Vol. 26: 51–60, 2001 | AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol | Published October 26

Potentially toxic epiphytic dinoflagellate assemblages on macroalgae in the NW Mediterranean

Magda Vila*, Esther Garcés, Mercedes Masó

Institut de Ciències del Mar, Passeig Marítim de la Barceloneta, 37–49, 08003 Barcelona, Catalonia, Spain

ABSTRACT: A potentially toxic epiphytic dinoflagellate assemblage on macroalgae was studied for 1 yr in a shallow protected rocky habitat in Palamós (Costa Brava, NW Mediterranean). The assemblage was monitored on 4 macroalgae: *Corallina elongata* (Rhodophyceae), *Dictyota dichotoma*, *Dilophus fasciola* and *Halopteris scoparia* (Phaeophyceae). The dominant dinoflagellates were *Ostreopsis* sp., and the accompanying species were *Coolia monotis* and *Prorocentrum lima*. The diatom *Coscinodiscus* sp. was an abundant component of the assemblage. *Ostreopsis* followed the same seasonal pattern on the 4 macroalgae selected. Substrate was not significantly different for the dinoflagellate assemblages. *Ostreopsis* was present both in the water column and in the sand concomitant with maximal cell densities on macroalgae. Small-scale sampling revealed that all the epiphytic organisms prefer slightly shaken habitats. While *Ostreopsis* sp. prefers shaken to slightly shaken waters, *Coolia monotis* prefers slightly shaken to calm ones. The dinoflagellate assemblage follows a clear seasonal pattern, achieving maximum cell concentration during spring and summer without significant relative changes in the species composition. The epiphytic assemblage was widespread along the Catalan coast and Majorca, although dinoflagellates were found to be more abundant in the Costa Brava. In Corsica, diatoms dominated the assemblage, whereas *Ostreopsis* sp. was a minor component.

KEY WORDS: *Ostreopsis* · *Coolia monotis* · *Prorocentrum lima* · Benthic dinoflagellates · Ciguatera fish poisoning

INTRODUCTION

Epiphytic (in close association with macroalgae) and benthic (in coral rubble, sand and detritus) dinoflagellates are relevant because the species *Gambierdiscus toxicus* causes ciguatera (Yasumoto et al. 1977, Adachi & Fukuyo 1979). Ciguatera, or ciguatera fish poisoning, is a human disease caused by the ingestion of contaminated marine finfish from tropical and subtropical regions, which results in gastrointestinal and neurological disorders and sometimes death. Polyether toxins (ciguatoxins and maitotoxins, among others) that are produced by marine epiphytic dinoflagellates (Steidinger 1983) may cause these symptoms. Macroalgae (and epiphytic assemblages of harmful dinoflagellates) are eaten by herbivorous fish, which then become toxic. Thus, the toxins are biologically concentrated within the food chain (Steidinger & Baden 1984). A dinoflagellate assemblage in the genera *Gambierdiscus*, *Ostreopsis*, *Coolia*, *Prorocentrum* and *Amphidinium* (Ballantine et al. 1985, Carlson & Tindall 1985, Bomber & Aikman 1989, Bourdeau et al. 1995, Faust 1995) has also been reported in ciguatera-endemic areas. In particular, *Prorocentrum lima*, *P. concavum*, *Ostreopsis siamensis* and *O. ovata* have been implicated in ciguatera fish poisoning based on distribution, toxicity to mice and the presence of a fat-soluble toxic fraction (Yasumoto et al. 1980, Nakajima et al. 1981). These organisms form epiphytic communities associated with coral reefs, or rather with macroalgae attached to coral surfaces. These assemblages may vary in species composition and cell concentration

*E-mail: magda@icm.csic.es

between sites (Tindall & Morton 1998). The mixed association of toxic dinoflagellates may contribute to the polymorphism of the clinical features of ciguatera (Yasumoto et al. 1987).

Ostreopsidaceae species are widespread in most epiphytic and benthic dinoflagellate communities from ciguatera-endemic regions of the world (35° N to 35° S). Thus, the geographic distribution of *Ostreopsis siamensis*, *O. lenticularis* and *O. ovata* is similar to that of *Gambierdiscus toxicus* (Tindall & Morton 1998), with 2 notable exceptions: *O. siamensis* and *O. ovata* have been reported in the Mediterranean Sea (Taylor 1979, Tognetto et al. 1995). Nevertheless, data are limited on the incidence of *Ostreopsis* in the waters of the Mediterranean Sea and on the magnitude of potentially toxic epiphytic dinoflagellate assemblage attached to macroalgae.

In this study, we quantified epiphytic dinoflagellate assemblages on the Catalan coast, NW Mediterranean. The potentially toxic epiphytic dinoflagellate assemblage associated with macroalgae was examined during an annual cycle in a rocky habitat. In addition, small-scale spatial variability and middle-scale spatial distribution were analysed to shed some light on the epiphytic dinoflagellate assemblages in the NW Mediterranean.

The dominant dinoflagellate *Ostreopsis* sp. could not be assigned to any described species. Thus, a brief description of the species with scanning electron microphotographs is included for further considerations.

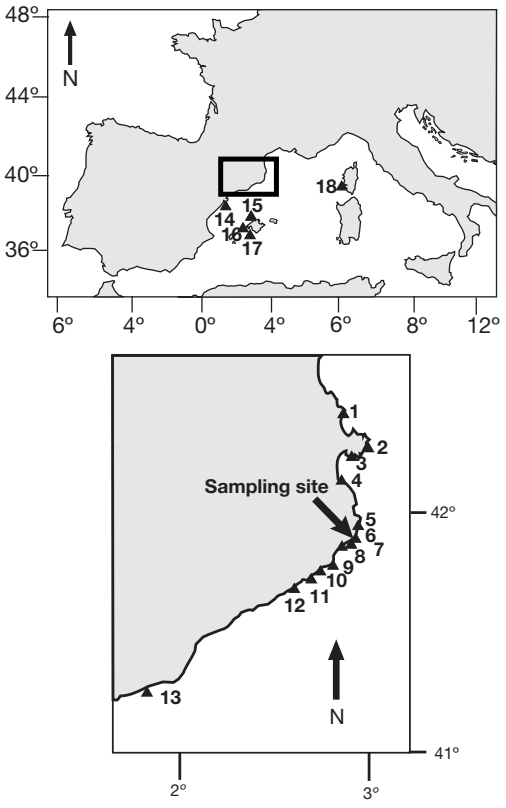

Fig. 1. Study area and stations sampled. 1: Cala Garbet; 2: Portlligat; 3: Canyelles Grosses; 4: Cala Portitxol; 5: Cala Pedrosa; 6: La Foradada; 7: Palamós (sampling site); 8: Gran de Palamós; 9: Cala Canyet; 10: Santa Cristina d'Aro; 11: El Xuclador; 12: Sant Pol de Mar; 13: Vallcarca; 14: Riu Sènia; 15: Deià; 16: Cala Fornells; 17: Portals Vells; 18: Campomoro; 1–14 in Catalonia; 15–17 in Majorca; 18 in Corsica

MATERIAL AND METHODS

Sampling sites. Epiphytic dinoflagellates on selected macroalgae (Rhodophyceae and Phaeophyceae) were quantified for 1 yr (July 1997 to July 1998). The sampled macroalgae grow in multispecies assemblages attached to stones in the infralittoral. Macroalgal specimens (in triplicate) were collected weekly at 20 to 40 cm depths during summer (July and August 1997) and monthly during the rest of the year. The sampling site was a shallow protected rocky habitat in Palamós (Catalan sea, NW Mediterranean). During the summers of 1997 and 1998, a more extensive study was carried out. Fourteen stations were sampled, mainly along the Costa Brava (northern Catalan coast) and in 2 other Mediterranean areas (Majorca and Corsica) (Fig. 1).

Sampling methods. A subsample (15 to 20 g fresh weight [FW]) was carefully cut and placed with tweezers in a small glass bottle containing 10 ml of formaldehyde-filtered seawater. Since the macroalgae community is dynamic and the species composition varies throughout the year, at every sampling date the available macroalgae were taken to cover the annual cycle. The main macroalgae analysed were *Corallina elongata* (Rhodophyceae), *Dictyota dichotoma*, *Dilophus fasciola* and *Halopteris scoparia* (Phaeophyceae). *C. elongata* is present throughout the year; thus, we have studied the seasonal patterns in this macroalga. In addition, other macroalgae were collected sporadically, mainly during the cold season, when the 4 target species were very scarce. The additional macroalgae sampled were *Jania corniculata*, *Pterocladia capillacea*, *Laurencia* gr. *obtusa*, *Rissoella verruculosa*, *Ceramium ciliatum*, *Peyssonnelia squamaria* (Rhodophyceae), *Dictyopteris membranacea*, *Padina pavonica* (Phaeophyceae) and *Ulva* sp. (Chlorophyceae). Surface water (0.5 m) and sediment samples were collected in 150 and 50 ml bottles and preserved with formaldehyde (1% final concentration) for dinoflagellate examination. Nutrient samples were taken and frozen immediately and analysed for nitrate, nitrite, ammonia, phosphate and silicate as described by Grasshoff et al. (1983). Temperature and salinity were measured.

Once in the laboratory, macroalgae bottles were shaken vigorously for 1 min to dislodge the epiphytic organisms. Macroalgae were removed and the sample was settled for 6 h in 10 ml counting chambers. An appropriate area of the chamber was then scanned (Throndsen 1995) for epiphytic organism counting at 63 to 200× magnification using a Leica-Leitz DM-IL inverted microscope (Leica Mikroskopie und Systeme GmbH, Wetzlar). Samples were examined and counted for epiphytic microalgal species. When high densities of organisms were found in the sample, only a subsample was examined. Macroalgae were processed for fresh weight (FW) and dry weight (DW) measurements. DW was measured after the macroalgae were dried in an oven at 60°C. FW and DW were highly correlated (regression analysis, $r^2 > 0.98$). Thus, we worked with FW, as is usual in other studies. One-way ANOVA was performed to test differences between the 4 macroalgae for each epiphyte dinoflagellate (4×4) (STATISTICA for Windows, Statsoft, Tulsa, OK). The analysis was done during the warmer months to avoid seasonal interactions. Water samples for phytoplankton quantification were settled for 24 h in 50 ml counting chambers and they were then examined as above. Sediment samples (around 30 g) were sonicated in filtered seawater for 10 to 15 s and sieved. The 20 to 135 µm fraction was examined in an inverted microscope, as described above.

In April 1998, small-scale samples were taken at Stn 7 to study the spatial variability of epiphytic organisms on *Corallina elongata* in relation to hydrodynamism. Three hydrodynamic regimens were defined: shaken, slightly shaken and calm. Shaken regimens were observed in sites where macroalgae were directly hit by waves (high hydrodynamism); calm regimens corresponded to sites where macroalgae were protected from the waves by rock barriers (low hydrodynamism); and the slightly shaken regimens were intermediate. Macroalgal samples were collected from the 3 habitats and processed as described above. Three sites were sampled at each habitat and 3 replicates were analysed. An ANOVA nested design was used ($3 \times 3 \times 3$) (STATISTICA for Windows).

During summer 1997 and 1998, 14 stations were sampled, mainly along the Costa Brava (northern region of the Catalan coast, Fig. 1) to limit the geographical distribution. Samples were also taken from Corsica (summer 1998) and Majorca (Balearic Islands, summer 2000) and qualitatively examined for the epiphytic assemblage to determine the extent of the phenomenon in the NW Mediterranean.

Identification of dinoflagellates. Samples were fixed with 4% glutaraldehyde for scanning electron microscopy. One millilitre of fixed sample was filtered through a 13 mm diameter and 0.8 µm pore size Nuclepore PC polycarbonate membrane filter (Costar, Europe Ltd, Badhoevedorp). Samples were washed in distilled water and dehydrated in an ethanol series (30, 50, 70, 80, 90, 100%) at 4°C, critical point dried with CO_2 and examined under a Hitachi S-570 scanning electron microscope (Nissei Sangyo Co. Ltd, Tokyo; modified from Faust et al. 1996).

RESULTS

Taxonomy of the epiphytic assemblages

Natural populations of benthic dinoflagellates and diatoms formed a mucilaginous matrix on the macroalgal thallus and aggregated therein (Fig. 2A,B). Cells remained motile within the matrix and loosely linked to macroalgae, as revealed by light microscopy. When the epiphyte assemblage was dense, the brownish mucilaginous matrix covering the surface of the algae was visible to the naked eye.

The dinoflagellate epiphyte assemblage on macroalgae comprised *Coolia monotis*, *Prorocentrum lima* and especially *Ostreopsis* sp. The highest *Ostreopsis* sp. concentration was 596×10^3 cells g^{-1} FW macroalga on *Halopteris scoparia* in July 1997, or 6270×10^3 cells g^{-1} DW macroalgae. *P. mexicanum* and *P. emerginatum* were occasionally recorded as minor components of the community. The benthic diatom *Coscinodiscus* sp. was an abundant constituent, sometimes accompanied by other diatoms such as *Striatella* sp. and *Cylindrotheca closterium*. Polychaete and crustacean larvae were often observed.

The morphological features of the dominant species, *Ostreopsis* sp., do not match those described elsewhere (Fukuyo 1981, Norris et al. 1985, Quod 1994, Faust & Morton 1995, Faust et al. 1996, Faust 1999). *Ostreopsis* cells were usually quite large, pointed towards the sulcus in apical view and compressed anteroposteriorly (about 22 µm). The dorsoventral diameter (length) was 63 to 90 µm (average 75 µm) and the transdiameter (width) 34 to 56 µm (average 45 µm) (Fig. 2C,D,E). The pore plate (Po) was about 10 µm long. On the epitheca, the 1′ plate was large and in contact with plates Po/2′, 3′, 1″, 2″, 6″ and 7″. The external part of the thecal plates was covered with 1 size of pores (0.1 to 0.2 µm diameter).

There may be confusion in the literature about the morphology of *Ostreopsis siamensis* and *O. lenticularis*. *O. siamensis*, which was first described by Schmidt (1902), was redescribed by Fukuyo (1981), when he also described *O. lenticularis* and *O. ovata*. *O. siamensis* and *O. lenticularis* were similar in size but differed in the presence of dissimilar thecal pore sizes. *O. siamensis* was found to have 1 size of thecal pore

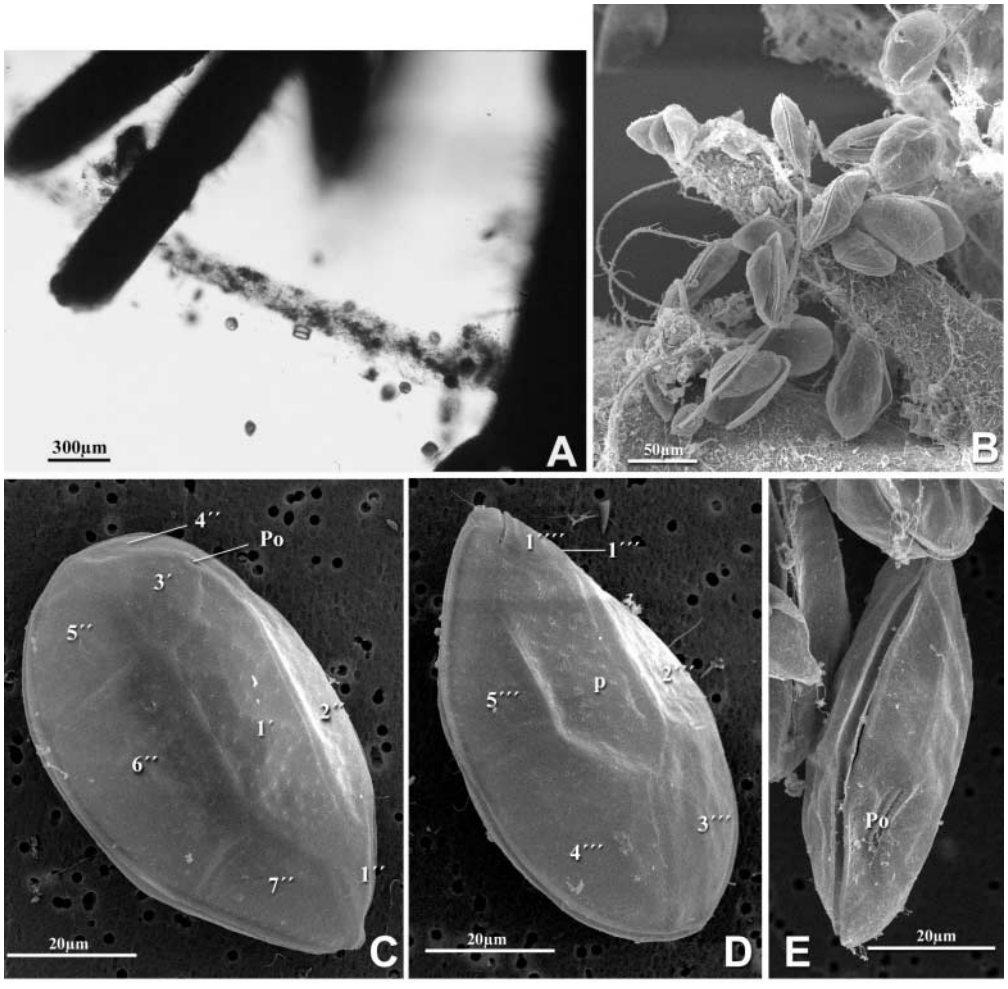

Fig. 2. Mucilaginous matrix of epiphytic dinoflagellates on a macroalga observed under (A) light microscope and (B) scanning electron microscope (SEM). *Ostreopsis* sp. cells viewed with SEM in (C) epithecal view, (D) hypothecal view and (E) left lateral view

whereas *O. lenticularis* had 2 sizes (Fukuyo 1981). *O. ovata* was smaller and had 1 size of thecal pores. In contast, Faust et al. (1996) found that *O. siamensis* was bigger than *O. lenticularis* and had 2 sizes of thecal pores, while *O. lenticularis* had 1 pore size. Our species description agrees with the *O. siamensis* (in cell size and number of pore sizes) described by Fukuyo (1981), but not with that described by Faust et al. (1996). It also agrees with *O. ovata* in the number of pore sizes, although this organism is smaller than our species. Thus, given the confusion, it was not assigned any specific name. Taxonomical studies and genetic assays are in progress (A. Penna in press).

Preferred habitat and seasonal variability

The temporal variability in the physico-chemical characteristics of the study site (Stn 7) is shown in Fig. 3A. Water temperature showed marked seasonality (range 11.5 to 26.3°C). The study site received, during periods of rain, freshwater from a small river. Accordingly, salinity oscillated between 37.2 and 38.1 psu. Nutrient concentrations ranged from 0.11 to 0.86 µM for phospate, 0.76 to 7.74 µM for DIN and 0.17 to 4.51 µM for silicate. A clear temporal variation was not observed. However, discrete high concentrations of DIN and silicate were observed during winter and spring.

The concentration of *Ostreopsis* sp. in 3 habitats (attached on macroalgae, in the water column and in sand) at Stn 7 is shown in Fig. 3B. *Ostreopsis* sp. was also the dominant species in the water column and sand. The 3 habitats showed a clear seasonal pattern, with high biomass from late winter until late summer. *Ostreopsis* sp. in the water column achieved high cell concentrations ($>10^4$ cells l^{-1}) concomitant with maximal cell densities on macroalgae (10^4 to 10^5 cells g^{-1} FW). Cell densities in the water column and on macroalgae were positively and significantly corre-

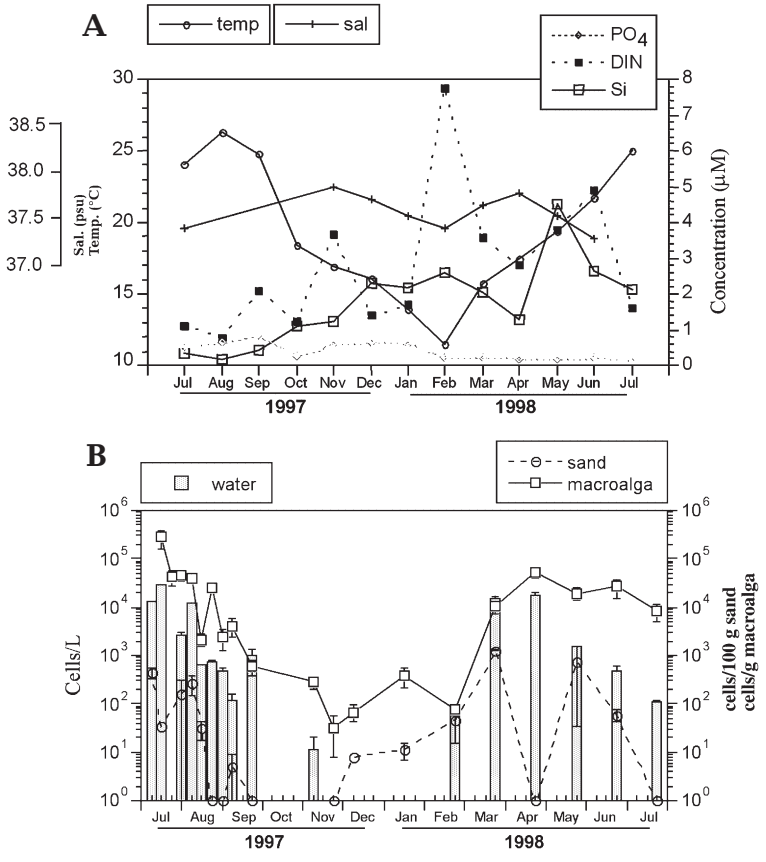

Fig. 3. (A) Temporal physico-chemical properties of the study area. Nutrient samples from July to September 1997 were taken approximately 200 m away from the sampling site. Sal: salinity; Temp: temperature. (B) Seasonal abundance of *Ostreopsis* sp. in the macroalgae, in the water column and on the sediment at the sampling site. Cells on macroalgae are the averaged values for the 4 macroalgae sampled. Bars indicate SE. Missing bars correspond to macroalgae that were not sampled. FW: fresh weight

lated (n = 18, Pearson's r = 0.82, $p < 0.001$), especially for *Corallina elongata* (n = 11, Pearson's r = 0.79, $p < 0.05$) and *Halopteris scoparia* (n = 8, Pearson's r = 0.91, $p < 0.05$). Cell densities in sand followed the same seasonal pattern (although this was not statistically significant, since we worked near the detection limit) as in the water column and on macroalgae. There were few *Coolia monotis* and *Prorocentrum lima* in the water column (maximum cell concentrations 4600 and 330 cells l^{-1}, respectively) and they were mostly absent from sand, except on some sampling days (cell concentrations <2 cells g^{-1}).

Ostreopsis followed the same seasonal pattern on the 4 substrates selected (*Corallina elongata*, *Dictyota dichotoma*, *Dilophus fasciola* and *Halopteris scoparia*) (Fig. 4A). Maximum concentrations were found from March to September. Substrate (macroalgae) was not significant (ANOVA, $p > 0.05$) for *Ostreopsis* sp., *Prorocentrum lima* and *Coolia monotis*, but was significant for *Cocinodiscus* sp. (ANOVA, $p < 0.05$). The analysis was performed during the warmest months to avoid seasonal interactions. Table 1 presents the epiphytic dinoflagellates on additional macroalgal species examined all year round. The epiphytic assemblage of dinoflagellates was the same, with *Ostreopsis* sp. the most abundant species *Coscinodiscus* also reached high cell numbers (Table 1).

The distribution of potentially toxic epiphytic dinoflagellate and *Coscinodiscus* assemblages on *Corallina elongata* (*Ostreopsis* sp., *Coolia monotis*, *Prorocentrum lima* and *Coscinodiscus* sp.) and monthly relative species abundance are shown in

Table 1. Epiphytic assemblage composition in macroalgae (other than 4 target species) from the sampling site (Palamós) year round. Average epiphytic cell concentration on macroalgae (cells g^{-1} fresh weight [FW]) and relative abundance (%). Os: *Ostreopsis* sp.; Co: *Coolia monotis*; Pl: *Prorocentrum lima*; Cs: *Coscinodiscus* sp.

Macroalgae	Season	n	Average (cells g^{-1} FW)				Relative abundance (%)			
			Os	Co	Pl	Cs	Os	Co	Pl	Cs
Pterocladia capillacea	Summer	2	2591	90	647	3574	38	1	9	52
Jania corniculata		1	1181	47	331	189	68	3	19	11
Laurencia gr. *obtusa*	Autumn	2	867	0	397	785	42	0	19	38
Laurencia gr. *obtusa*		3	1410	4	35	16	96	0	2	1
Pterocladia capillacea	Winter	4	156	0	1	0	99	0	1	0
Ulva sp.		1	109	0	5	34	74	0	4	23
Risoella verruculosa		1	0	0	0	0				
Jania corniculata		4	242	5	2	124	65	1	0	33
Pterocladia capillacea		3	133	1	1	39	76	1	1	23
Ceramium ciliatum	Spring	3	74147	4681	55	11190	82	5	0	12
Dictyopteris membranacea		3	47976	9557	238	16529	65	13	0	22
Laurencia gr. *obtusa*		3	134512	11801	130	6740	88	8	0	4
Peyssonnelia squamaria		3	4209	1030	46	2354	55	13	1	31
Padina pavonica		2	56404	33825	481	25475	49	29	0	22

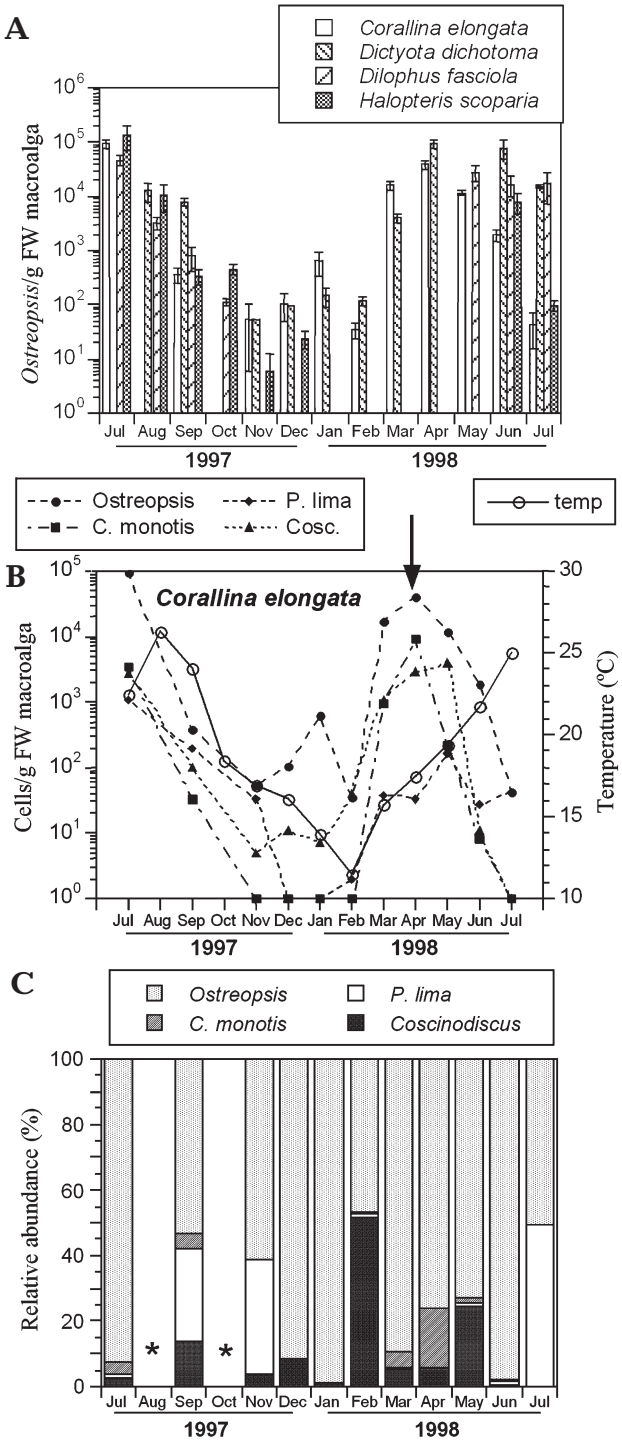

Fig. 4. (A) Seasonal abundance of epiphyte *Ostreopsis* sp. on the 4 macroalgae: *Corallina elongata*, *Dictyota dichotoma*, *Dilophus fasciola* and *Halopteris scoparia*. (B) Seasonal abundance and (C) percentage of the major epiphytic organisms (*Ostreopsis* sp., *C. monotis*, *P. lima* and *Coscinodiscus* sp.) on *C. elongata* from July 1997 to July 1998. Asterisks indicate the months in which the macroalgae were not collected. Arrow indicates the day on which sampling was intensive to record the spatial variability associated with 3 hydrodynamic regimens

Fig. 4B and C, respectively. The 4 epiphytic species followed the same seasonal pattern (n = 34, Pearson's r = 0.87, $p < 0.05$). Although *Ostreopsis* sp. was the most dominant species, the relative abundances varied (Fig. 4C). For instance, in April *C. monotis* achieved 18% relative abundance in *C. elongata*, whereas it reached 64% in the other substrate, *Dictyota dichotoma* (absolute abundance of 143×10^3 cells g^{-1} FW). *P. lima* occasionally achieved high relative abundance (28 to 49%), which does not reflect high cell densities (<200 cells *P. lima* g^{-1} FW). On the contrary, it is caused by low densities on the whole epiphytic community (80 to 700 cells g^{-1} FW).

The spatial variability of the epiphytic assemblage on *Corallina elongata* is shown in Fig. 5 (April 1998). The photophilic communities of *C. elongata* are typical of shaken or turbulent environments. In the area sampled, macroalgae were scarce in the calm area (where fine sandy deposition was observed) and abundant in slightly shaken or shaken areas. Cell densities of all epiphytic organisms were the highest in slightly shaken sites. The dominant species were *Ostreopsis* sp. and *Coolia monotis*, whereas cell densities on *Coscinodiscus* sp. (<10^4 cells g^{-1} FW) and *Prorocentrum lima* were very low (<10^2 cells g^{-1} FW). *Ostreopsis* sp. was more abundant than *C. monotis* in shaken sites (75 vs 19%) and less abundant in calm sites (8 vs 81%). In slightly shaken sites, the preferred habitat for *Ostreopsis* sp. and *C. monotis*, they were co-dominant (41 vs 51%) (Fig. 5). Differences among the 3 regimens (shaken, slightly shaken and calm) were significant (ANOVA, $p < 0.05$), but those within groups (3 sites, 3 replicates) were not significant (ANOVA, $p > 0.05$).

Cell abundances are shown from the broader sampling area along Costa Brava in Table 2. The epi-

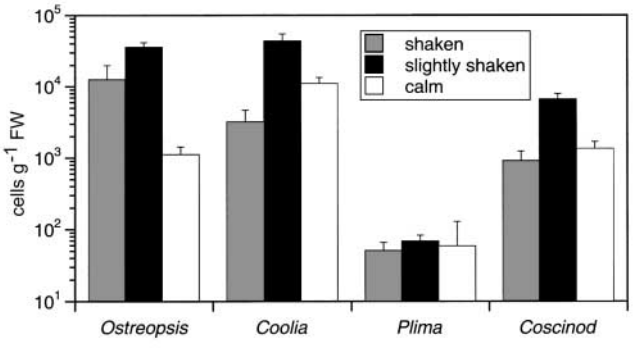

Fig 5. Epiphytic averaged densities (*Ostreopsis* sp., *Coolia monotis*, *Prorocentrum lima* and *Coscinodiscus*) on *Corallina elongata* in 3 hydrodynamic regimens (shaken, slightly shaken and calm). Samples were taken in the sampling station in April 1998 (when cell densities where high, see arrow in Fig 4B)

phytic assemblage (4 microalgal species enumerated) is shown on various macroalgae. Dinoflagellates were most abundant at Stns 6 to 11 (which are the nearest to the sampled site; Fig. 1). A similar assemblage (on the fourth main species) was found in Majorca, in contrast with Corsica, where diatoms dominated the community and *Ostreopsis* sp. was a minor component.

DISCUSSION

The epiphytic dinoflagellate assemblage in the Mediterranean: comparison with other places

This is not the first report of the genus *Ostreopsis* in the Mediterranean Sea. Taylor (1979) described an association of *Ostreopsis siamensis*, *Coolia monotis*,

Table 2. Epiphytic assemblage composition in macroalgae from other stations in the NW Mediterranean. Average epiphytic cell concentration on macroalgae (cells g^{-1} FW) and relative abundance (%). See Table 1 for species abbreviations. –: absent; +: present; ++: low abundance; +++: high abundance; ++++: very high abundance. *Diatoms other than *Coscinodiscus*

Macroalgae	Stn	Abundance (cells g^{-1} FW)				Relative abundance (%)			
		Os	Co	Pl	Cs	Os	Co	Pl	Cs
Catalonia, summer 1997 and 1998									
Phaeophyceae									
Dictyota dichotoma	1	312	37	73	0	74	9	17	0
Dictyota dichotoma	2	228	2151	554	196	7	69	18	6
Halopteris scoparia	2	0	63	98	0	0	39	61	0
Dilophus fasciola	3	414	61	97	170	56	8	13	23
Halopteris scoparia	3	10	10	105	0	8	8	85	0
Halopteris scoparia	4	0	0	0	1	0	0	0	100
Dictyota dichotoma	5	0	104	367	389	0	12	43	45
Dictyota dichotoma	6	17939	76	284	132	97	0	2	1
Halopteris scoparia	6	7	0	20	0	25	0	75	0
Dictyota dichotoma	8	159	26	53	160	40	7	13	40
Halopteris scoparia	8	282	0	311	256	33	0	37	30
Dilophus fasciola	9	0	342	683	506	0	22	45	33
Dilophus fasciola	10	10917	363	60	30	96	3	1	0
Dictyota dichotoma	11	21	10	7573	2195	0	0	77	22
Halopteris scoparia	11	129	39	741	107	13	4	73	11
Dictyota dichotoma	12	0	40	71	0	0	36	64	0
Dictyota dichotoma	13	6	18	0	0	26	74	0	0
Dictyota dichotoma	14	0	0	0	0	0	0	0	0
Rhodophyta									
Corallina elongata	1	0	0	7	0	0	0	100	0
Corallina elongata	2	4	59	28	4	4	63	29	4
Corallina elongata	3	25	18	55	0	25	19	56	0
Corallina elongata	4	0	36	7	58	0	36	7	57
Corallina elongata	5	0	0	2	1	11	0	56	33
Corallina elongata	6	77136	1322	1542	331	96	2	2	0
Jania + *Corallina*	9	24	416	559	309	2	32	43	24
Jania corniculata	10	6543	239	84	6	95	3	1	0
Corallina elongata	11	1090	88	651	158	55	4	33	8
Corallina elongata	12	0	3	0	0	0	100	0	0
Corallina elongata	14	103	0	6	0	94	0	6	0
Majorca, summer 2000									
Phaeophyceae									
Halopteris scoparia	15	–	–	+	++				
Dictyopteris membranacea	15	++++	++	+	+				
Dilophus fasciola	15	+++	++	++	–				
Padina pavonica	15	++++	++	++	++				
Halopteris scoparia	16	+++	–	+	++++				
Halopteris scoparia	17	–	–	+	–				
Dictyota + *Dilophus*	17	++	+	+	+				
Corsica, summer 1998									
Phaeophyceae + Rhodophyta						Other diatoms*			
Mixed sample (*Corallina*, *Jania*, *Dictyota*, *Halopteris*...)	18	++	–	–	–	++++			

Oxyrrhis marina and *Amphidinium* sp. in the NW Mediterranean (Vilefranche-sur-Mer). The occurrence of *O. ovata* in the water column was also documented in the Thyrrhenian Sea (Tognetto et al. 1995). Moreover, low cell concentrations of *Ostreopsis* cf. *siamensis* (<200 cells l^{-1}) have been recorded in Andalusia (Mamán et al. 2000) and Catalonia (Vila et al. 2001). An association dominated by *C. monotis* and low concentrations of *Ostreopsis* sp. were reported in other Mediterranean localities (Ganzirri Lagoon, Sicily) (Gangemi 2001). However, long-term epiphytic associations had not been quantified in the Mediterranean Sea. In this study, the epiphytic microscopic assemblage mentioned in the previous section was also detected in samples from Majorca and Corsica. Thus, this association is probably common in the NW Mediterranean Sea.

A similar association, consisting of *Ostreopsis siamensis*, *O. lenticularis*, *O. ovata*, *Prorocentrum lima*, *P. compressum* and *Coolia monotis*, has been recorded in northern New Zealand. The dominant species, *O. siamensis*, accounted for 64 to 85% of the total epiphytic flora during summer (Chang et al. 2000). Although *Gambierdiscus toxicus* and *O. lenticularis* are co-dominant in many tropical regions (Bagins et al. 1985, Ballantine et al. 1985, Carlson & Tindall 1985, Gillespie et al. 1985, Bomber & Aikman 1989), *G. toxicus* has been recorded only once (and in extremely low concentrations) in northern New Zealand (Chang et al. 2000) and never on the Catalan coast. There may be a latitudinal gradient that implies different species composition within the benthic association. What is certain is that these epiphytic assemblages are not restricted to tropical and subtropical waters but are present in temperate water as well.

The epiphytic and benthic dinoflagellates from New Zealand seem to be associated with the lipid-soluble toxins detected in shellfish from the studied area. However, the link between toxins and the presence of *Ostreopsis siamensis* is not yet clear (Chang et al. 2000). The epiphytic community toxicity in Catalonia had been tested by injecting the extract (intraperitoneally) into mice (modified from the Association of Official Analytic Chemists 1980, Yasumoto et al. 1980). The organic fraction was not toxic, whereas the water-soluble fraction killed the mice in 20 min. The symptoms observed in mice were not PSP symptoms. Instead, they were reminiscent of neurotoxic symptoms (E. Cacho pers. comm.). Signs of paralytic shellfish poisoning (PSP) were also not detected by HPLC analysis (J. M. Franco pers. comm.). Our preliminary results suggest that the toxin is present in the water-soluble fraction, in disagreement with the study carried out in New Zealand and in agreement with Tindall et al. (1990), who identified a water-soluble toxin very similar to maitotoxin (ostreotoxin) in *O. lenticularis*. However, the specific toxicity of the Mediterranean epiphytic community requires further research.

Preferred habitat and seasonal variability

Dinoflagellates in this study were epiphytic on macroalgae, and low densities were detected in the water column and on the sediments. Numerous species of macroalgae host significant numbers of epiphytic dinoflagellates. They include members of Rodophyta, Phaeophyta, Chlorophyta and Cyanophyta (Tindall & Morton 1998). The macroalgae tested in this study, which correspond mainly to Rodophyta and Phaeophyta, supported high densities of epiphytic dinoflagellates. The highest density detected for *Ostreopsis* sp. in this study was 5.9×10^5 cells g^{-1} FW in *Halopteris scoparia* during July 1997. To our knowledge, this is one of the highest densities of epiphytic species ever reported. For example, the highest density of *Ostreopsis lenticularis* was estimated to be 2.35×10^5 cells g^{-1} FW on the macroalga *Dictyota* at Laurel Reef, Puerto Rico (Ballantine et al. 1985) and that of *Gambierdiscus toxicus* was estimated to be 5.0×10^5 cells g^{-1} FW on *Jania* in a Gambier Island reef (Yasumoto et al. 1980). At Virgin Islands, *Coolia monotis* density was 1.2×10^6 cells g^{-1} FW macroalgae and that of *Prorocentrum mexicanum* was 1.5×10^6 cells g^{-1} FW macroalgae (Carlson & Tindall 1985). However, the maximum densities of epiphytic species commonly range from 10^2 to 10^4 cells g^{-1} FW macroalgae (Tindall & Morton 1998). Significant differences in epiphytic densities between macroalgae were not detected in this study, in agreement with Taylor (1985), Lobel et al. (1988) and Bomber et al. (1989), who stated that epiphytic dinoflagellates prefer 3-dimensional, flexible, high-surface area algae, like the macroalgae sampled in this study, rather than a particular macroalgal species or phylum.

Cell densities around 10×10^3 to 20×10^3 cell l^{-1} were sometimes recorded during warm months in the water column at the sampling site (Stn 7). However, bloom concentrations of *Ostreopsis* were never detected in the water column and *Ostreopsis* was very scarce on the sediment. The positive and significant correlation of *Ostreopsis* sp. concentrations in the water column and sediment with those on macroalgae probably indicates that the former were resuspended or released from the surface of macroalgae. The presence of *Ostreopsis* sp. in the water column on the Catalan coast has been well documented since the beginning of routine monitoring in 1995 (Vila et al. 2001). *Ostreopsis* sp. in the water column has occasionally been detected, but in cell densities lower than 100 cells l^{-1}. High densities of *Ostreopsis* sp. were observed only in Garraf harbour

(5×10^3 to 78×10^3 cells l^{-1}) during autumn 1997 (near Stn 13), concomitant with wrested macroalgae that were floating during the sampling days, and in Blanes harbour (98×10^3 cells l^{-1}) on October 27, 1997 (near Stn 10), after a heavy storm. Thus, the preferred habitat of *Ostreopsis* sp. in the Catalan sea is epiphytic on macroalgae. In coral reef areas, these organisms were mostly associated with macroalgae located between 0.5 and 3 m (Ballantine et al. 1985, Carlson & Tindall 1985, Bomber & Aikman 1989), but macroalgae attached to mangrove roots and dead coral pavement did not support high numbers of dinoflagellates (Carlson & Tindall 1985). In contrast, dead corals colonised by algal turf to various extents had higher epiphytic dinoflagellate densities than macroalgal substrates in Mayotte Island (SW Indian Ocean) (Grzebyk et al. 1994, Quod 1994).

The dinoflagellate assemblage on macroalgae follows a clear seasonal pattern in response to several factors, probably the same factors that trigger spring growth of macroalgae in the area (Ballesteros 1992): increase in temperature and irradiance at the beginning of spring, calmer sea (the spatial variability due to hydrodynamic regimens is significant) and availability of substrate. No significant correlations were observed between epiphytic organisms and water temperature or nutrients. Gillespie et al. (1985) showed that periodicity in the densities of *Gambierdiscus toxicus* was not directly linked to temperature. They found the maximum density in water at temperatures near 20°C and before the maximum temperature was reached. Here, no clear seasonal pattern in the relative abundance of epiphytic organisms was observed (e.g., substitution of dinoflagellates vs diatoms). The dominant dinoflagellate was mostly *Ostreopsis* sp., although *Coolia monotis* and *Prorocentrum lima* occasionally achieved high absolute and relative numbers. The mechanisms that trigger species abundance are unclear, but changes in the hydrodynamic regime many be involved. Spatial variability (Fig. 5) indicates that although *C. monotis* and *Ostreopsis* sp. are better adapted to slightly shaken environments, *C. monotis* outnumbers *Ostreopsis* sp. in calm waters; however, the former is excluded by *Ostreopsis* sp. in shaken waters.

In autumn, the input of external energy from storms and rains exerts a negative effect on the macroalgae, which simplify the macroalgal community structure and reduce the biomass (Ballesteros 1992). These conditions may also affect all epiphytic organisms. Differences in cell concentration during warm and cold months may also result from the carrying capacity of macroalgae (Lobel et al. 1988), which varies according to hydrodynamic characteristics (Tindall & Morton 1998). Each species of macroalga has a characteristic surface area or space, which, once occupied, can support no additional cells. In high turbulence conditions, this space is limited to the surface layer, whereas in stagnant conditions dinoflagellates multiply to fill all the spaces within the macroalgal canopy. In highly turbulent conditions, when no additional cells can be supported by macroalgae, dinoflagellates continuously migrate to adjacent areas. Bomber et al. (1989) suggested that the presence of *Prorocentrum* spp., *Coolia monotis* and *Ostreopsis siamensis* in the water column is due to vertical migration, which facilitates cell redistribution and concentration. Thus, the epiflora does not strictly depend on a given macroalga because under certain circumstances (e.g., macroalga death) they can migrate and colonise other algae.

The high cell concentrations recorded during summer for the 4 target macroalgae are attributed to the less shaken and more stable water environments, similar to the Type II system described by Tindall & Morton (1998). On the other hand, the low cell concentrations recorded during winter months, which are probably associated with more shaken fluxes, can be compared to the Type I system.

In conclusion, the epiphytic associations of *Ostreopsis* sp., *Coolia monotis*, *Prorocentrum lima* and *Coscinodiscus* sp. are characteristic along the Catalan coast, NW Mediterranean. Further research in other areas of the Mediterranean Sea is required to define the spatial distribution and to determine whether such an association exists.

Acknowledgements. We thank M. Delgado, E. Ballesteros and collaborators for sampling assistance, E. Ballesteros for the taxonomical identification of macroalgae, S. Fraga for comments and suggestions on scanning electron microscopy protocols and *Ostreopsis* taxonomy, and E. Cacho and J. M. Franco for toxicity analysis. J. M. Fortuño, R. Fernández and R. Ventosa provided technical assistance. The authors thank J. Camp for critical reading of the manuscript and his support of our work. Financial support was provided by the ACA (Departament de Medi Ambient, Generalitat de Catalunya) and CSIC.

LITERATURE CITED

Adachi R, Fukuyo Y (1979) The thecal structure of a marine toxic dinoflagellate *Gambierdiscus toxicus* gen. et sp. nov. collected in a ciguatera-endemic area. Bull Jpn Soc Sci Fish 45:67–71

Association of Official Analytic Chemists (1980) Standard mouse bioassay for paralytic shellfish toxins. In: Horwitz W (ed) Official methods of analysis, 13th edn. Association of Official Analytic Chemists, Washington, p 298–299

Bagins R, Bennet J, Prieur C, Legrand AM (1985) The dynamics of three toxic benthic dinoflagellates and the toxicity of ciguateric surgeonfish in French Polynesia. In: Anderson DM, White AW, Baden DG (eds) Toxic dinoflagellates. Elsevier Science Publisher, New York, p 177–182

Ballantine DL, Bardales AT, Tosteson TR, Durst HD (1985) Seasonal abundance of *Gambierdiscus toxicus* and *Ostre-*

opsis sp. in coastal waters of southwest Puerto Rico. Proc 5th Int Coral Reef Congress, Tahiti 4:417–422
Ballesteros E (1992) Els vegetals i la zonació litoral: espècies, comunitats i factors que influeixen en la seva distribució. Institut d'Estudis Catalans, Barcelona
Bomber JW, Aikman KE (1989) The ciguatera dinoflagellates. Biol Oceanogr 6:291–311
Bomber JW, Rubio MG, Norris DR (1989) Epiphytism of dinoflagellates associated with ciguatera: substrate specificity and nutrition. Phycologia 28:360–368
Bourdeau P, Durand-Clement M, Ammar M, Fessard V (1995) Ecological and toxicological characteristics of benthic dinoflagellates in a ciguateric area (Saint Barthelemy: French West Indies). In: Lassus P, Arzul G, Erard E, Gentien P, Marcaillou C (eds) Harmful marine algal blooms. Lavoisier, London, p 133–137
Carlson RD, Tindall DR (1985) Distribution and periodicity of toxic dinoflagellates in the Virgin Islands. In: Anderson DM, White AW, Baden DG (eds) Toxic dinoflagellates. Elsevier, New York, p 171–176
Chang FH, Shimizu Y, Hay B, Stewart R, Mackay G, Tasker R (2000) Three recently recorded Ostreopsis spp. (Dinophyceae) in New Zealand: temporal and regional distribution in the upper North Island from 1995 to 1997. NZ J Mar Freshw Res 34(1):29–39
Faust MA (1995) Benthic, toxic dinoflagellates: an overview. In: Lassus P, Arzul G, Erard E, Gentien P, Marcaillou C (eds) Harmful marine algal blooms. Lavoisier, London, p 847–854
Faust MA (1999) Three new *Ostreopsis* species (Dinophyceae): *O.marinus* sp. nov., *O. belizeanus* sp. nov., and *O. carribeanus* sp. nov. Phycologia 38(2):92–99
Faust MA, Morton SL (1995) Morphology and ecology of the marine dinoflagellate *Ostreopsis labens* sp. nov. (Dinophyceae). J Phycol 31:456–463
Faust MA, Morton SL, Quod JP (1996) Further SEM study of marine dinoflagellates: the genus *Ostreopsis* (Dinophyceae). J Phycol 32:1053–1065
Fukuyo Y (1981) Taxonomical study on benthic dinoflagellates collected in Coral Reefs. Bull Jpn Soc Sci Fish 47(8): 967–978
Gangemi E (2001) Dinoflagellati nocivi nelle fitocenosi di un ecosistema litorale della Sicilia Orientale (Pantano Grande — Lagune di Capo Peloro, Me). PhD thesis, Università di Messina, Sicily
Gillespie NC, Holmes MJ, Burke JB, Doley J (1985) Distribution and periodicity of *Gambierdiscus toxicus* in Queensland, Australia. In: Anderson DM, White AW, Baden DG (eds) Toxic dinoflagellates. Elsevier, New York, p 183–188
Grasshoff K, Ehrhardt M, Kremling K (1983) Methods of sea water analysis. Ed Verlag Chemie, Weinheim
Grzebyk D, Berland B, Thomassin BA, Bossi C, Arnoux A (1994) Ecology of ciguateric dinoflagellates in the coral reef complex of Maynotte Island (S.W. Indian Ocean). J Exp Mar Biol Ecol 178:51–66
Lobel PS, Anderson DM, Durand-Clement M (1988) Assessment of ciguatera dinoflagellate populations: sample variability and algal substrate selection. Biol Bull 175:91–101
Mamán L, Fernández L, Ocaña A, Marco JJ, Morales J, Caballos M, Márquez I, Aguilar M (2000) Seguimiento de fitoplancton tóxico en la costa de Andalucía durante los años 1997 y 1998. In: Márquez I (ed) VI Reunión Ibérica de Fitoplancton Tóxico y Biotoxinas Sevilla, Junta de Andalucía, p 41–49
Nakajima I, Oshima Y, Yasumoto T (1981) Toxicity of benthic dinoflagellates in Okinawa. Bull Jpn Soc Sci Fish 47: 1029–1033
Norris DR, Bomber JW, Balech E (1985) Benthic dinoflagellates associated with ciguatera from Florida Keys. I. *Ostreopsis heptagona* sp. nov. In: Anderson DM, White AW, Baden DG (eds) Toxic dinoflagellates. Elsevier, New York, p 39–44
Penna A, Gangemi E, Vila M, Giacobbe MG, Garcés E, Florean S, Androni F, Magnani M (in press) A preliminary survey of Ostreopsidaceae (Dinophyta) in the Mediterannean: molecular and morphological analyses of different isolates. Phycologia 40(Suppl):51
Quod JP (1994) *Ostreopsis mascarensis* sp. nov. (Dinophyceae), dinoflagellé toxique associé à la ciguatera dans l'Océan Indien. Cryptogamie. Algol 15(4):243–251
Schmidt J (1902) Flora of Koh Chang. Contrition to the knowledge of the vegetation in the Gulf of Siam. Part IV. Peridiniales. J Bot 23:212–218
Steidinger KA (1983) A re-evaluation of toxic dinoflagellate biology and ecology. In: Round FE, Chapman D (ed) Progress in phycological research. New York, Elsevier, p 147–188
Steidinger KA, Baden DG (1984) Toxic marine dinoflagellates. In: Spectot DL (ed) Dinoflagellates. Academic Press, New York, p 201–261
Taylor FJR (1979) A description of the benthic dinoflagellate associated with maitotoxin and ciguatoxin, including observations on Hawaiian material. In: Taylor DL, Seliger HH (eds) Toxic dinoflagellate blooms. Elsevier North Holland, New York, p 71–76
Taylor JFR (1985) The distribution of the dinoflagellate *Gambierdiscus toxicus* in eastern Caribbean. In: Salvat B (ed) Proc 5th Int Coral Reef Congress, Moorea, p 423–428
Throndsen J (1995) Estimating cell numbers. In: Hallegraeff GM, Anderson DM, Cembella AD (eds) Manual on harmful marine microalgae. IOC Manuals and Guides No. 33. UNESCO, Paris, p 63–80
Tindall DR, Miller DM, Tindall PM (1990) Toxicity of *Ostreopsis lenticularis* from the British and United States Virgin Islands. In: Graneli E, Sundström B, Edler L, Anderson DM (eds) Toxic marine phytoplankton. Elsevier, New York, p 424–429
Tindall DR, Morton SL (1998) Community dynamics and physiology of epiphytic/benthic dinoflagellates associated with Ciguatera. In: Anderson DA, Cembella AD, Hallegraeff GM (eds) Physiological ecology of harmful algal blooms. Springer-Verlag, Berlin, p 293–313
Tognetto L, Bellato S, Moro I, Andreoli C (1995) Occurrence of *Ostreopsis ovata* (Dinophyceae) in the Tyrrhenian Sea during summer 1994. Bot Mar 38:291–295
Vila M, Camp J, Garcés E, Masó M, Delgado M (2001) High resolution spatio-temporal detection of HABs in confined waters of the NW Mediterranean. J Plankton Res 23(5): 497–514
Yasumoto T, Nakajima I, Bagins R, Adachi R (1977) Finding of a dinoflagellate as a likely culprit of ciguatera. Bull Jpn Soc Sci Fish 43:1021–26
Yasumoto T, Oshima Y, Murakami Y, Nakajima I, Bagnis R, Fukuyo Y (1980) Toxicity of benthic dinoflagellates found in coral reef. Bull Jpn Soc Sci Fish 46(1):327–331
Yasumoto T, Seino N, Murakami Y, Muata M (1987) Toxins produced by benthic dinoflagellates. Biol Bull 172: 128–131

Editorial responsibility: David Caron, Los Angeles, California, USA

Submitted: April 18, 2001; Accepted: July 16, 2001
Proofs received from author(s): October 17, 2001