Botanica Marina 51 (2008): 449–463 © 2008 by Walter de Gruyter • Berlin • New York. DOI 10.1515/BOT.2008.067

Seasonal variability in *Dinophysis* spp. abundances and diarrhetic shellfish poisoning outbreaks along the eastern Adriatic coast

Živana Ninčević-Gladan*, Sanda Skejić, Mia Bužančić, Ivona Marasović, Jasna Arapov, Ivana Ujević, Natalia Bojanić, Branka Grbec, Grozdan Kušpilić and Olja Vidjak

Institute of Oceanography and Fisheries, Šetalište I. Meštrovića 63, 21000 Split, Croatia,
e-mail: nincevic@izor.hr

* Corresponding author

Abstract

Annual dynamics and ecological characteristics of the genus *Dinophysis* spp. and associated shellfish toxicity events were studied from 2001 to 2005 during monitoring fieldwork in the coastal waters of the eastern Adriatic Sea. Analysis of the seasonal occurrence of *Dinophysis* species identified *D. acuminata* and *D. sacculus* as typical spring species, *D. caudata*, *D. fortii* and *D. rotundata* as summer and late summer species and *D. tripos* as a winter species. The highest abundances occurred when there were large differences between surface and bottom temperatures and salinities. *D. caudata*, *D. sacculus* and *D. rotundata* abundances had significant relationships with Δt, while the highest abundances of *D. acuta*, *D. fortii* and *D. tripos* were associated with high Δs values. Much higher abundances of *D. caudata* and *D. fortii* in offshore compared to inshore waters of the northern Adriatic Sea and the significant inverse relationship of these species' abundances with salinity suggested the possibility of their transport by Italian river-influenced coastal waters towards the eastern Croatian coast during the summer season and under stratified conditions. Toxicity events occurred more frequently in the more eutrophicated northern Adriatic Sea than in the southern Adriatic Sea and mostly succeeded the rainfall periods. Diarrhetic shellfish poisoning (DSP) toxin profile analyses identified okadaic acid and yessotoxin as the main DSP toxins occurring in Croatian waters.

Keywords: Adriatic Sea; algal toxins; *Dinophysis* spp.; shellfish toxicity.

Introduction

Dinoflagellates of the cosmopolitan genus *Dinophysis* (Sournia 1986) occur in various coastal waters (Hallegraeff 2003), including those of the Adriatic Sea (France and Mozetič 2006). The ecology and ecophysiology of *Dinophysis* species are poorly understood, mostly because they occur in low abundance, but also due to difficulties in culturing. Research on *Dinophysis* species increased greatly after they were linked to algal biotoxins and diarrhetic shellfish poisoning (DSP) (Yasumoto et al. 1980). The main dinoflagellates causing DSP belong to genera *Prorocentrum* and *Dinophysis*. In contrast to *Prorocentrum*, *Dinophysis* blooms are rare, but they can induce poisoning even at low cell densities (Bruno et al. 1998).

Sedmak and Fanuko (1991) reported the presence of several *Dinophysis* species in the Adriatic Sea. Previous research conducted in the northern Adriatic Sea and along the western coast showed that *Dinophysis* distribution follows a strong seasonal pattern (Sidari et al. 1995a, Bernardi Aubry et al. 2000, Vila et al. 2001). In eastern Mediterranean waters, increased abundances of *Dinophysis* spp. can be detected at different times of the year (Koukaras and Nikolaidis 2004). Seawater temperature and water column stability seem to be the most important factors controlling their abundances (Delmas et al. 1992, Bernardi Aubry et al. 2000, Koukaras and Nikolaidis 2004).

The occurrence of shellfish poisoning in the Adriatic Sea was first noted in 1989 in the northern part (Boni et al. 1992, Della Loggia et al. 1993, Marasović et al. 1998). Toxic compounds other than the more frequently detected okadaic acid (OA) and dinophysistoxin-1 (DTX-1) were found to be involved in DSP phenomena (Pavela-Vrančić et al. 2002).

This study analyses data obtained during the harmful algal bloom (HAB) monitoring program on shellfish resources in Croatian coastal waters of the eastern Adriatic Sea from 2001 to 2005. The purpose of this program was (1) to find out which *Dinophysis* species appear in Croatian coastal waters and to determine their seasonal and spatial occurrence patterns based on the 5-year study period, and (2) to determine DSP toxin profiles occurring at Croatian shellfish farms. On the basis of the results, we describe the dynamics of *Dinophysis* species and DSP toxicity occurrence along the eastern coast of the Adriatic Sea, both of which have been poorly reported so far.

Materials and methods

Investigated area

The area investigated comprised five different shellfish farming zones along the eastern Adriatic coast and station VB located in the eastern part of Kaštela Bay in the middle Adriatic Sea (Figure 1).

In the northern Adriatic Sea, the first area investigated is located along the western coast of the Istrian peninsula, comprising stations IP1 (11 m) and IP2 (8 m) located

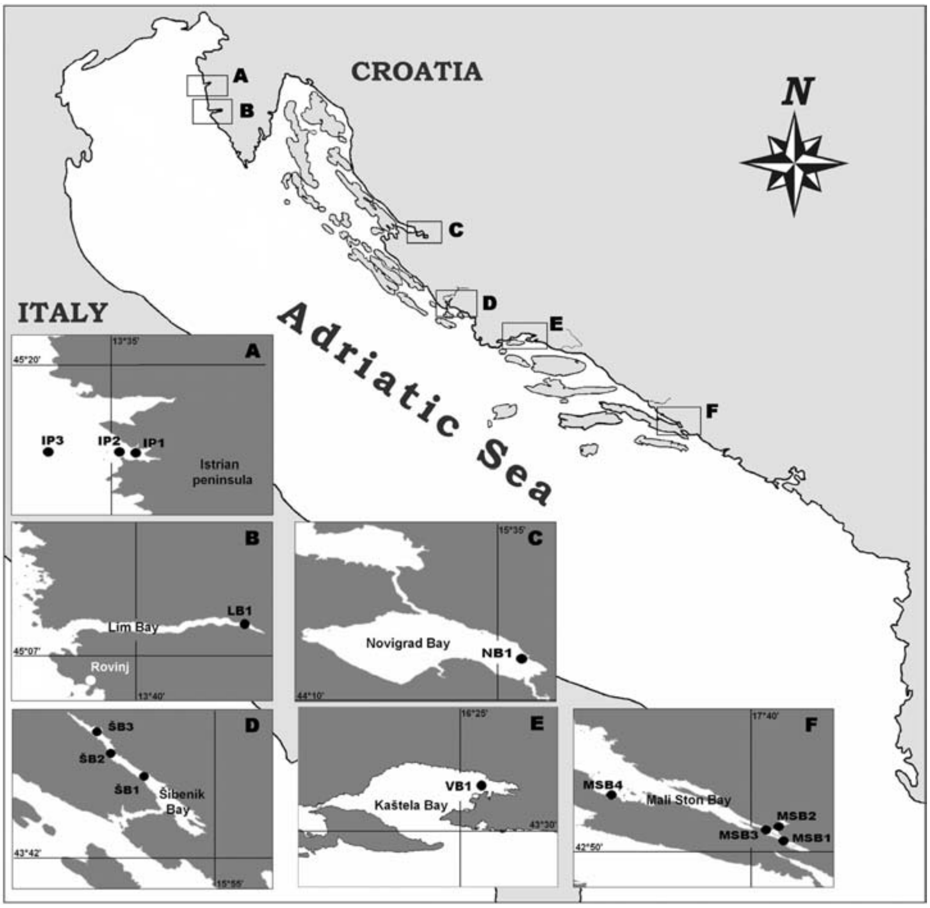

Figure 1 The study area, showing six investigated areas and 13 sampling stations along the eastern Adriatic coast.

in a shellfish farming area, and IP3 (18 m) located in wild shellfish stocks (Figure 1A). The shellfish farm is located 4–5 km south of the Mirna River estuary, without significant freshwater or sewage water inflow, and is therefore convenient for shellfish farming. The second area investigated is a shellfish farm placed in the very narrow, land incised, 600 m long Lim Bay (Figure 1B). The maximum depth of 33 m is at the entrance of the Bay. Station LB1 (14 m) is located in the shallow inner part, which is the most productive area due to the strong influence of ground waters enriched with organic matter. The third area investigated, containing station NB1 (10 m) is located in the land incised and enclosed Novigrad Bay, and is influenced by the River Zrmanja estuary (Figure 1C). A stratified water column with a sharp and shallow halocline characterizes this area. Nutrient concentrations and distributions indicate oligotrophic conditions in the bay (Burić et al. 2004).

In the mid Adriatic Sea, stations ŠB1 (8 m), ŠB2 (11 m), and ŠB3 (17 m) are located in the Šibenik Bay (Figure 1D). This area, influenced by the Krka River estuary, is one of the most productive bays along the east Adriatic coast, with freshwater present in the surface layer throughout the entire area. Station VB (18 m) is located in a shallow, semi-enclosed, eastern part of the larger Kaštela Bay in the mid Adriatic Sea (Figure 1E). This locality is characterized by reduced water exchange with the larger Bay area, and increased nutrient concentrations originating from anthropogenic pollution (Zore-Armanda 1980). Phytoplankton blooms are known to occur sporadically (Marasović and Vukadin 1982, Marasović et al. 1991, 1995). Data obtained from this station were analyzed separately due to different sampling methodology and absence of the shellfish farms in the adjacent waters.

In the South Adriatic Sea, sampling stations are located in Mali Ston Bay, which is one of the most important shellfish farming areas in Croatia (Figure 1F). The bay is located at the end of the Neretva Channel. The outer part is under the occasional influence of the River Neretva, whose influence in the inner part of the bay is weak (Vukadin 1981). Four sampling stations MSB1, MSB2, MSB3, and MSB4 are located in this Bay, with depths from 7 m to 8 m at all stations.

Field surveys

Field surveys were conducted from January 2001 to December 2005. In the colder part of the year (from November to March), sampling was performed on a monthly basis. In the warmer period from April to October, sampling was carried out every 2 weeks, but only the higher abundances were used in the analysis. At shellfish farms, phytoplankton samples were collected with a plankton net (mouth diameter 29 cm, mesh size 20 μm) towed vertically from near bottom to the surface. The results were expressed as the number of cells m^{-2}. According to an earlier study by Sidari et al. (1995b) and our previous field experiences, net sampling is more suitable for the detection of low abundance species, such as *Dinophysis* spp. Since cells tend to aggregate at different depths in the water column, we believe that it is more accurate to express their abundance per m^2 rather than dividing their abundances by volume of filtered water, which could lead to the underestimation of their density. Abundances per m^2 were calculated as N×P×F, where N is the number of organisms in the counting chamber, P is a proportion of total volume to subsample volume and F is net factor calculated as 1 m^2 divided by net area ($r^2\pi$).

Apart from the vertical net hauls, additional phytoplankton samples were taken with a 1.7 l Niskin bottle in the surface layer in 2001.

In 2001 at station VB in Kaštela Bay, phytoplankton samples were taken with a 1.7 l Niskin bottle at 0, 5, 10, and 18 m depths. Samples were fixed with glutaraldehyde solution (final concentration of 0.5%). Phytoplankton species were identified and counted under an inverted microscope (Olympus IX50, Tokyo, Japan) according to the Utermöhl method (Utermöhl 1958). For *Dinophysis* spp. abundance determination, the whole chamber bottom was scanned for cells. Chlorophyll *a* concentrations were determined fluorometrically from 90% acetone extracts (Strickland and Parsons 1972) with a Turner (Sunnyvale, CA, USA) TD 700 laboratory fluorometer. Temperature and salinity in the surface and bottom layers were recorded with a YSI (Yellow Springs, OH, USA) Model 63 handheld pH, salinity and temperature probe. At station VB in Kaštela Bay, temperature and salinity were measured with a CTD probe "Idronaut" (Brugherio, Italy). Oxygen concentrations were determined using the Winkler method (Strickland and Parsons 1972). Nutrients concentrations at station VB in Kaštela Bay were determined colorimetrically on an Auto-Analyzer-3 (Bran and Luebbe, Norderstedt, Germany) according to Grasshoff (1976).

Evaluation of DSP toxicity presence

At shellfish farms, mussel samples for evaluation of DSP toxicity presence were collected from ropes at 1–5 m depth. DSP toxicity was analyzed using the mouse bioassay method according to Yasumoto et al. (1985). Shellfish hepatopancreases (20 g) were extracted with acetone and diethyl ether. Evaporated extracts were dissolved in 1% Tween 60, followed by dilution of the sample (1 ml) for use in the test. Three replicate tests were performed. A test was regarded as toxic for humans if the result was positive within 5 h (Marcaillou-Le Baut et al. 1985, Yasumoto et al. 1985).

Determination of toxins in shellfish

Samples that induced a positive mouse bioassay were analyzed for toxin profiles (okadaic acid, OA; carboxyyessotoxin, COOHYTX; 45-hydroxyyessotoxin, 45-OHYTX; yessotoxin, YTX; homo yessotoxin, homoYTX; 45-hydroxyhomoyessotoxin, 45-OHhomoYTX; gimnodimine; spirolid; dinophysistoxin-1, DTX 1; dinophysistoxin-2, DTX 2; pectenotoxin-2, PTX2; pectenotoxin-6, PTX6; pectenotoxin-2 secoacid, PTX2 secoacid; azaspiracids, AZAs) in Centro Ricerche Marine di Cesenatico (Italy).

In 2001, DSP positive samples were investigated only for concentrations of OA using the PP2A (Protein-Phosphatase Assay) (Holmes 1991, Tubaro et al. 1996). In 2004 and 2005, positive samples were investigated further for lipophilic toxins using liquid chromatography tandem mass spectrometry (LC-MS/MS; Quilliam et al. 2001). LC-MS/MS analyses were performed using a 1200 dm^{-3} triple quadrupole mass spectrometer (Varian Inc., Walnut Creek, CA, USA).

Meteorological data

Regional precipitation data along the west coast of the Istrian peninsula (Rovinj) used in the analyses were obtained from the State Hydrographic Institute.

Statistical data analysis

The Bray-Curtis coefficient was used to analyze similarity between stations and sampling occasions based on fourth root transformed abundance data set of seven most abundant *Dinophysis* species (PRIMER 5, version 5.2.9, Plymouth Marine Laboratory, Plymouth, UK) (Clarke and Warwick 2001). Two-dimensional multidimensional scaling (MDS) ordination was used to illustrate the relationships between stations and between sampling occasions, with superimposed bubble plots representing different abundances of *Dinophysis* species.

An analysis of the similarities between sampling years was carried out using hierarchical cluster analysis (CA) based on a similarity matrix of fourth root transformed averaged abundances.

Correlation analysis between *Dinophysis* spp. abundances and environmental factors was performed using the statistical package StatSoft Inc. (2000) STATISTICA for Windows (StatSoft Inc., Tulsa, USA). Differences in temperature and salinity values between different layers were expressed as Δt and Δs, respectively. At stations located in shellfish farms where phytoplankton was sampled by vertical net hauls, Δt and Δs were calculated between surface and bottom layer values. In Vranjic Bay, where sampling was performed by Niskin bottle at defined depths, Δt and Δs were calculated between the sampled depth and the nearest neighboring depth.

Results

Hydrographic parameters

At stations along the western Istrian coast, mean monthly temperature ranged from 8.5°C in February to 25.9°C in August. Differences between temperature in the surface and in the bottom layer first appeared either in May or June, with maximum duration until August (Figure 2). In Lim Bay, mean monthly temperature ranged from 9.9°C to 26.1°C in February and August, respectively, and Δt >3°C was detectable from May to August. At stations in Novigrad and Šibenik Bays, mean monthly temperatures ranged from 6.8°C in January to 26.1°C in August, with Δt >3°C present from May to August. At stations in Mali

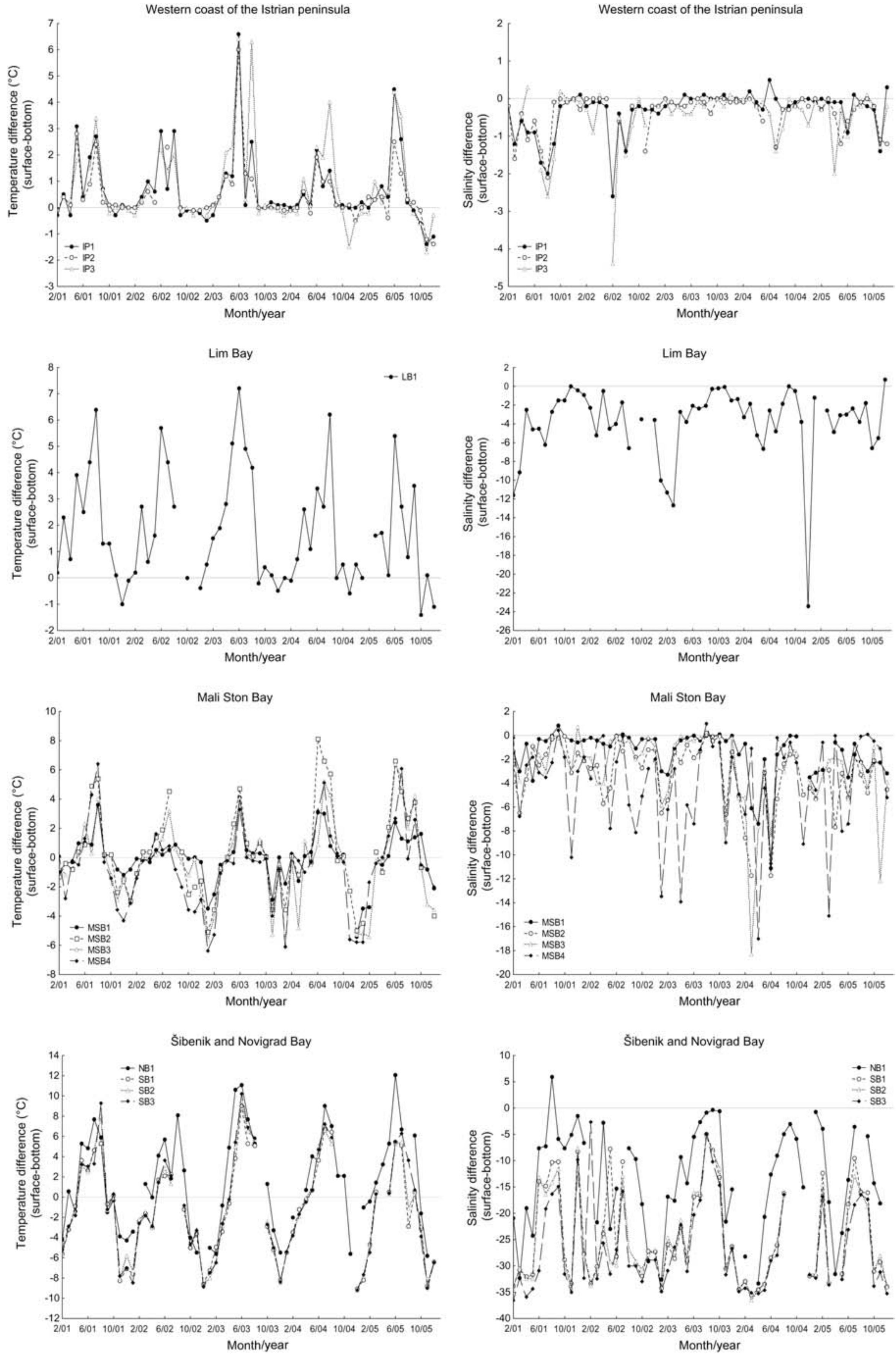

Figure 2 Differences between surface and bottom temperature and salinity values at investigated stations from 2001 to 2005 (see Figure 1 for locations).

Ston Bay, the lowest mean monthly temperature of 7.0°C was recorded in January, and the highest value of 24.8°C in August. Differences between surface and bottom layer temperatures existed for a very short period, probably due to the shallow depth of this area. Novigrad, Šibenik, and Mali Ston Bays were characterized by inverse thermal stratification during winter (Figure 2).

Mean monthly salinity along the western Istrian coast ranged from 36.6 to 38.5 in July and January, respectively. In Lim Bay, mean monthly salinity varied from 31.2 to 38.6 in February and January, respectively. In Novigrad Bay, mean monthly salinity ranged from 14.5 to 37.1 in March and May, respectively. The lowest salinity values were recorded in Šibenik Bay, which has a permanent halocline (Figure 2). Mean monthly salinity ranged from 4.7 to 38.5. In Mali Ston Bay, mean monthly salinity varied from 31.5 to 38.1.

In almost the entire area investigated, the lowest salinities were recorded during the colder period of the year, except at stations located along the western coast of the Istrian peninsula, which had minimum surface salinities during summer.

High oxygen concentrations exceeding 6.0 ml l^{-1} were recorded in the surface layer during spring and winter at all stations. Annual mean values were somewhat higher in the areas influenced by river estuaries, such as Novigrad and Šibenik Bays.

At station VB in Kaštela Bay during 2001, salinity varied from 34.4 to 38.4, with lower values in the surface layer during spring. Temperatures ranged from 11.0°C to 23.1°C, with a thermal stratification in July and August. Oxygen concentrations ranged from 3.31 to 7.18 ml l^{-1}.

Dinophysis spp. seasonal and spatial distribution

From 2001 to 2005, 13 *Dinophysis* species were found along the eastern Adriatic coast: *D. caudata* Saville-Kent, *D. fortii* Pavillard, *D. sacculus* Stein, *D. acuminata* Claparède *et* Lachmann, *D. rotundata* Claparède *et* Lachmann, *D. acuta* Ehrenberg, *D. tripos* Gourret, *D. hastata* Stein, *D. ovum* Schütt, *D. parva* Schiller, *D. rapa* (Stein) Balech, *D. diegensis* Kofoid and *D. odiosa* (Pavillard) Tai *et* Skogsberg. The most frequently occurring species in the net samples was *D. caudata*. In total, seven *Dinophysis* species were regularly recorded in the samples, and were therefore chosen for the analysis of seasonal and spatial distribution: *D. acuminata*, *D. sacculus*, *D. rotundata*, *D. fortii*, *D. acuta*, *D. caudata* and *D. tripos* (Figure 3).

MDS ordination (Figure 4) showed that *Dinophysis acuminata* occurred during spring, followed by *D. sacculus* in late spring and summer. *D. caudata* occurred in summer, followed by *D. fortii* and *D. rotundata* in late summer and autumn. Among all species investigated, *D. acuta* occurred in lowest abundances. This species was present in a low abundance during the whole year but higher abundances occurred in spring and autumn periods. *D. tripos* was present in low abundance in all seasons, but was most abundant during the colder part of the year.

Similarities between different years identified by hierarchical clustering of seven *Dinophysis* species showed two groups classified at the 86.84% similarity level. The first group comprised 2001, 2002, 2004, and 2005. 2003 was an outlier due to the low abundances of *Dinophysis* species, likely a consequence of unusually high temperatures and low precipitation observed during that period. The highest similarity was recorded between 2004 and 2005 (89.62%) and between this cluster and 2001 (88.40%).

MDS representation of similarity between stations based on *Dinophysis* spp. occurrence revealed their more frequent presence and higher abundances in the eutrophicated northern Adriatic Sea. The majority of *Dinophysis* species reached highest abundances in Lim Bay (Figure 5). *D. tripos* occurred in highest densities in

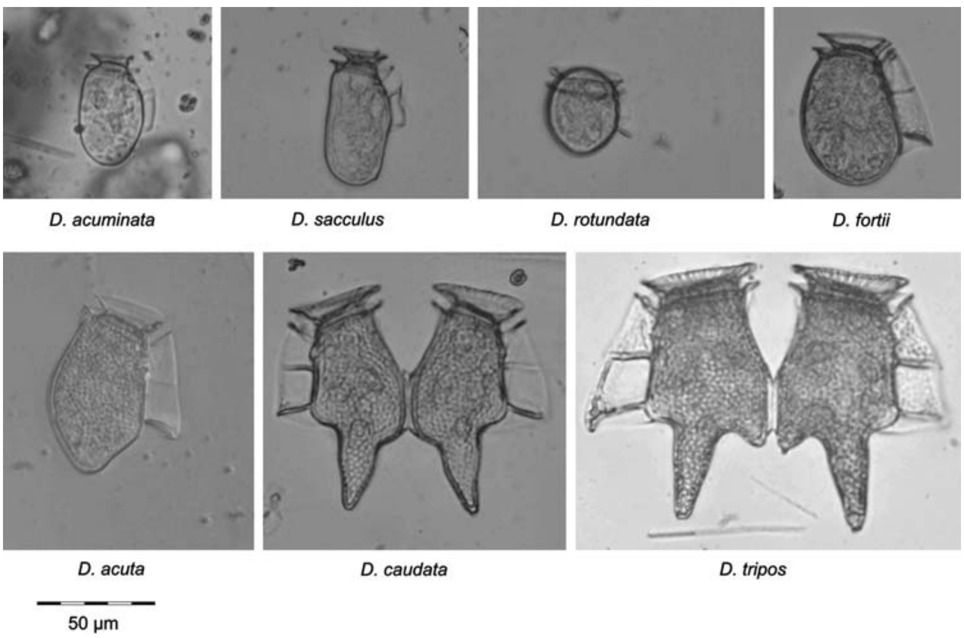

Figure 3 Seven *Dinophysis* spp. chosen for analysis of seasonal and spatial distribution: light micrographs of fixed cells.

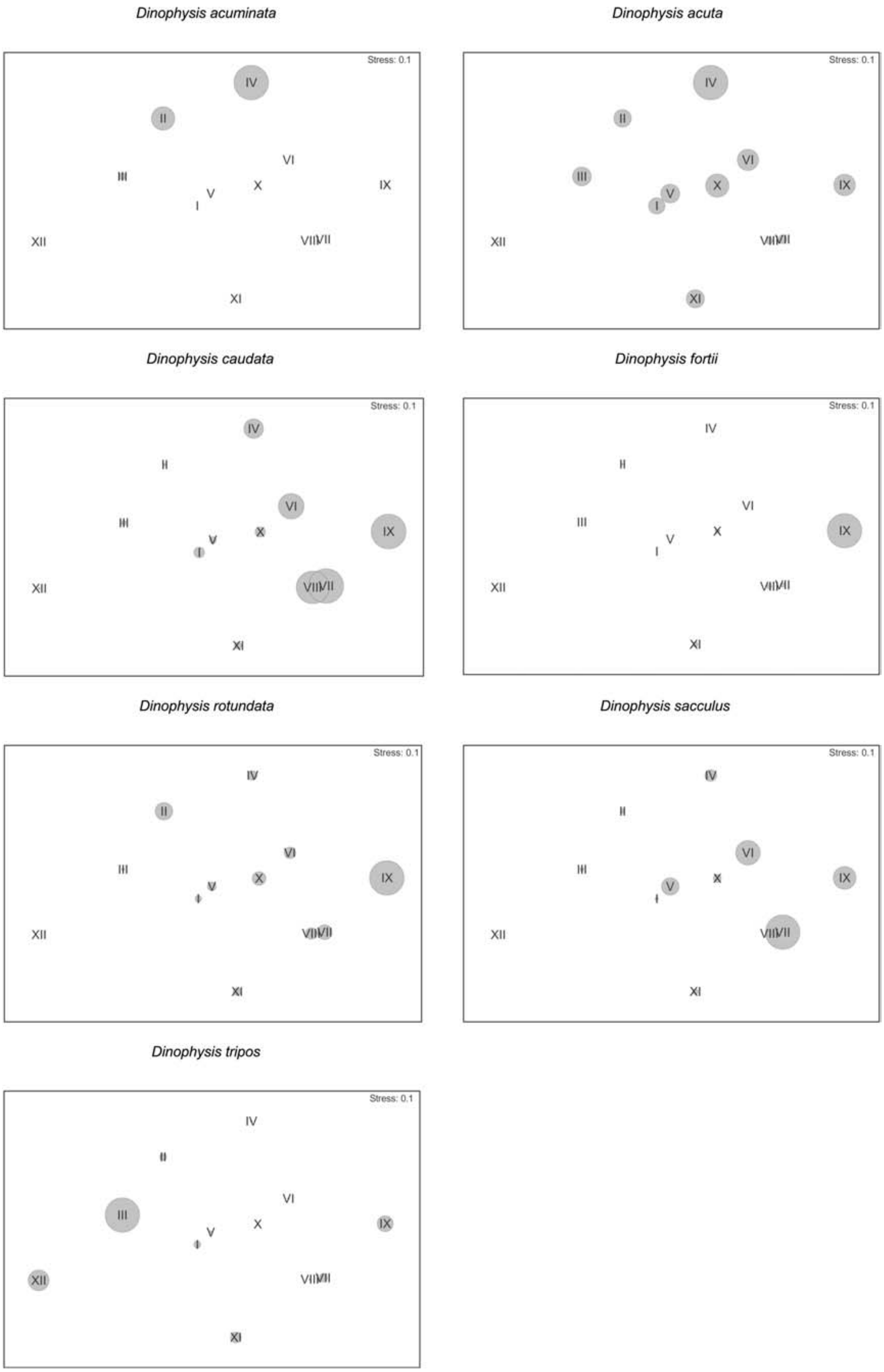

Figure 4 MDS ordinations of the sampling months (denoted by Roman numerals, I is January, XII is December) with abundances of seven *Dinophysis* species represented as superimposed bubbles increasing in size with increasing abundances of *D. acuminata*, *D. acuta*, *D. caudata*, *D. fortii*, *D. rotundata*, *D. sacculus* and *D. tripos.*

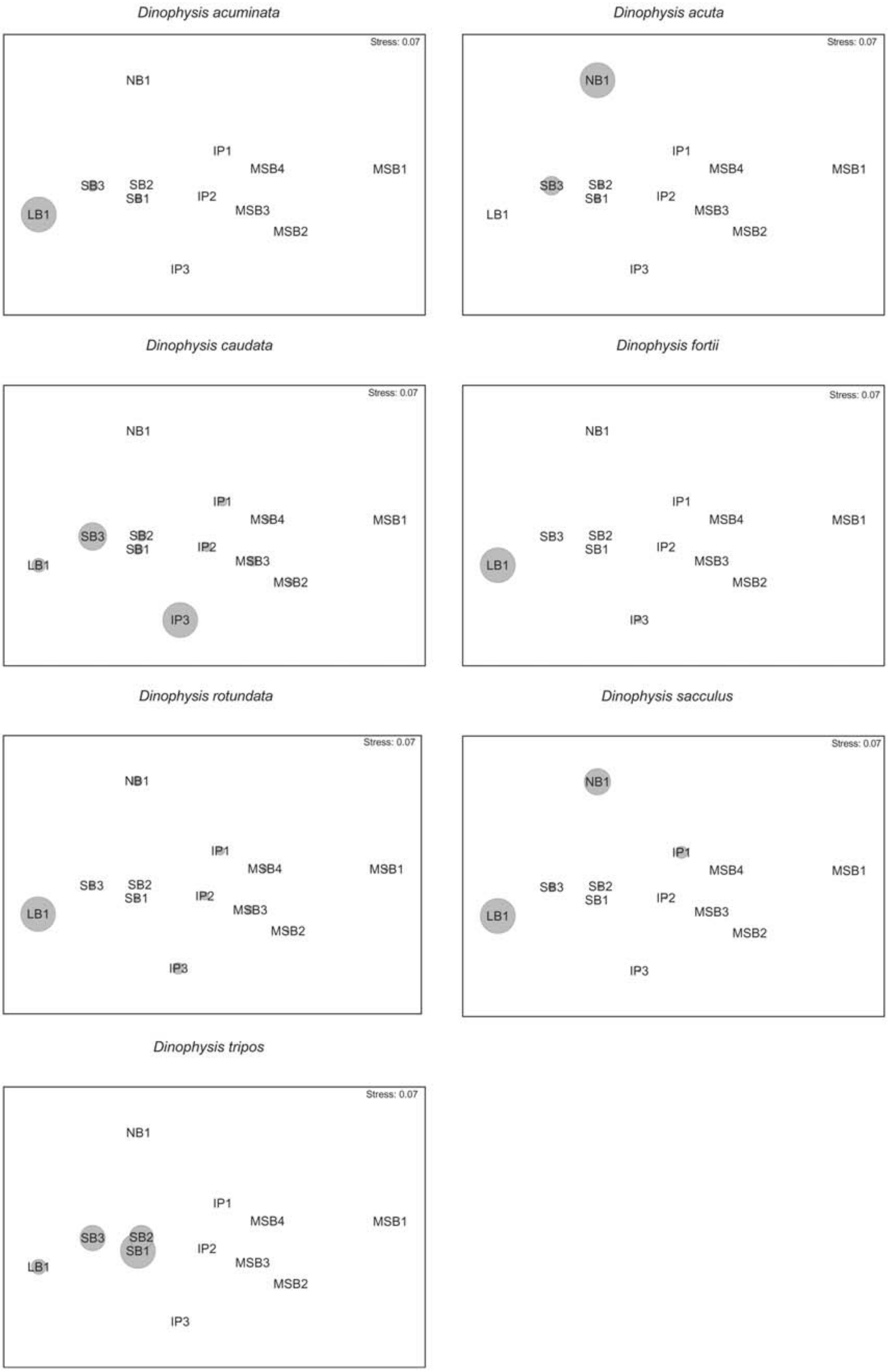

Figure 5 MDS ordinations of the sampling stations based on abundances of seven *Dinophysis* species represented as superimposed bubbles increasing in size with the increasing abundances of *D. acuminata*, *D. acuta*, *D. caudata*, *D. fortii*, *D. rotundata*, *D. sacculus* and *D. tripos*.
IP, Istrian peninsula; LB, Lim Bay; NB, Novigrad Bay; ŠB, Šibenik Bay; MSB, Mali Ston Bay; see Figure 1.

Šibenik Bay. *D. acuta* appeared in estuarine areas, such as Novigrad and Šibenik Bays. The highest dissimilarity was recorded between stations LB1 in Lim Bay (with maximum abundance of *Dinophysis* spp.) and station MSB1 in Mali Ston Bay where *Dinophysis* species occurred rarely and in low abundances.

Relationship between *Dinophysis* species and abiotic factors

Dinophysis species abundances had significant relationships with Δt and Δs. *D. caudata*, *D. sacculus*, and *D. rotundata* developed highest abundances when Δt values were high, while higher abundances of *D. fortii*, *D. acuta*, and *D. tripos* were associated with high Δs (Table 1). The highest abundances of *D. tripos* occurred in Šibenik Bay (Figure 5), which has a permanent halocline (Figure 2).

Off the western coast of the Istrian peninsula, *Dinophysis caudata* and *D. sacculus* abundances were significantly correlated with both Δt and Δs, probably resulting from the simultaneous presence of temperature and salinity differentials between surface and bottom in this area (Figure 2).

In Mali Ston Bay, *Dinophysis fortii* abundance was negatively correlated with Δt, a result of the species occurrence during the colder part of the year when there is an inverse thermocline in that area as a consequence of freshwater inflow.

Bottle samples from station VB revealed the presence of *Dinophysis sacculus* blooms in spring and autumn in the surface layer, with maximum abundance of 3.3×10^3 cells l^{-1}. The proportion of *D. sacculus* in total phytoplankton abundance ranged from 0.05% to 0.94%. *D. sacculus* abundance was significant negatively correlated with salinity and positively correlated with Δs, oxygen, and nitrate concentrations (Table 2), but there was no significant correlation between temperature and chlorophyll *a*.

Mouse bioassay and *Dinophysis* diversity during DSP toxicity events

The mouse bioassay was positive in June 2001 at station IP2 located on the western coast of the Istrian peninsula. DSP toxicity occurrence and *Dinophysis* diversity at IP2 are shown in Figure 6. *D. rotundata*, *D. sacculus*, and *D. caudata* were present prior to and during toxicity occurrence. Bottle samples revealed the presence of *D. rotundata* in the surface layer in February and June, with 1600 and 740 cells l^{-1}, respectively (Figure 6A).

DSP toxicity was not recorded during 2002 and 2003. In 2004, DSP toxicity occurred in July and August at stations IP1 (Figure 7A) and IP2 (Figure 7B) off the western Istrian coast and station MSB2 in Mali Ston Bay (Figure 7C). Species associated with DSP toxicity were *Dinophysis caudata* (which was the most abundant), *D. rotundata*, and *D. fortii*.

In 2005, significant DSP toxicity events occurred throughout summer and autumn along the Istrian coast (Figure 8), in Lim, Novigrad, and Šibenik Bays (Figure 9) and in Mali Ston Bay (Figure 10). *Dinophysis* species linked to toxicity events were: *D. caudata*, *D. fortii*, *D. rotundata*, *D. sacculus*, and *D. tripos*.

Along the western coast of the Istrian peninsula in 2005, toxicity events were recorded after an intensive rainfall period with unusually low salinity and precipitation quantities considerably exceeding the long-term average.

DSP toxin profile

In 2001, low concentrations of OA (0.03 and 0.05 μg g^{-1} hepatopancrease at stations IP1 and IP2, respectively) were recorded in DSP positive samples. A toxin profile determined for positive mouse bioassay samples in 2004 and several positive samples in 2005 revealed presence of both OA and yessotoxins (Tables 3 and 4). The amounts of these toxins individually were below concentrations dangerous to human health, but if combined, they might yield positive results, as confirmed by the positive mouse bioassay. High concentrations of OA in shellfish occurred after a *Dinophysis caudata* bloom and

Table 2 Pearson's correlation coefficients between *Dinophysis sacculus* abundances and the main environmental parameters at the VB station in Kaštela Bay.

Salinity	Δs	Oxygen	Nitrate
-0.70**	0.53*	0.37*	0.46**

*p<0.05; **p<0.005.

Table 1 Pearson's correlation coefficients between *Dinophysis* species abundances and temperature and salinity difference between surface and bottom layers, i.e., Δt and Δs, respectively.

Investigated area	Species	n	Δt	Δs
Western coast of	*Dinophysis caudata*	164	0.22*	0.27*
Istrian peninsula	*Dinophysis sacculus*	164	0.24*	0.24*
	Dinophysis fortii	47	ns	0.40*
Lim Bay	*Dinophysis caudate*	47	0.31*	ns
Novigrad Bay	*Dinophysis sacculus*	47	0.35*	ns
Šibenik Bay	*Dinophysis acuta*	153	ns	0.19*
	Dinophysis fortii	153	ns	0.24*
	Dinophysis rotundata	153	0.22*	ns
Mali Ston Bay	*Dinophysis caudate*	218	0.20*	ns
	Dinophysis fortii	218	-0.22*	ns
All investigated	*Dinophysis acuta*	638	ns	0.23*
stations	*Dinophysis tripos*	638	ns	0.20*

Significant: *p<0.05; ns: not significant.

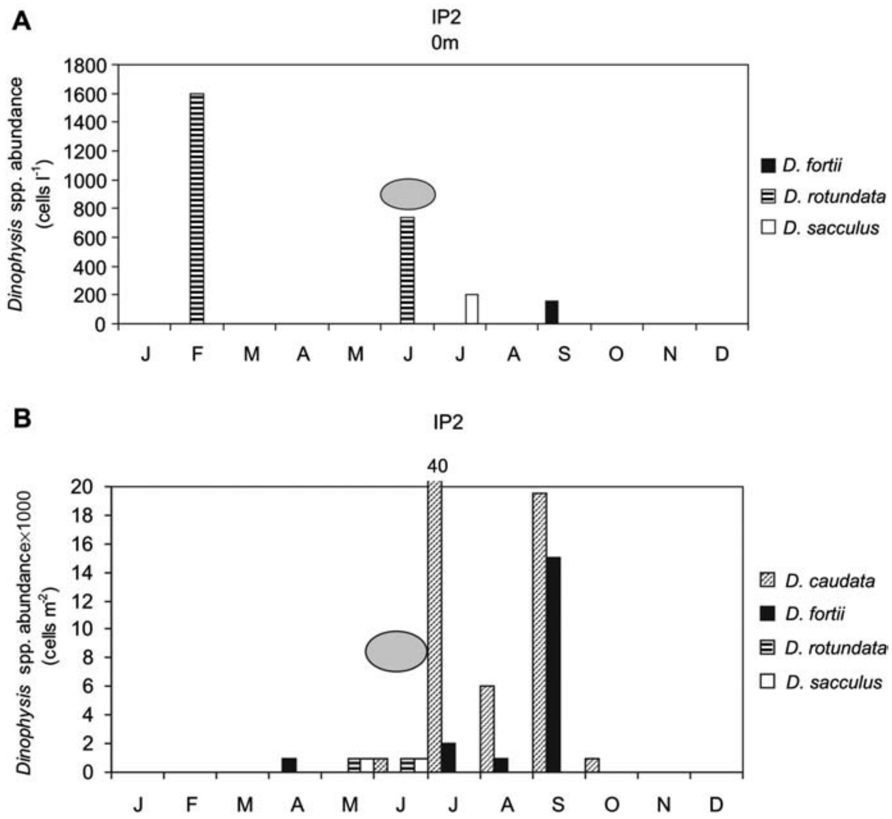

Figure 6 Mean *Dinophysis* spp. abundances at station IP2 during DSP toxicity events in 2001.
(A) Abundances from the surface layer. (B) Abundances from the net samples. Gray ovals depict periods of DSP toxicity events. See Figure 1 for location of station IP2.

these concentrations were associated with the presence of *D. caudata*, *D. rotundata*, *D. fortii*, and *D. sacculus*, while the presence of yessotoxin may be attributed to presence of *Lingulodinium polyedrum* (F. Stein) J.D. Dodge (Figures 7–10).

Discussion

Annual dynamics of *Dinophysis* species in the eastern Adriatic Sea

Dinophysis species occurrence in the Adriatic Sea has been reported regularly during the last decade (Giacobbe et al. 1995, Caroppo et al. 1999, Bernardi Aubry et al. 2000, Caroppo 2001, Pavela-Vrančić et al. 2002, France and Mozetič 2006). Ours is the first study to evaluate the seasonal and spatial pattern of *Dinophysis* bloom dynamics along the Croatian coast over an extended time scale and under varying hydrographic conditions. In the period from 2001 to 2005, 13 *Dinophysis* species were identified, among which, seven were recorded regularly: *D. acuta*, *D. acuminata*, *D. caudata*, *D. fortii*, *D. rotundata*, *D. sacculus* and *D. tripos*. Several authors report that the *Dinophysis* species represent only a small fraction of total phytoplankton assemblage (Maestrini 1998, Bernardi Aubry et al. 2000). This conclusion was largely confirmed in our study, but an exception was noted at stations located along the western coast of the Istrian peninsula, where *D. rotundata* contributed up to 64% to the total phytoplankton population. Under these conditions, with *Dinophysis* species dominating the community, these phytoplankters can probably induce shellfish toxicity even if present in low abundances. This shows that during monitoring activities both *Dinophysis* species presence and their contribution to total phytoplankton community should be taken into consideration.

Although *Dinophysis* species occurred mostly during warmer parts of the year, they differed in timing of appearance. *D. acuminata* and *D. sacculus* appeared in spring and early summer, while *D. caudata* was characteristic of the summer period. *D. fortii* and *D. rotundata* occurred in late summer, while *D. tripos*, contrary to much of the published data (Sidari et al. 1995a, Blanco et al. 1998, Maestrini 1998, Bernardi Aubry et al. 2000), appeared in the winter period. High abundances of *D. sacculus* in the Adriatic and Mediterranean Seas during spring and summer periods have already been reported (Bernardi Aubry et al. 2000, Giacobbe et al. 2000, Caroppo 2001). An intensive bloom of *D. acuminata* in eastern Mediterranean coastal waters was recorded during winter and spring (Koukaras and Nikolaidis 2004). In the North Sea, *D. acuminata* bloomed during late summer, while *D. acuta* was more abundant in spring and late summer (Klöpper et al. 2003). In Maizuru Bay (Japan), *D. acuminata* had maximum occurrence during spring and autumn (Nishitani et al. 2002). These various seasonal appearances in different geographic areas presumably reflect differences in physical and chemical conditions of

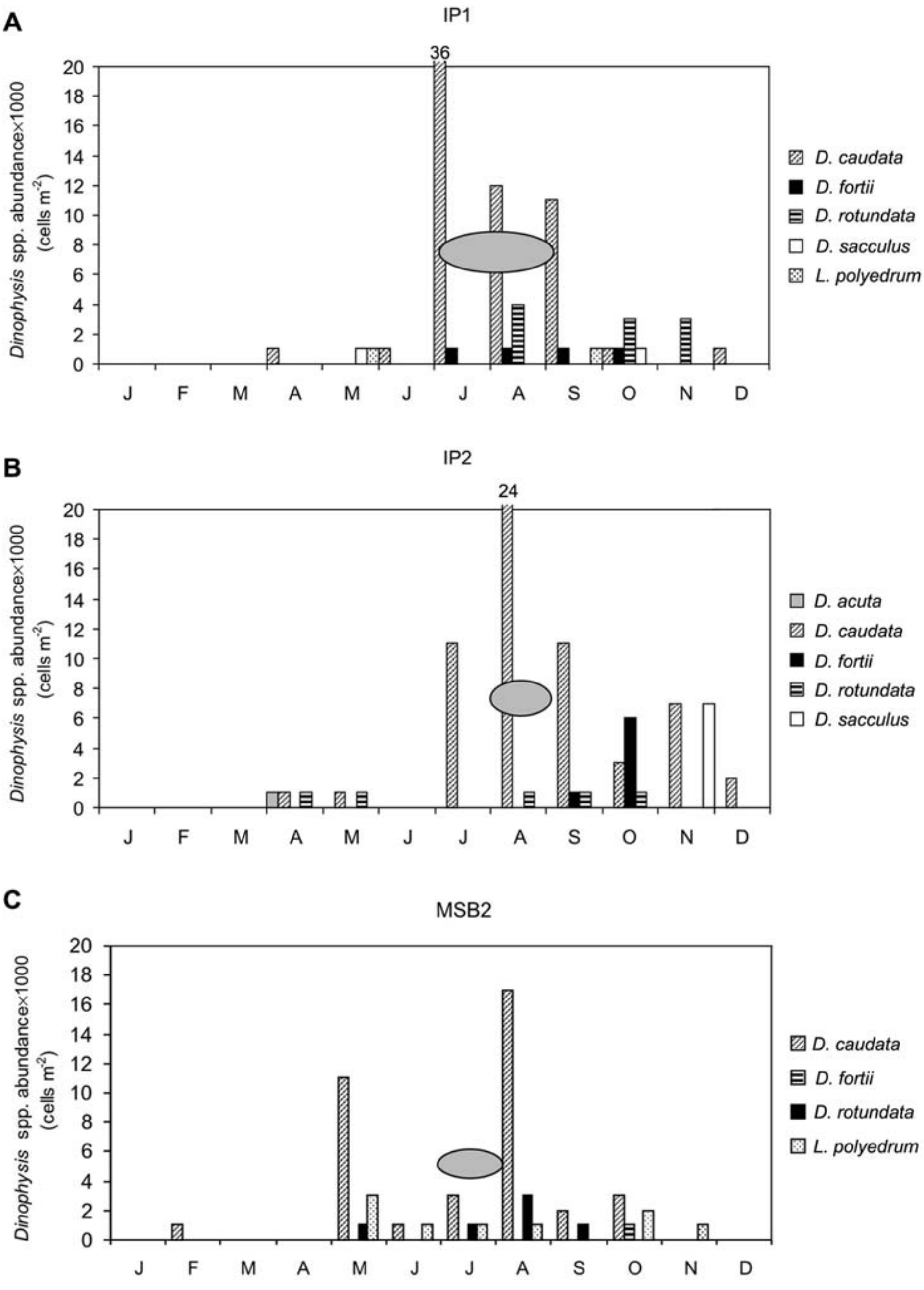

Figure 7 Mean *Dinophysis* spp. abundances at stations during DSP toxicity events in 2004.
(A) Station IP1. (B) Station IP2. (C) Station MSB2. Gray ovals depict periods of DSP toxicity events. See Figure 1 for locations of stations.

the seawater. Temperature is frequently reported as an important factor influencing *Dinophysis* spp. distribution (Delmas et al. 1992, Cabrini et al. 1995, Reguera et al. 1995, Bernardi Aubry et al. 2000, Koukaras and Nikolaidis 2004). In our study, the most significant positive relation with temperature was recorded for *D. caudata* and *D. sacculus.* According to Maestrini (1998), the role of temperature is expressed as an increasing stratification of the water column, rather than as a direct effect on *Dinophysis* spp. In this study, we obtained significant relationships between *Dinophysis* spp. abundances and Δt and Δs. *D. sacculus*, *D. caudata*, and *D. rotundata* developed higher abundances in the warmer part of the year when Δt was high as a result of thermal stratification, which commonly occurs from May to September in the Adriatic Sea (Buljan and Zore-Armanda 1976). Significant relationships with Δs were calculated for *D. acuta*, *D. fortii*, and *D. tripos*, which developed highest abundances in Šibenik Bay, an area characterized by a permanent halocline. *D. sacculus* abundance had a significant relationship with Δt in Novigrad Bay, and a significant relationship with Δs at station VB in Kaštela Bay, indicating that the abundance of this species is related to pycnocline presence rather than the origin of stratification in the water column. These findings support the generally accepted theory that stability of the water column is a critical condition for accumulation of *Dinophysis* cells and its dense patchy distribution.

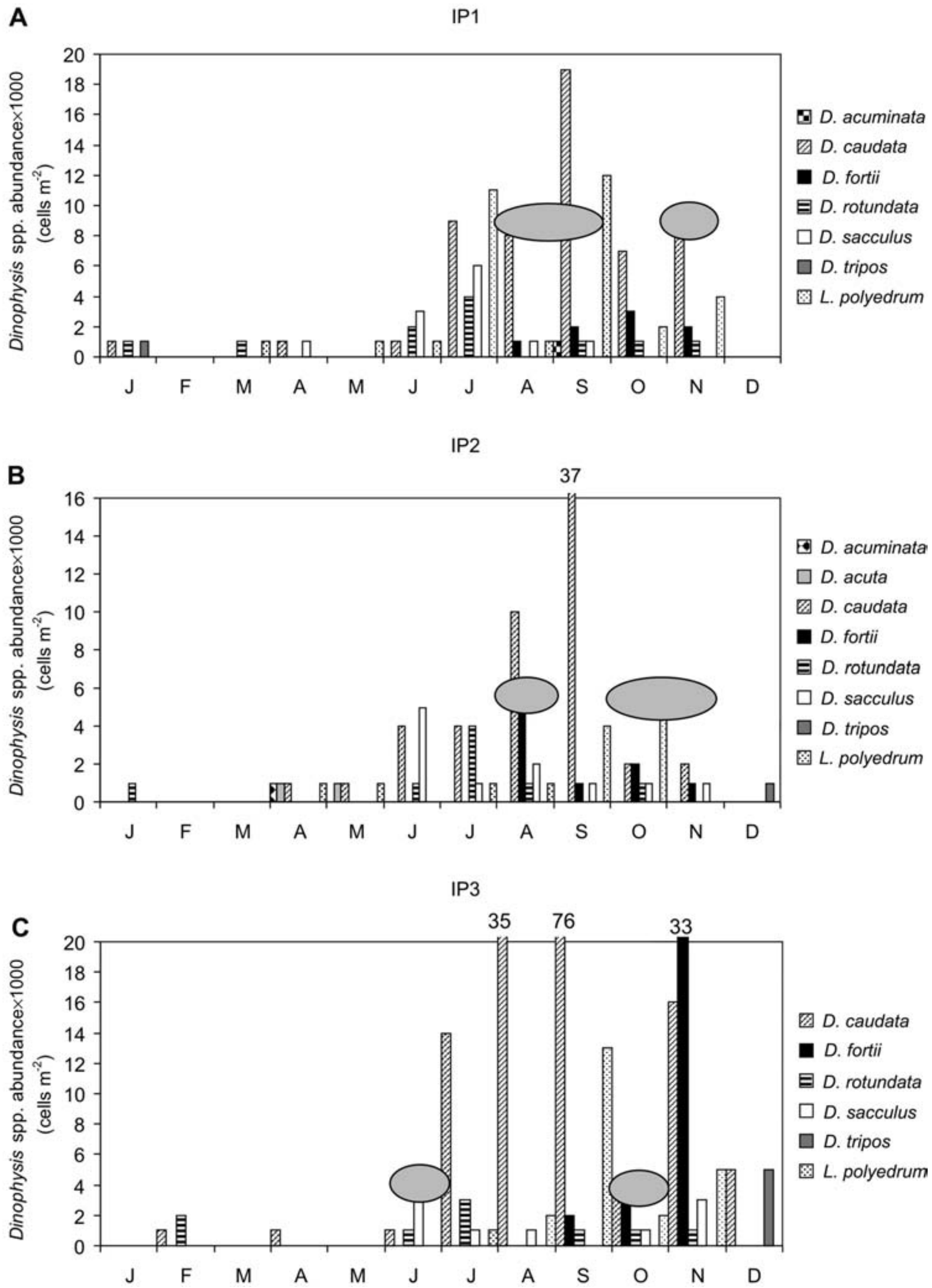

Figure 8 Mean *Dinophysis* spp. abundances at stations off the Istrian coast where DSP toxicity events were recorded in 2005. (A) Station IP1. (B) Station IP2. (C) Station IP3. Gray ovals depict periods of DSP toxicity events. See Figure 1 for locations of stations.

Negative correlation between salinity and *Dinophysis sacculus* abundance occurred in bottle samples collected in the semi-enclosed Kaštela Bay (Table 2). High affinity of *D. sacculus* for semi-enclosed basins, estuaries, and lagoons has already been reported (Giacobbe et al. 1995, Zingone et al. 1998). *D. sacculus* is an autotrophic species, and its significant abundance correlation with nitrate (that we recorded in the Kaštela Bay at station VB) has been previously reported by Giacobbe et al. (1995) and Caroppo (2001). Although accumulation on the pycnocline explains the formation of dense populations, it does not show which nutrients and environmental factors sustain growth. The majority of *Dinophysis* species are mixotrophic and the relative importance of photosynthesis, dissolved organic nutrients, and feeding are still largely unknown (Burkholder et al. 2006).

Dinophysis species and DSP toxicity events

Based on our results, five *Dinophysis* species were positively associated with toxicity events in Croatian waters of the Adriatic Sea: *D. fortii*, *D. rotundata*, *D. caudata*, *D. sacculus*, and *D. tripos.* DSP shellfish contamination events related to the presence of *D. fortii*, *D. tripos*, *D. caudata*, and *D. sacculus* in the northern Adriatic Sea were previously reported by Boni et al. (1992). Shellfish toxicity events mostly occurred in the northern Adriatic Sea which is periodically under significant influence of

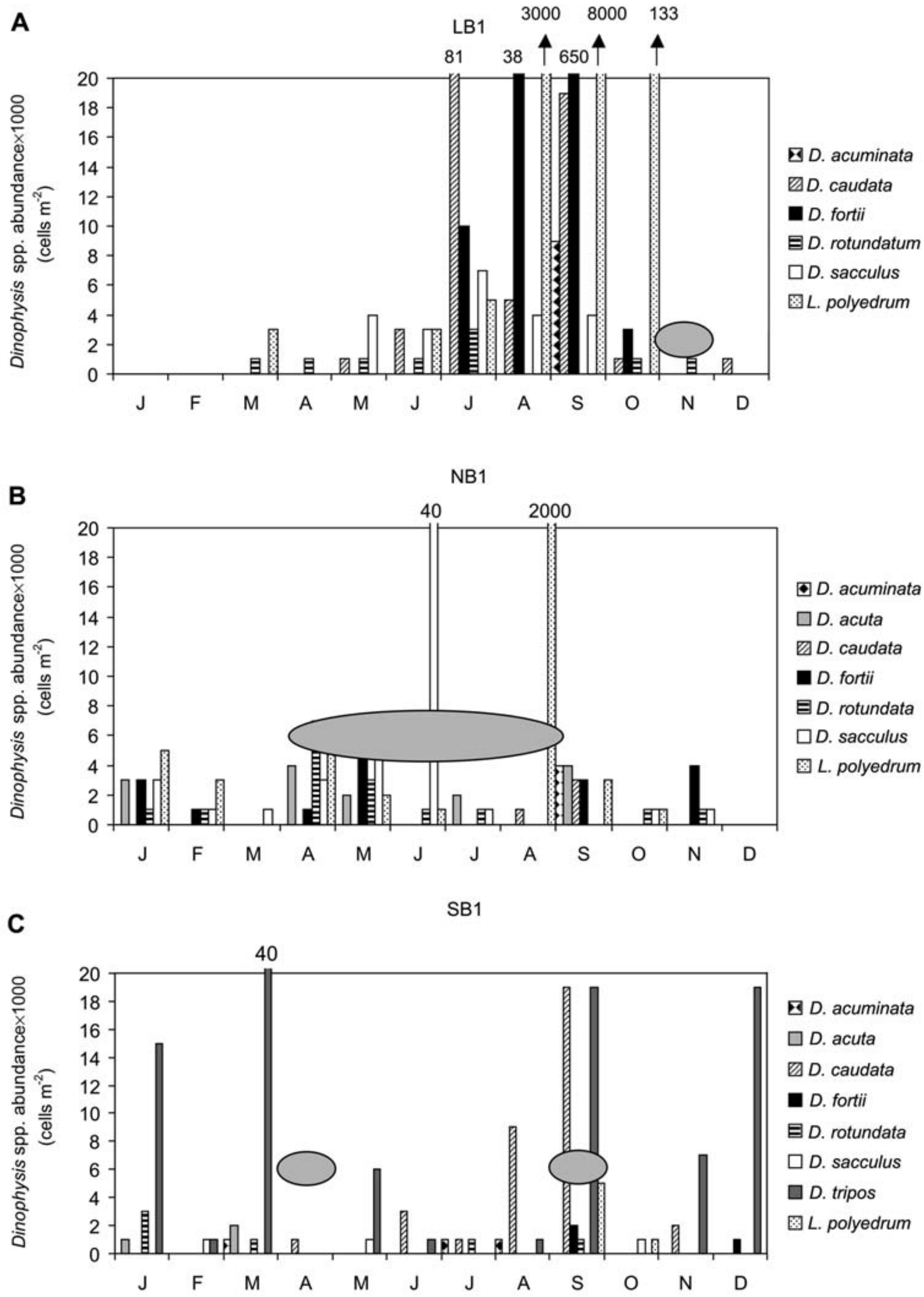

Figure 9 Mean *Dinophysis* spp. abundances in Lim, Novigrad and Šibenik Bays where DSP toxicity events were recorded in 2005. (A) Station LB1. (B) Station NB1. (C) Station SB1. Gray ovals depict periods of DSP toxicity events. See Figure 1 for locations of stations.

the River Po (Degobbis et al. 2000). The northern Adriatic Sea is more eutrophicated than the mid and southern Adriatic Sea, supporting the theory that eutrophication processes stimulate algal blooms (Hallegraeff 1995). Much higher abundances of *D. caudata* and *D. fortii* were recorded at offshore station IP3 than at inshore stations IP1 and IP2, suggesting an offshore origin of the bloom. During the summer season when a stratified water column occurs, less saline waters from the Italian rivers flow above the pycnocline not only along the western coast, but also towards the northern and eastern coasts (Orlić 1989). It is possible that this water mass favors *Dinophysis* spp. growth or even spreading to the eastern part.

During the 5-year investigation period, we observed differences in the frequency of toxicity events. In 2002 and 2003, there were no events of DSP shellfish poisoning; in 2001 and 2004 there were a few toxicity events, while in 2005, a year characterized by unusually high precipitation quantities, significant DSP toxicity events were recorded. Toxicity events mostly succeed intensive rainfall periods, which cause increased river drainage that elevates nutrient input, leading to phytoplankton blooms. Vale and Sampayo (2003) report that rainfall is an extremely important factor in controlling *in situ* blooms of toxic *Dinophysis* spp. and, consequently, toxicity implications for shellfish.

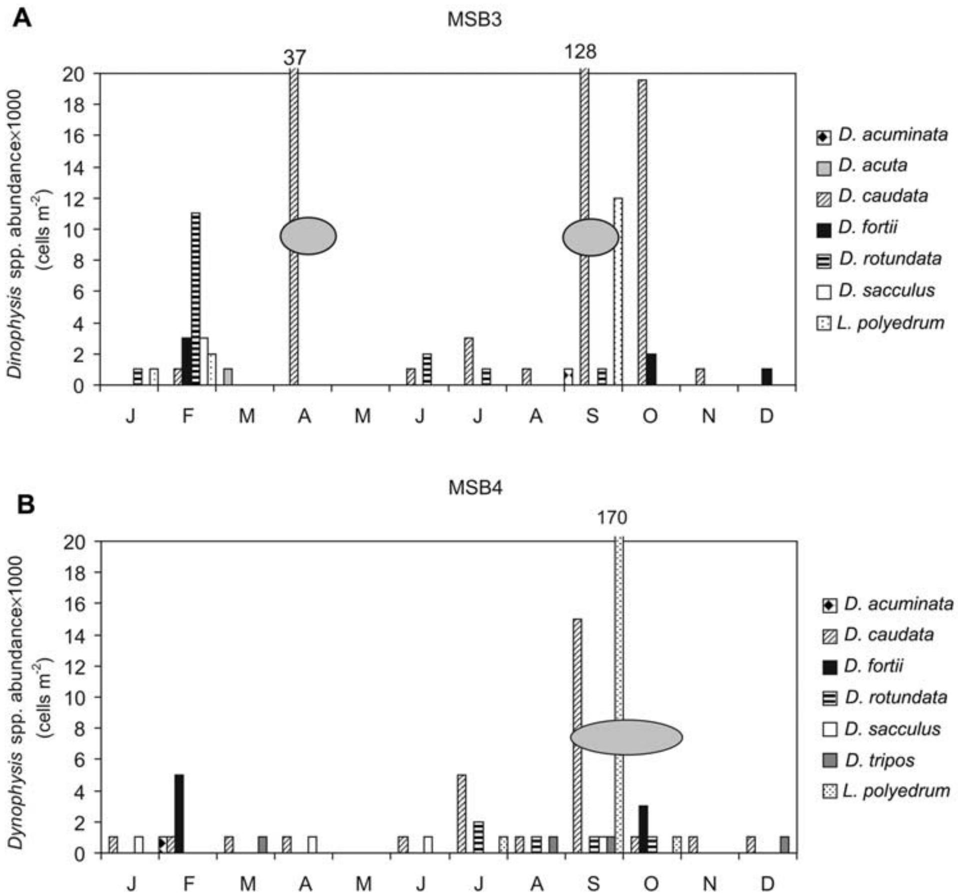

Figure 10 Mean *Dinophysis* spp. abundances at stations in Mali Ston Bay where DSP toxicity events were recorded in 2005. (A) Station MSB3. (B) Station MSB4. Gray ovals depict periods of DSP toxicity events. See Figure 1 for locations of stations.

Table 3 LC-MS/MS analysis of DSP positive samples in 2004.

Station	Date	OA (μg kg⁻¹)	COOHYTX (mg kg⁻¹)	45-OHYTX (mg kg⁻¹)	YTX (mg kg⁻¹)	homoYTX (mg kg⁻¹)
IP1	Jul 24	nd	0.029	0.013	0.203	0.016
IP1	Aug 24	185	0.041	0.018	0.144	0.038
IP2	Aug 24	nd	0.042	0.024	0.202	0.045

See Figure 1 for station locations. Okadaic acid, OA; carboxyyessotoxin, COOHYTX; 45-hydroxyyessotoxin, 45-OHYTX; yessotoxin, YTX; homo yessotoxin, homoYTX; not detected, nd.

Table 4 LC-MS/MS analysis of DSP positive samples in 2005.

Station	Date	OA (μg kg⁻¹)	COOHYTX (mg kg⁻¹)	45-OHYTX (mg kg⁻¹)	YTX (mg kg⁻¹)	homoYTX (mg kg⁻¹)
SB1	Apr 12	nd	nd	nd	nd	nd
MSB3	Apr 12	nd	nd	nd	nd	nd
NB1	Apr 14	nd	nd	0.009	0.110	0.010
SB1	Sep 21	nd	nd	nd	nd	nd
IP2	Oct 11	68	0.056	nd	0.035	0.036
IP3	Oct 27	nd	nd	nd	nd	nd
IP2	Oct 27	60	0.034	nd	0.012	nd

See Figure 1 for station locations. nd, not detected. See legend to Table 3 for toxin abbreviations.

DSP toxin profiles showed that OA and yessotoxin are the main DSP toxins in Croatian waters of the eastern Adriatic Sea. Besides OA, which has previously been reported (Orhanović et al. 1996, Marasović et al. 1998), our results confirm an earlier suggestion that yessotoxin is present (Marasović et al. 1998, Pavela-Vrančić et al. 2002). The presence and high cell densities of *Lingulodinium polyedrum* during DSP toxicity events in the northern Adriatic Sea indicate the involvement of this species in yessotoxin appearance, as previously reported for this water body (Tubaro et al. 1998, Draisci et al. 1999).

Conclusions

The present study emerging from a 5-year data set demonstrated: (1) that seasonal cycles of *Dinophysis* species in Croatian waters were initiated by *D. acuminata* in spring followed by *D. sacculus* in late spring and summer and *D. caudata* in summer, continuing with *D. fortii* and *D. rotundata* in late summer and ending with *D. tripos* in winter, (2) that toxicity events occurred more frequently in the more eutrophicated northern Adriatic Sea than in the southern Adriatic Sea, (3) the possible offshore origin of the bloom in the northern Adriatic Sea and the influence of less saline waters from the Italian coast on *Dinophysis* species spreading towards the eastern Adriatic waters, (4) that *D. fortii*, *D. rotundata*, *D. caudata*, *D. sacculus*, and *D. tripos* were species associated with DSP toxicity events in Croatian waters, (5) the existence of significant relations between *Dinophysis* spp. and low salinity, and between *D. sacculus* and nitrate concentration, and (6) the presence of yessotoxin in addition to OA as the main DSP toxins in Croatian waters.

Acknowledgements

The authors thank A. Milandri (Centro Ricerche Marine di Cesenatico, Italy) for toxin analysis. Standard for quantification of homoYTX was kindly provided by Prof. Ernesto Fattorusso (University of Naples, Italy) and standards for 45-OH-YTX and COOH-YTX by Prof. Gian Paolo Rossini (University of Modena, Italy). We would also like to thank M. Šiško for technical help in preparation of illustrations. The comments of the anonymous reviewers are greatly appreciated. The Croatian Ministry of Science, Education and Sports through the grants 001-0010501-0848 and 001-0013077-0845 supported this research.

References

Bernardi Aubry, F., A. Berton, M. Bastianini, R. Bertaggia, A. Baroni and G. Socal. 2000. Seasonal dynamics of *Dinophysis* in coastal waters of the NW Adriatic Sea (1990–1996). *Bot. Mar. 43*: 423–430.

Blanco, J., A. Morono, Y. Pazos, J. Maneiro and J. Marino. 1998. Trends and variations of the abundance of main PSP and DSP producing species in the Galician Rias: environmental and biological influences. *In*: (B. Reguera, J. Blanco, M.L. Fernández and T. Wyatt, eds.) *Harmful algae*. Xunta de Galicia and IOC of UNESCO, Santiago de Compostela. pp. 204–207.

Boni, L., L. Mancini, A. Milandri, R. Poletti, M. Pompei and R. Viviani. 1992. First cases of diarrhetic shellfish poisoning in the Northern Adriatic Sea. *In*: (R.A. Vollenweider, R. Marchetti and R. Viviani, eds.) *Marine coastal eutrophication*. Sci. Total Environ. Suppl. Elsevier, Amsterdam. pp. 419–426.

Bruno, M., F. Autumneni, E. Marchiori. A. Cosentino, M. Ercolessi, P. Ammazzalorso, C. Mengarelli and C. Caroppo. 1998. Fattori fisici ambientali e dinamica del genere *Dinophysis.* Campionamento nelle acque del mare Adriatico. *Ambiente Risorse Salute 63*: 44–47.

Buljan, M. and M. Zore-Armanda. 1976. Oceanographical properties of the Adriatic Sea. *Oceanogr. Marine Biol. Annu. Rev. 14*: 11–98.

Burić, Z., K. Caput and D. Viličić. 2004. Distribution of diatom *Cocconeis scutellum* in the karstic estuary (Zrmanja, eastern Adriatic Sea). *Biol., Bratislava 59*: 1–7.

Burkholder, J.M., R.V. Azanza and Y. Sako. 2006. The ecology of harmful dinoflagellates. *In*: (E. Granéli and J.T. Turner, eds.) *Ecology of harmful algae*. Springer, Berlin. pp. 53–66.

Cabrini, M., B. Cataletto, P. Ganis, I. Pecchiar and S. Fonda Umani. 1995. Relationship between toxic phytoplankton and environmental factors in the Gulf of Trieste: multifactorial analysis. *In*: (P. Lassus, G. Arzul, E. Erard, P. Gentien and C. Marcaillou, eds.) *Harmful marine algal blooms: technique et documentation*. Lavoisier, Intercept Ltd., Paris. pp. 139–144.

Caroppo, C. 2001. Autoecology and morphological variability of *Dinophysis sacculus* (Dinophyceae: Dinophysiaceae) in a Mediterranean lagoon. *J. Mar. Biol. Assoc. UK 81*: 11–21.

Caroppo, C., R. Congestri and M. Bruno. 1999. On the presence of *Phalacroma rotundatum* in the southern Adriatic Sea (Italy). *Aquat. Microb. Ecol. 17*: 301–310.

Clarke, K.R. and R.M. Warwick. 2001. *Change in marine communities: an approach to statistical analysis and interpretation*. 2nd edition. PRIMER-E Ltd, Plymouth. Chapter 5, pp. 1–12.

Degobbis, D., R. Precali, I. Ivančić, N. Smodlaka, D. Fuks and S. Kveder. 2000. Long-term changes in the northern Adriatic ecosystem related to anthropogenic eutrophication. *Int. J. Environ. Pollut. 13*: 1–6.

Della Loggia, R., M. Cabrini, P. Del Negro, G. Honsell and A. Tubaro. 1993. Relationship between *Dinophysis* spp. in seawater and DSP toxins in mussels in the Northern Adriatic Sea. *In*: (T.J. Smayda and Y. Shimizu, eds.) *Toxic phytoplankton blooms in the sea.* Elsevier, Amsterdam. pp. 483–488.

Delmas, D., A. Herbland and S.Y. Maestrini. 1992. Environmental conditions which lead to increase in cell density of the toxic dinoflagellates *Dinophysis* spp. in nutrient-rich and nutrient-poor waters of the French Atlantic coast. *Mar. Ecol. Prog. Ser. 89*: 53–61.

Draisci, R., E. Ferretti, L. Palleschi, C. Marghiafava, R. Poletti, A. Ceredi and M. Pompei. 1999. High levels of yessotoxin in mussels and presence of yessotoxin and homoyessotoxin in dinoflagellates of the Adriatic Sea. *Toxicon 37*: 1187–1193.

France, J. and P. Mozetič. 2006. Ecological characterization of toxic phytoplankton species (*Dinophysis* spp., Dinophyceae) in Slovenian mariculture areas (Gulf of Trieste, Adriatic Sea) and the implications for monitoring. *Mar. Pollut. Bull. 52*: 1504–1516.

Giacobbe, M.G., F. Oliva, R. La Ferla, A. Puglisi, E. Crisafi and G. Maimone. 1995. Potentially toxic dinoflagellates in Mediterranean water (Sicily) and related hydrobiological condition. *Aquat. Microb. Ecol. 9*: 63–68.

Giacobbe, M.G., A. Penna, A. Ceredi, A. Milandri, R. Poletti and X. Yang. 2000. Toxicity and ribosomal DNA of the dinoflagellate *Dinophysis sacculus* (Dinophyta). *Phycologia 39*: 177–182.

Grasshoff, K. 1976. Methods of seawater analysis. Verlag Chemie, Weinheim. pp. 307.

Hallegraeff, G.M. 1995. Harmful algal blooms: a global overview. *In*: (G.M. Hallegraeff, D.M. Anderson and A.D. Cembella, eds.) *Manual on harmful marine microalgae*. UNESCO Publishing, Paris. pp. 1–22.

Hallegraeff, G.M. 2003. Harmful algal blooms: a global overview. *In*: (G.M. Hallegraeff, D.M. Anderson and A.D. Cembella, eds.) *Manual on harmful marine microalgae*. UNESCO Publishing, Paris. pp. 25–49.

Holmes, C.F.B. 1991. Liquid chromatography-linked protein phosphatase bioassay: a highly sensitive marine bioscreen for okadaic acid and related diarrhetic shellfish toxins. *Toxicon 29*: 469–477.

Klöpper, S., R. Scharek and G. Gerdts. 2003. Diarrhetic shellfish toxicity in relation to abundance of *Dinophysis* spp. in the German Bight near Helgoland. *Mar. Ecol. Prog. Ser. 259*: 93–102.

Koukaras, K. and G. Nikolaidis. 2004. *Dinophysis* blooms in Greek coastal waters (Thermaikos Gulf, NW Aegean Sea). *J. Plankton Res. 26*: 445–457.

Maestrini, S.Y. 1998. Bloom dynamics and ecophysiology of *Dinophysis* spp. *In*: (D.M. Anderson, A.D. Cembella and G.M. Hallegraeff, eds.) *Physiological ecology of harmful algal blooms*. NATO ASI Series, Vol. G41, Springer-Verlag, Berlin. pp. 243–265.

Marasović, I. and I. Vukadin. 1982. Red tide in the Vranjic basin (Kaštela Bay). *Biljeske-Notes 48*: 1–8.

Marasović, I., M. Gačić, V. Kovačević, N. Krstulović, G. Kušpilić, T. Pucher-Petković, N. Odžak and M. Šolić. 1991. Development of the red tide in the Kaštela Bay (Adriatic Sea). *Mar. Chem. 32*: 375–385.

Marasović, I., Ž. Ninčević and N. Odžak. 1995. The effect of temperature on blooms of *Lingulodinium polyedra* and *Alexandrium minutum* in Kaštela Bay. *In*: (P. Lassus, G. Arzul, P. Gentien and C. Marcaillou, eds.) *Harmful marine algal blooms: technique and documentation*. Lavoisier Intercept Ltd, Paris. pp. 187–192.

Marasović, I., Ž. Ninčević, M. Pavela-Vrančić and S. Orhanović. 1998. A survey of shellfish toxicity in the Central Adriatic Sea. *J. Mar. Biol. Assoc. UK 78*: 745–754.

Marcaillou-Le Baut, C., D. Lucas and L. Le Dean. 1985. *Dinophysis acuminata* toxin status of toxicity bioassay in France. *In*: (D.M. Anderson, A.W. White and D.G. Baden, eds.) *Toxic dinoflagellates*. Elsevier Press, New York. pp. 485–488.

Nishitani, G., H. Sugioka and I. Imai. 2002. Seasonal distribution of species of the toxic dinoflagellate genus *Dinophysis* in Maizuru Bay (Japan), with comments on their autofluorescence and attachment of picophytoplankton. *Harmful Algae 1*: 253–264.

Orhanović, S., Ž. Ninčević, I. Marasović and M. Pavela-Vrančić. 1996. Phytoplankton toxins in the central Adriatic Sea. *Croatica Chem. Acta 69*: 291–303.

Orlić, M. 1989. Salinity of the North Adriatic. A fresh look at some old data. *Boll. Oceanol. Teor. Applic. 7*: 219–228.

Pavela-Vranči, M., V. Meštrović, I. Marasović, M. Gillman, A. Furey and K.J. James. 2002. DSP toxin profile in the coastal waters of the central Adriatic Sea. *Toxicon 40*: 1601–1607.

Quilliam, M.A., P. Hess, C. Dell'Aversano. 2001. Recent developments in the analysis of phycotoxins by liquid chromatography-mass spectrometry. *In*: (W.J. deKoe, R.A. Sampson, H.P. van Egmond, J. Gilbert and M. Sabino, eds.) *Mycotoxins and phycotoxins in perspective at the turn of the millenium*. W.J. deKoe, Wageningen. pp. 383–391.

Reguera, B., I. Bravo and S. Fraga. 1995. Autoecology and some life history stages of *Dinophysis acuta* Ehrenberg. *J. Plankton Res. 17*: 999–1015.

Sedmak, B. and N. Fanuko. 1991. Occurrence of *Dinophysis* spp. and toxic shellfish in the Northern Adriatic. *J. Appl. Phycol. 3*: 289–294.

Sidari, L., S. Cok, M. Cabrini, A. Tubaro and G. Honsell. 1995a. Temporal distribution of toxic phytoplankton in the Gulf of Trieste (Northern Adriatic Sea) in 1991 and 1992. *In*: (P. Lassus, G. Arzul, E. Erard, P. Gentien and C. Marcaillou, eds.) *Harmful marine algal blooms. Technique et documentation.* Lavoisier, Intercept Ltd, Paris. pp. 231–236.

Sidari, L., P. Nichetto, S. Cok, S. Sosa, A. Tubaro, G. Honsell and R. Della Loggia. 1995b. Phytoplankton detection and DSP toxicity: methodological considerations. *J. Appl. Phycol. 7*: 163–166.

Sournia, A. 1986. *Atlas du phytoplancton marin. Introduction, cyanophycées, dictyochophyées, dinophycées et raphidophycées*. Vol. I. Editions du CNRS, Paris. pp. 219.

Strickland, J.D.H. and T.R. Parsons. 1972. A practical handbook of seawater analysis. *Bull. Fish. Res. Bd. Can. 167*: 1–310.

Tubaro, A., C. Florio, E. Luxich, S. Sosa, R. Della Loggia and T. Yasumoto. 1996. A protein phosphatase 2A inhibition assay for a fast and sensitive assessment of ocadaic acid contamination in mussels. *Toxicon 34*: 743–752.

Tubaro, A., L. Sidari, R. Della Loggia and T. Yasumoto. 1998. Occurrence of yessotoxin-like toxins in phytoplankton and mussels from northern Adriatic Sea. *In*: (B. Reguera, J. Blanco, M.L. Fernández and T. Wyatt, eds.) *Harmful algae*. Xunta de Galicia and IOC of UNESCO, Santiago de Compostela. pp. 470–472.

Utermöhl, H. 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *Mitt. Int. Ver. Theor. Angew. Limnol. 9*: 1–38.

Vale, P. and M.A.M. Sampayo. 2003. Seasonality of diarrhetic shellfish poisoning at a coastal lagoon in Portugal: rainfall patterns and folk wisdom. *Toxicon 41*: 187–197.

Vila, M., J. Camp, E. Garcés, M. Maso and M. Delgado. 2001. High resolution spatio-temporal detection of potentially harmful dinoflagellates in confined waters of the NW Mediterranean. *J. Plankton Res. 23*: 497–514.

Vukadin, I. 1981. Hydrographic properties of Mali Ston Bay and the adjacent Sea. *In*: (J. Roglić and M. Meštrov, eds.) *Proceedings of the Symposium on Mali Ston Bay*, Dubrovnik, Croatia, 12–24 November 1981. pp. 52–65 (in Croatian).

Yasumoto, T., Y. Oshima, W. Sugawara, Y. Fukuyo, H. Oguri, T. Igarashi and N. Fujita. 1980. Identification of *Dinophysis fortii* as the causative organism of diarrhetic shellfish poisoning. *Bull. Jap. Soc. Sci. Fish. 46*: 1405–1411.

Yasumoto, T., M. Murata, Y. Oshima, M. Sano, G. Matsumoto and J. Clardy. 1985. Diarrhetic shellfish toxins. *Tetrahedron 41*: 1019–1025.

Zingone, A., M. Montresor and D. Marino. 1998. Morphological variability of the potentially toxic dinoflagellate *Dinophysis sacculus* (Dinophyceae) and its taxonomic relationships with *D. pavillardii* and *D. acuminata*. *Eur. J. Phycol. 33*: 259–273.

Zore-Armanda, M. 1980. Some dynamic and hydrographic properties of the Kaštela Bay. *Acta Adriat. 21*: 55–74.

Received 14 April, 2008; accepted 12 September, 2008