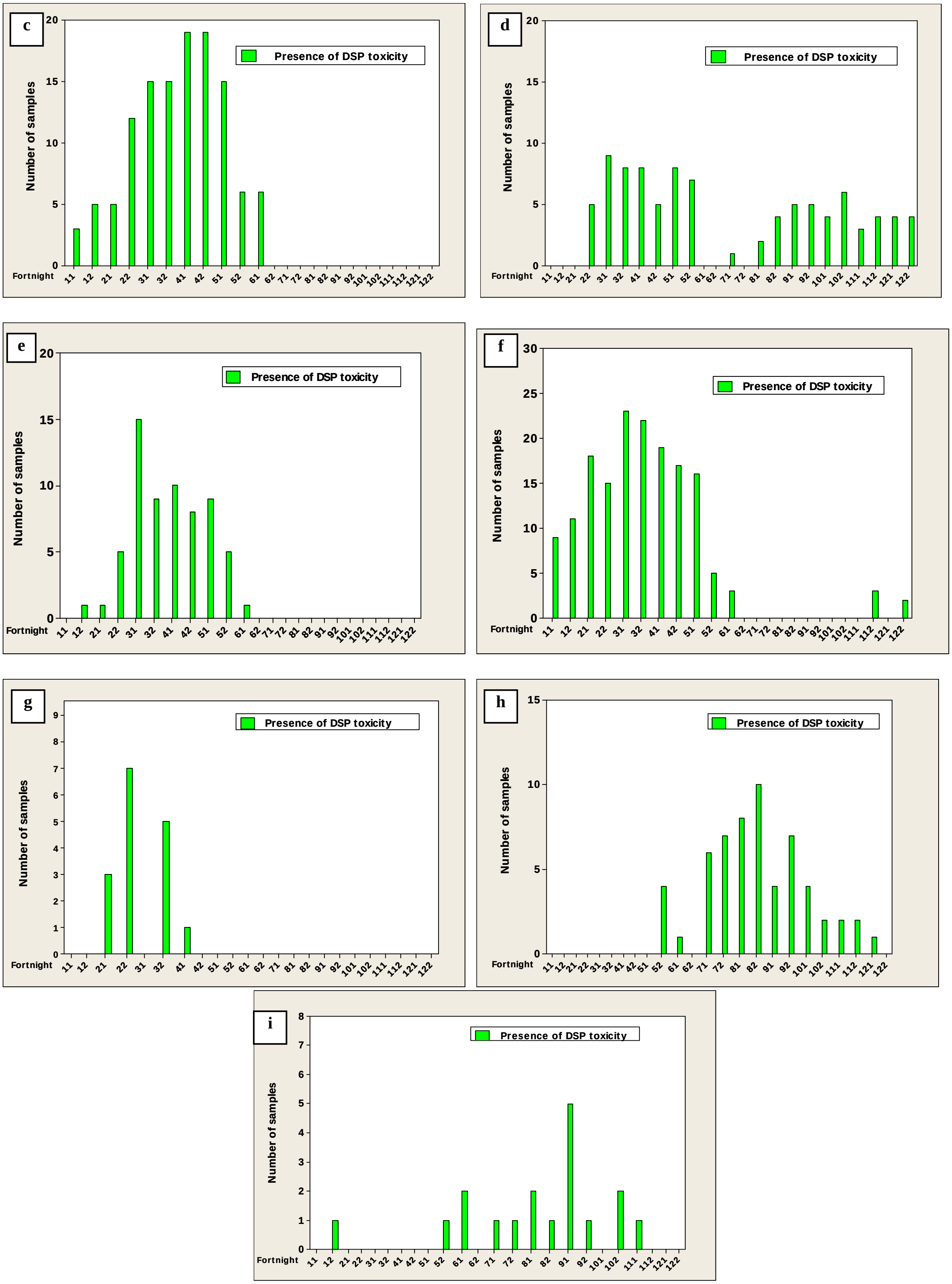


Fig. 2. (Continued), c) Thessaloniki (Thermaikos west coasts), d) Thessaloniki (Thermaikos east coasts), e) Imathia, f) Pieria, g) Preveza, h) Kavala and i) Fthiotida, during the years 2003-2008.

Time periods with DSP regulatory closures during the years 2003-2008

Graphical representation of the DSP toxic episodes using the time periods when regulatory closures were imposed, either due to presence of DSP toxicity in mussels or due to cell counts of *Dinophysis* spp. exceeding the regulatory limits, depicts to a great extent the fact that the majority of the winter – spring DSP toxic episodes follow and/or concur time-wise with increased cell abundances of the genus *Dinophysis*. Figure 3 presents such indicative diagrams of time periods when regulatory closures are in force for DSP toxic episodes. In the case of sampling points Drepano of the West Attiki Prefecture (Fig. 3a) and Halastra-Kavoura of the Thessaloniki Prefecture (West coasts of Thermaikos Gulf, fig. 3b), a time concurrence of the two causes of regulatory closures is evident in winter and spring periods. On the other hand, no such correlation can be observed in production areas of prefectures where the rule is the occurrence of "summer – autumn" DSP toxic episodes, e.g. in the sampling points Keramoti of the Kavala Prefecture (Fig. 3c) and Molos of the Fthiotida Prefecture (Fig. 3d).

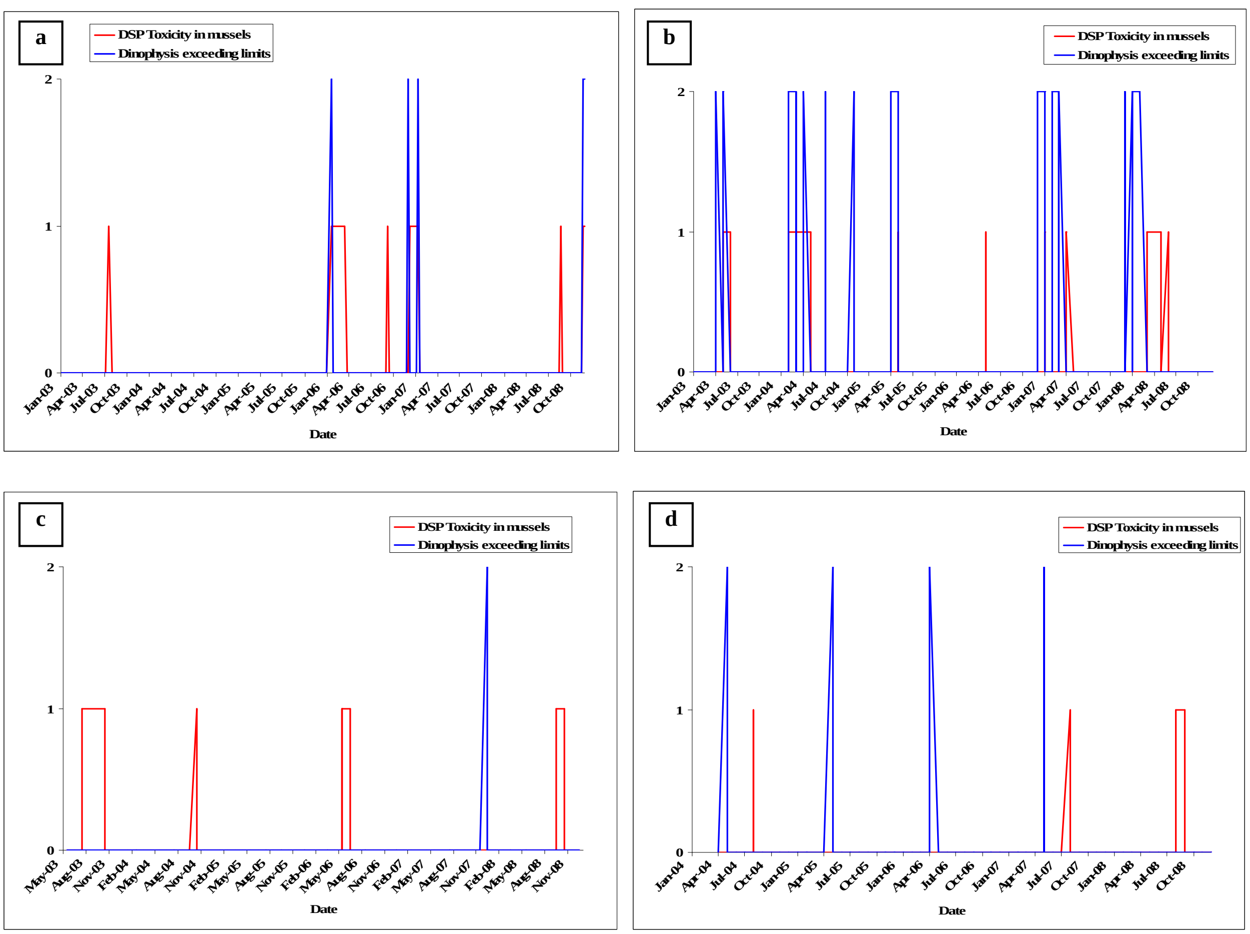
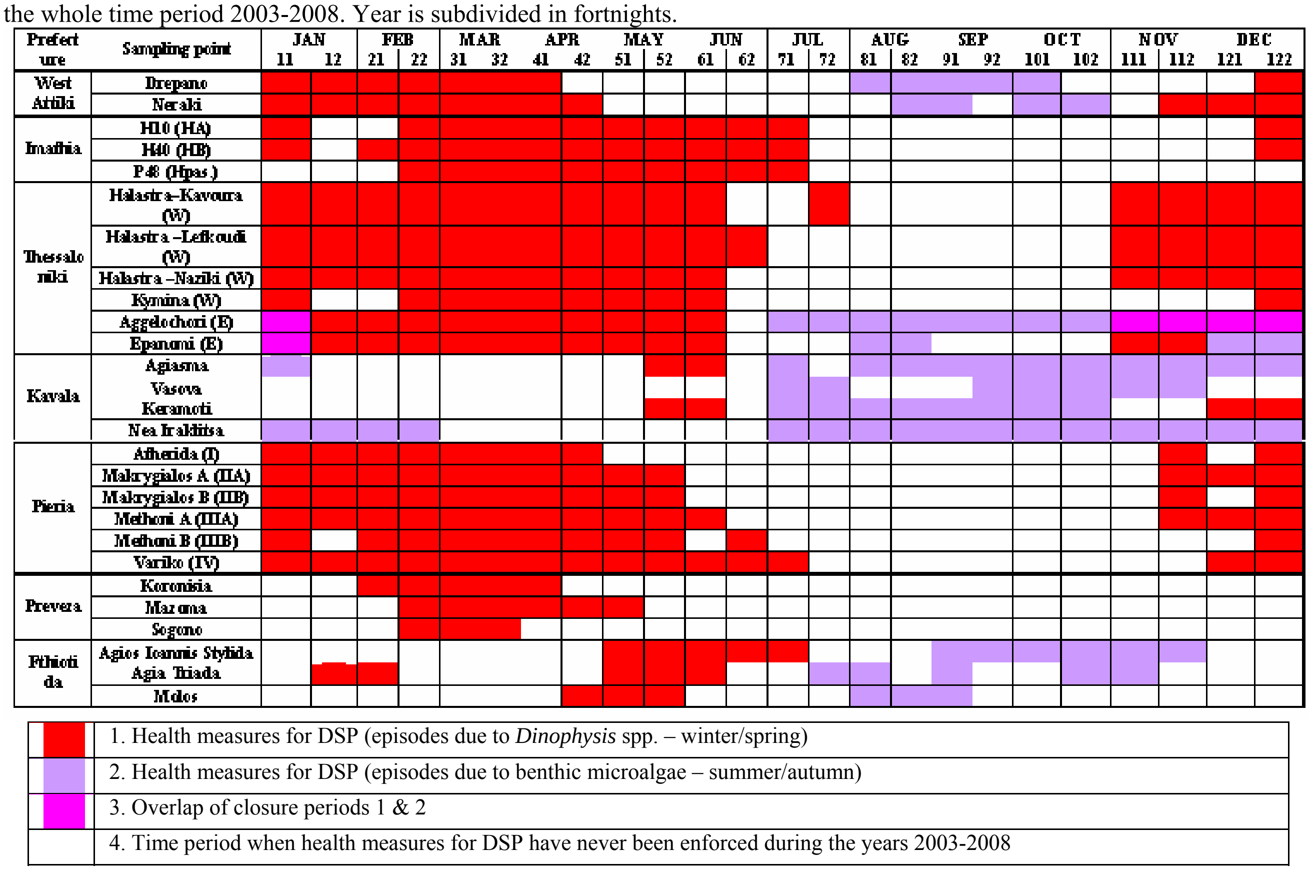


Fig. 3. Time periods according to cause of regulatory closures for DSP toxic episodes at the sampling points: (a) Drepano/ Prefecture of West Attiki, (b) Halastra-Kavoura, Prefecture of Thessaloniki, (c) Keramoti/Prefecture of Kavala and (d) Molos/ Prefecture of Fthiotida.

Temporal distribution of preventive health measures (closure periods) enforced due to cell concentrations of *Dinophysis* spp. exceeding the regulatory limits and/or due to presence of DSP toxicity in mussels, in all prefectures and their individual sampling points included in the study during the period 2003-2008 is shown in Table 2. It is evident that periods of DSP toxic episodes' occurrence present regionally characteristic particularities during the year. The most important are described below:

a) Production areas of West Attiki Prefecture: both "winter – spring" as well as "summer – autumn" DSP toxic episodes are observed. No health measures have been enforced between May and July during the study period.

Table 2. Depiction of preventive health measures (closure periods) enforced in the production areas included in the study during the whole time period 2003-2008. Year is subdivided in fortnights.



b) Production areas of Thessaloniki Prefecture: two distinct trends are observed, according to geographical localization. No “summer – autumn” DSP toxic episodes have ever been recorded in the sampling points located in the west side of the Gulf of Thessaloniki and Thermaikos Gulf (Kavoura, Lefkoudi, Naziki, Kymina) and no health measures have been enforced between July and October, with one exception in the second fortnight of July in Kavoura.

The situation is quite different in sampling points located in the east side of the Gulf of Thessaloniki and Thermaikos Gulf (Aggelochori and Epanomi), where both “winter – spring” as well as “summer – autumn” DSP toxic episodes are observed. In the sampling point Aggelochori, distribution of enforced health measures is more widespread covering almost the whole year, with the only exception being the second fortnight of June. In the sampling point Epanomi time periods without enforcement of health measures have occurred in the months July, September and October.

c) Production areas of Pieria Prefecture: time occurrence of health measures presents, more or less, similar trends to those of production areas of Thessaloniki Prefecture located in the west part of Thermaikos Gulf, without any records of “summer – autumn” DSP toxic episodes.

Overall during the whole study period in the sampling points of Pieria, no health measures for DSP toxic episodes have been enforced between the second fortnight of July and the first fortnight of November, whereas in some of the individual sampling points time periods free of health measures were more extended time-wise.

d) Production areas of Imathia Prefecture: trends with regard to time periods of regulatory closures are similar to those mentioned for production areas of both Thessaloniki Prefecture located in the west part of Thermaikos Gulf and Pieria Prefecture, without any records of “summer – autumn” DSP toxic episodes. No health measures for DSP toxic episodes have been enforced between the second fortnight of July and the first fortnight of December.

e) Production areas of Kavala Prefecture: the time period when health measures are enforced is completely different to those of the above production areas. DSP toxic episodes recorded are almost exclusively of the “summer – autumn” type. No health measures for DSP toxic episodes have been enforced in the sampling points located in the

Gulf of Keramoti (Keramoti, Agiasma and Vasova) between the second fortnight of January and the first fortnight of May, as well as during the second fortnight of June.

The situation is somewhat different in the production area of Nea Iraklitsa, located in the west part of the Gulf of Kavala, where duration of health measures was generally longer, with the only period free of health measures being between March and June.

f) Production areas of Fthiotida Prefecture: both “winter – spring” and “summer – autumn” DSP toxic episodes have been recorded, with relatively shorter durations compared to those of Thermaikos and Saronikos Gulf. Within the study period in all three production areas, no health measures for DSP toxic episodes have been enforced between the beginning of December until the first fortnight of January, as well as between mid-February and mid-April. Time periods free of health measures are longer in individual sampling points.

g) Production areas of Preveza Prefecture: within the years 2003-2008, regulatory closured were enforced between February and the second fortnight of May, without any records of “summer – autumn” DSP toxic episodes. Despite the fact that in the rest of the year no health measures have ever been imposed during the whole study period, it should be noted that the available data for these production areas are only indicative and very inadequate. The reason for this is that sampling was conducted very occasionally, without complete conformance to the weekly sampling frequency foreseen by the monitoring program.

Discussion

In the total of the six years studied (2003-2008) an evident periodicity was observed in each production area with regard to occurrence of DSP toxic episodes, regardless of the type of the “winter – spring” or “summer – autumn” character of the episodes.

Periodicity in the occurrence of DSP toxic episodes is a phenomenon recorded at a world-wide level. In Portugal, Vale and Sampayo (2003) concluded that starting from the year 1994 shellfish contamination with toxins of the DSP complex, and specifically OA, was repeated at a yearly basis and was mostly connected to presence of the microalgal species *Dinophysis* cf. *acuminata*. Their study showed that, in all years, concentrations of DSP toxins higher than 2 μg/g hepatopancreas were more frequently detected between June and September, less frequently in the adjacent months, May and October, but never detected between December and April.

These increased concentrations also coincided with high cell counts of the species *D. acuminata*, which is closely connected to the occurrence of DSP toxic episodes in shellfish. Recorded abundances exceeding 500 cells/l were distributed between April and September, whereas frequency of such counts was larger than 40% in May, June, July and September. Similar conclusions were reached by Nikolaidis et al. (2005), who investigated the presence of toxic and harmful microalgae during the time period of 2000-2004 in Greek coastal waters and especially in Thermaikos Gulf. During the recorded outbreaks of diarrhetic poisoning in 2000 and 2001 (Economou et al. 2007), and at the same time periods, high cell counts of *Dinophysis* were repeatedly recorded between February and April of each year, with peak abundances occurring in February.

Similar periodicity with regard to *Dinophysis* spp. growth has also been reported in other European countries (Bernardi Aubry et al. 2000; France and Mozetič 2006; Ninčević-Gladan et al. 2008), as well as world-wide (Koike et al. 2000; Morton et al. 2009; Swanson et al. 2010). The only deviation is the specific time when highest cell counts occur, which lies in different parts of the year, depending on geographical position and obviously on the individual climatological conditions of each area.

Species belonging to the genus *Dinophysis* are widely distributed and occur in tropical, subtropical, as well as temperate seas (Hallegraeff and Lucas 1988; van den Hoek et al. 1995). Changes in climatological parameters, however, such as wind intensity and direction, temperature and rainfall, can disturb water column conditions and can, therefore, have a direct or indirect impact on the structure of the phytoplankton community, as by definition plankton consists of organisms suspended in the water column (Estrada and Berdalet 1997). Hence, it is expected that regional climatological conditions can greatly influence, among other things, the occurrence of DSP toxic episodes.

Conclusion

Presence of DSP toxicity in mussels is connected to a large extent with the presence of high cell counts of the genus *Dinophysis*. This relationship only concerns the case of "winter – spring" DSP toxic episodes, whereas no such connection is observed in the case of "summer – autumn" DSP toxic episodes. The latter are attributed to other toxic microalgal species capable of producing lipophilic toxins, such as *Prorocentrum* spp. and *Ostreopsis* spp. With regard to spatial distribution of DSP toxicity in mussels, the most heavily contaminated areas are those located in Thermaikos and Saronikos Gulfs, whereas DSP toxic episodes in production areas of Kavala and Fthiotida seem to be milder in terms of both intensity and duration.

Of particular interest concerning duration and intensity of DSP toxic episodes are those production areas where both "winter – spring" and "summer – autumn" toxic episodes occur, as well as the fact that the latter type of episodes is observed only in certain areas.

For each prefecture, production area and/or sampling point, based on the data of six consecutive years (2003-2008) derived from the MRDF monitoring program, it is evident that within the year there are time periods in which health measures for lipophilic toxins have never been enforced, regardless of the episodes' intensity in individual years. These "ideal" time periods could be taken into account in each Greek production area, in purpose of rational management and effective scheduling of both mussel culture production and marketing of the product.

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