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Distribution of toxic dinoflagellates along the leaves of seagrass *Posidonia oceanica* and *Cymodocea nodosa* from the Gulf of Tunis

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Abstract: This study examined the first results about the occurrence of epiphytic dinoflagellate species in the Gulf of Tunis. Three potentially harmful marine species were found: *Prorocentrum lima* (Ehrenberg) Dodge 1975, *Ostreopsis siamensis* Schmidt, 1901 and *Coolia monotis* Meunier 1919. From June 2001 to May 2002, the annual distribution of these species was studied on the seagrass *Posidonia oceanica* (Linnaeus) Delile. In the area where *P. lima* was the most abundant species, two peaks were recorded in fall and spring. The maximum density of *O. siamensis* was recorded in August until September whereas the maximum density of *C. monotis* was recorded in September and May. The abundance of epiphytic dinoflagellates was higher from August to October. These species displayed host substratum preference on *Cymodocea nodosa* (Ucria) Ascherson where the cell abundance was higher than on *P. oceanica*, particularly for *P. lima*.

Résumé : *Distribution de Dinoflagellés toxiques sur les feuilles de* Posidonia oceanica *et* Cymodocea nodosa *dans le Golfe de Tunis*. Cette étude porte sur les premiers résultats relatifs à la présence de dinoflagellés épiphytes dans le Golfe de Tunis. Il s'agit de trois espèces potentiellement toxiques qui sont rencontrées sur les feuilles des phanérogames marines : *Prorocentrum lima* (Ehrenberg) Dodge 1975, *Ostreopsis siamensis* Schmidt, 1901 et *Coolia monotis* Meunier 1919. De juin 2001 à mai 2002, la distribution de ces espèces a été suivie au niveau des herbiers de *Posidonia oceanica* (Linnaeus) Delile dans la région de La Marsa (golfe de Tunis). *P. lima* y est l'espèce dominante, deux pics d'abondance ont été enregistrés au printemps et en automne. *O. siamensis* présente des concentrations maximales d'août à septembre alors que *C. monotis* prolifère en septembre et en mai. L'assemblage de ces microalgues épiphytes est caractérisé par une concentration élevée enregistrée à partir du mois d'août jusqu'en octobre. Ces microalgues, en particulier *P. lima*, sont beaucoup plus abondantes au niveau des herbiers à *Cymodocea nodosa* (Ucria) Asherson qui constituerait un substrat plus favorable.

Keywords: epiphytic dinoflagellates; harmful algae; *Prorocentrum lima*; *Ostreopsis siamensis*; *Coolia monotis*; *Posidonia oceanica*; *Cymodocea nodosa*; Gulf of Tunis

Introduction

Since 1989, blooms of harmful algae associated with fish mortalities were detected mainly in southern Tunisian coasts and in lagoons (Romdhane *et al*., 1998; Turki & El Abed, 2001a). The species were *Trichodesmium erythraeum* Ehrenberg (Cyanophyceae), *Gymnodinium cf maguelonnense* Biecheler 1939 (Dinophyceae), *Tetraselmis* sp. (Prasinophyceae); *Rhodomonas* sp. (Cryptophyceae). *G. maguelonnense*, morphologically similar to *Karenia mikimotoi* Hansen et Moestrup 2000 (syn: *Gymnodinium mikimotoi* Miyake and Kominami ex Oda 1935;

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Gyrodinium aureolum Hulburt 1957) and *Karenia brevis* Hansen and Moestrup 2000, were predominantly cytotoxic due to active oxygen radicals, that caused severe fish mortalities in aquatic farms and dystrophic crisis in southern Tunisian coasts, respectively, during the fall of 1991 and 1994 (Guelorget, 1992; Arzul et al., 1998; Jenkinson & Arzul, 2001). According to these latter authors, *G. cf. maguelonnense* was characterized by a high haemolytic activity that reduced survival time of sea bass in culture by 74% at 3000 cells.ml^{-1}. In 1995, a monitoring program on harmful phytoplanktonic species was implemented in Tunisian shellfish harvesting areas where toxic events caused by atypic toxins have been related by Krys & Kharrat (unpublished data).

Monitoring programs of benthic dinoflagellates are primarily implemented in subtropical zones characterized by endemic areas in coral reefs (Turquet et al., 1998; Heil et al., 1998). Pearce et al. (2001) found that harmful epiphytic species: *Prorocentrum* spp, *C. monotis* and *O. siamensis* on seagrass *Zostera mulleri* occurred also in the lagoons of the East coast of Tasmania. These benthic species associated with the more important member *Gambierdiscus toxicus* Adachi and Fukuyo 1979 are known to produce toxins associated with Ciguatera Fish Poisoning (CFP) which are transferable to the humans through the food chain (Yasumoto & Satabe, 1998). These syndromes have never been detected in Tunisia. However, Haro et al. (1993) reported some cases of Ciguatera intoxication after eating Mediterranean Sparidae fish coming from Marseille (France).

The objectives of the present study were to detect harmful epiphytic dinoflagellates attached on the phanerogam plants represented by *P. oceanica* and *C. nodosa* in the Gulf of Tunis and to examine their seasonal distributions.

Material and methods

The study area is located in the Gulf of Tunis (Fig. 1). Samples of the angiosperms were monthly collected from the seagrass *Posidonia* and *Cymodocea*, respectively, in the bay of La Marsa (36° 52' 40'' N, 11° 20' 40'' E). The occurrence of *P. oceanica* and *C. nodosa* in the Gulf of Tunis mainly depends upon the stability of the substratum (Ben Aleya, 1972). The occurrence of a cyclic pattern of sedimentology is demonstrated for the west coast of the Gulf of Tunis where neritic silting occurs via the sediment carried by rainfall during winter and spring. The extension of seagrass beds of *Posidonia* was affected by the turbidity and sedimentation and by the wide range of temperature and salinity changes. *Cymodocea* thrives more on unstable substrata while *Posidonia* thrives in areas of stable substrata or in certain alluvial areas where wave action prevents siltation. The ecological characteristics in the Gulf of Tunis was summarized in table 1. The sampling was done between June 2001 and May 2002: 12 *P. oceanica* and 6 *C. nodosa* were sampled every month. Water temperature and salinity were measured at the sampling station by using a conductimeter LF 340-A/SET WTW.

Samples were collected between 2 to 5 metres from the shore at a fixed station "La Marsa", where 100 to 300 grams of plants were harvested and transferred in bottles with 500 ml of filtered seawater. They were vigorously shaken and filtered on three successive sieves of 800 μm, 150 μm and 20 μm (Yasumoto et al., 1984). Then, the phanerogam plants were removed from the bottles and weighed with precision. The residue was transferred and the volume calculated. Sub samples of 1 to 2 ml were taken and filtered through a polycarbonate Millipore isopore membrane filters 5.0 μm TMTP, 25 mm diameter with addition of 1 ml of white calcofluor M2R (stock solution: 10 mg.ml^{-1} with a final concentration about 2 mg.l^{-1}). An epifluorescence microscope (Olympus BH2 equipped with reflected light fluorescence attachment BH2-RFC) was used for the visualisation of thecal plates and valvae of armoured and desmokont dinoflagellate species (Frietz & Triemer, 1985; Andersen & Kristensen, 1995). Cell abundance of dinoflagellate species was calculated as number of cells.100g^{-1} of wet weight (WW) phanerogams. According to the Poisson distribution, the counting number of 50 to 200 cells in a subsample was corresponding approximately to an acceptable precision of 15-30 % (at 95% confidence limits).

The epiphyte dinoflagellate species were identified using references and descriptions illustrated by Fukuyo (1981), Faust (1991) and Steidinger (1997).

Results

Three epiphytic dinoflagellate species were identified from the Gulf of Tunis: *Prorocentrum lima*, *Ostreopsis siamensis*

Table 1. Annual mean of the ecological parameters in the Gulf of Tunis (Kéfi Daly-Yahia, 1998)
Tableau 1. Moyennes annuelles des paramètres écologiques recueillis dans le Golfe de Tunis (Kéfi Daly-Yahia, 1998)

Air temperature	Rainfall	Tide range	N-NO3	P-PO4	Turbidity
20 °C	470 mm.an^{-1}	0.30 m	0.352 μmole.l^{-1}	0.189 μmole.l^{-1}	4.35 NTU

Figure 1. Location of the sampling station "La Marsa": map of the phanerogam seagrass distribution in the Golf of Tunis (Ben Aleya, 1972)

Figure 1. Localisation de la station de prélèvement « La Marsa » : carte de la distribution des phanérogames marines (Ben Aleya, 1972).

and *Coolia monotis*. *P. lima* was found almost yearly but it withstood, with *O. siamensis* and *C. monotis*, the range of temperatures between 20-27 °C and salinity of 37 (Figs. 2, 3). In New Zealand, *C. monotis* occurred in sediments at 10-30 °C and salinities of 20-34 and *O. siamensis* tolerates temperatures of 15-25 °C and salinities 28-34. According to Faust *et al*. (1999), optimum growth of *P. lima* occurred in the cool water season (26 °C) and salinity of 32. However, this species occurs in world-wide coastal areas.

The determination of these species was relatively easy after staining the cells by the calcofluor. *P. lima* was identified by its more or less oval form, the disposition of the valve, marginal pores, apical pore complex and a periflagellar area on the right valve. The mean length of the valves is 45.3 ± 4.8 μm; the mean width is 30.2 ± 4.5 μm (n = 30), the L/l ratio = 1.5. These dimensions are comparable with

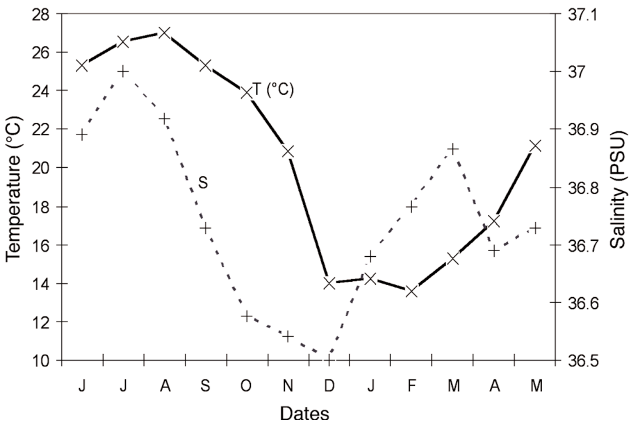

Figure 2. Annual variations of the water temperature and salinity in the sampling station "La Marsa".

Figure 2. Variations annuelles de la température de l'eau et de la salinité à la station de prélèvement "La Marsa".

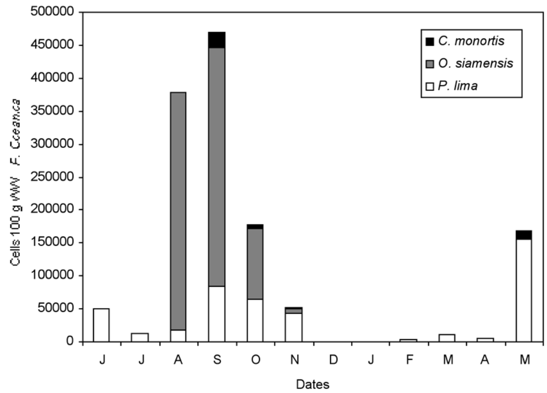

Figure 3. Annual variation of cell abundance of epiphytic dinoflagellates on *P. oceanica*: *P. lima*, *O. siamensis* and *C. monotis*.

Figure 3. Variations annuelles de la densité des dinoflagellés épiphytes sur *P. oceanica* : *P. lima*, *O. siamensis* and *C. monotis*.

several strains of *P. lima* from the Pacific and Atlantic areas and certain lagoons of the subtropical zones (Faust, 1991). *O. siamensis* was recognisable by its pyriform shape. This species could be confused with *O. lenticularis* but we confirmed the presence of one type of the valvar pores in *O. siamensis* as in Fukuyo (1981). Mean dorso-ventral diameter and the transverse diameter were respectively about 71.3 ± 7.7 μm and 45.8 ± 7.3 μm (n = 25). Confusions could be made between small cells of *O. siamensis* and those of *C. monotis* which the identification was possible by its oval form in dorsal or ventral face, and dissymmetrical form in side view. The dimensions reported for this species were slightly greater than those found by Fukuyo (1981), the mean total length is 41.2 ± 5.2 μm; the mean dorso-ventral diameter is 29.7 ± 3.8 μm and the mean cingular diameter is 38.2 ± 5.6 μm (n = 25).

Among this epiphytic dinoflagellate assemblage, *P. lima* was the dominant species all along the year on *Posidonia oceanica*. Cell abundance varies from 1.8×10^2 to 1.6×10^5 cells.100g^{-1} WW *Posidonia*, with 2 peaks registered during the year: from September to November and from May to June (Fig. 3). *O. siamensis* was seasonally distributed during the annual cycle. Cell density varied from 0 to 3.6×10^5 cells.100g^{-1} WW *Posidonia* with a maximum reported from August to September (Fig. 3).

C. monotis, like *O. siamensis*, was not abundant at all seasons, with two peaks recorded in September and May (Fig. 3). In the *Posidonia* seagrass bed ecosystem, August to October appeared to be a favourable period for the development of dinoflagellate species which density varied from 2 to 4×10^5 cells.100g^{-1} WW *Posidonia* (Fig. 3).

The distribution of the epiphytic dinoflagellates found on *Posidonia* seagrass was different compared to that occurred on *C. nodosa* (Fig. 4). In fact, concentrations were much more higher on the *Cymodocea* seagrass except for *O. siamensis*. However, cell abundance of *P. lima* reached 2.2×10^6 cells.100g^{-1} WW *Cymodocea* in July 2002. Seasonal densities varied, respectively, for *O. siamensis* from 0 to 7.8×10^4 cells.100g^{-1} WW *Cymodocea* and for *C. monotis* from 0 to 8.4×10^4 cells.100g^{-1} WW *Cymodocea*.

Discussion

Epiphytic dinoflagellates present on mediterranean marine phanerogams in the bay of La Marsa (Gulf of Tunis) were characterized by their biodiversity lower than the one found in coral reef ecosystems located in tropical zone. Turquet et al. (1998) identified 20 species, mainly represented by *Prorocentrum* spp., of which 13 were harmful in these endemic areas. In the bay of La Marsa, *P. lima* concentrations were higher than those found on the different macroalgal species in coral reefs where the maximum was estimated at 1.2×10^5 cells.100g^{-1} WW macroalgae (Heil et al., 1998). This perennial microalga was the most abundant dinoflagellate found on the two phanerogam species. The suitable period for the development of dinoflagellate populations on the *Posidonia* seagrass beds was almost the same for the populations composed of *G. toxicus*, *Prorocentrum* spp. and *Ostreopsis* spp. in coral reef ecosystem at La Réunion Island where Turquet et al. (1998) indicated that the favourable period extended from September to October.

These cosmopolitan species, largely widespread in coastal

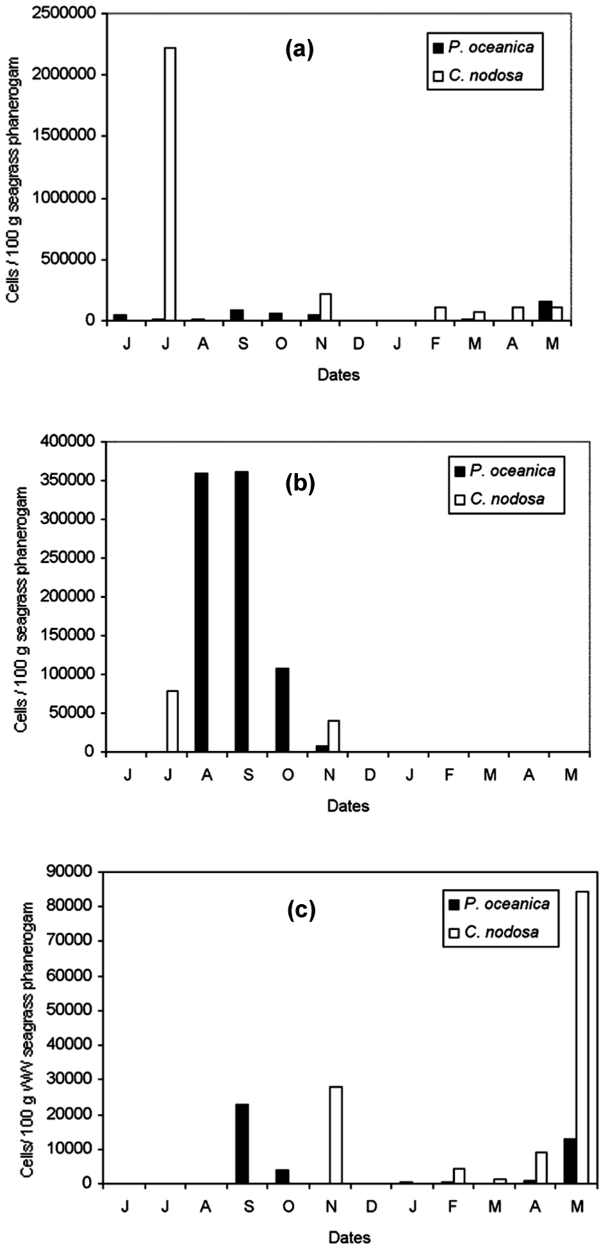

Figure 4. Distribution of the epiphytic dinoflagellates associated with seagrass phanerogam: *P. lima* (a), *O. siamensis* (b) and *C. monotis* (c).

Figure 4. Distribution des dinoflagellés épiphytes au niveau des phanérogames marines: *P. lima* (a), *O. siamensis* (b) and *C. monotis* (c).

zones, are harmful to marine fauna (Faust et al., 1999). Several toxins were identified from cultures of *P. lima* (okadaic acid and its derivatives, a water-soluble toxin characterized by a very fast action in mouse bioassay test, and a nitrogen toxin or prorocentrolid). According to Yasumoto (1990) in Faust et al. (1999), the toxin associated with *P. lima* enters the food chain as Diarrheic Shellfish Poisoning, rather than as a ciguatoxin, and constitutes a complex toxin which can be classified into lipid and water soluble polyether compounds. *O. siamensis* is known to produce polytoxins causing a positive bioassay test with a cell number estimated to 3.2×10^7 (Rhodes et al., 2000). Cooliatoxin was not yet detected in *C. monotis*, but analogs of unknown polyether compounds extracted from different batch culture (total cell number 2.1×10^7) resulted in mouse deaths.

As these epiphytic species are known to produce toxic compounds as evidenced by mouse bioassays, they were among the harmful microalgae species specifically noted during routine phytoplankton monitoring program of the shellfish harvesting areas implemented in Tunisia since 1995. Densities of *P. lima* and *O. siamensis* higher than 500 cells.l^{-1} were respectively noted in August and May in the Lake of Tunis and August and September in the Lake of Bizerte (Turki & El Abed, 2001b). These are the same periods identified in the present study when the maximum cell abundance of these species was recorded on *Posidonia* vegetation.

Since 1995, mouse bioassay tests carried by the Institut Pasteur of Tunis indicate the presence of an unknown biotoxin in the routine monitoring program (Kharrat, comm. pers.). Consequently, this study related to the distribution of epiphytic dinoflagellates assemblages on phanerogam vegetation, where several shellfish species find their preferential substratum, since they could be a source of biotoxin contamination of these filter-feeding bivalves. Nevertheless, isolation and growth of culture of these epiphytic dinoflagellate species have to be established to characterize the specificity of these toxins and their effects on shellfish species when the harmful algal bloom populations appear in the Gulf of Tunis.

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References

Andersen P., Kristensen H.S. 1995. Rapid and precise identification and counting of thecate dinoflagellates using epifluorescense microscopy: Harmful Marine Algal blooms. In: *Proceedings of the Sixth International Conference on Toxic Marine Phytoplankton* (P. Lassus, G. Arzul, E. Erard-Le Denn, P. Gentien and C. Marcaillout-Le Baut eds.), pp. 713-718. Lavoisier Publishing: Paris.

Arzul G., Bodennec G., Gentien, P., Bornens P., Crassous M.P. 1998. The effect of dissolved oxygen on the haemolytic property of *Gymnodinium* ichtyotoxins: Harmful algae. In: *Proceedings of the Eighth International Conference on harmful algae* (B. Reguera, J. Blanco, M.L. Fernandez and T. Wyatt eds.), pp. 611-614. Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO 1998: Spain.

Ben Aleya H. 1972. Répartition et conditions d'installation de *Posidonia oceanica* Delile et *Cymodocea nodosa* Asherson dans le golfe de Tunis. *Bulletin de l'Institut Océanographique de la Pêche, Salammbô*, **2**: 331-416.

Faust M.A. 1991. Morphology of ciguatera-causing *Prorocentrum lima* (Pyrrophyta) from widely different sites. *Journal of Phycology,* **27**: 642-648.

Faust M.A., Larsen J., Moestrup O. 1999. Potentially toxic phytoplankton. 3. Genus *Prorocentrum* (Dinophyceae). ICES Identification Leaflets for Plankton. International Council for the exploration of the sea. *Leaflet,* **184**, 24 pp.

Fritz L., Triemer R.E. 1985. A rapid simple technique utilising Calcofluor White M2R for the visualization of Dinoflagellate thecal plates. *Journal of Phycol*ogy, **21**: 662-664.

Fukuyo Y., 1981. Taxonomical study on benthic Dinoflagellates collected in coral reefs. *Bulletin of the Japanese Society of Scientific Fisheries*, **47**: 967-978.

Guelorget O. 1992. Sinistre AST. C'était bien un *Gymnodinium* toxique. *Aqua Revue,* **41**: 21-23.

Haro de L., Treffot M., Jouglard J., Perringue C. 1993. Trois cas d'intoxication ciguatérique après ingestion de Sparidae de Méditerranée. *Ichtyophysiologica Acta*, **16**: 133-146.

Heil C., Bird P., Dennison W.C. 1998. Macroalgal habitat preference of ciguatera dinoflagellates at Heron island, a coral cay in the southeastern great barrier reef, Australia: Harmful algae. In: *Proceedings of the Eighth International Conference on harmful algae* (B. Reguera, J. Blanco, M.L. Fernandez and T. Wyatt eds.), pp. 52-53. Xunta de Galicia and Inter-governmental Oceanographic Commission of UNESCO 1998: Spain.

Jenkinson I.R., Arzul G. 2001. Mitigation by cysteine compounds of rheotoxicity, cytotoxicity and fish mortality caused by the dinoflagellates, *Gymnodinium mikimotoi* and *G. cf maguelonnense*: Harmful algal blooms. In: *Proceedings of the Ninth International Conference on harmful algae blooms* (G.M. Hallegreaff, S.I. Blackburn, C.J. Bolch and Lewis R.J. eds.), pp. 461-464. Intergovernmental Oceanographic Commission of UNESCO 2001: France.

Kéfi Daly-Yahia O. 1998. Le phytoplankton de la baie de Tunis (analyse systématique, biogéographique, quantitative et synécologique des diatomées et des dinoflagellés). Thèse de Doctorat de Biologie Animale, Faculté des Sciences de Tunis, 330 pp.

Pearce I., Marshall J., Hallegraeff G.M. 2001. Toxic temperate epiphytic dinoflagellates in coastal Lagoons off the east coast of Tasmania. Harmful algal blooms. In: *Proceedings of the Ninth International Conference on harmful algae blooms* (G.M. Hallegreaff, S.I. Blackburn, C.J. Bolch and Lewis R.J. eds.), pp. 54-57pp. Intergovernmental Oceanographic Commission of UNESCO 2001: France.

Rhodes L., Adamson J., Toshiyuki S., Lyn B., Ian G. 2000. Toxic marine epiphytic dinoflagellates, *Ostreopsis siamensis* and *Coolia monotis* (Dinophyceae) in New Zealand. *New Zealand Journal of Marine and Freshwater research*, **34**: 371-383.

Romdhane, M.S., Eilertsen, H.C., Kéfi Daly-Yahia, O., Daly-Yahia, M.N. 1998. Toxic dinoflagellate blooms in Tunisian lagoons: causes and consequences for aquaculture: Harmful algae. In: *Proceedings of the Eighth International Conference on harmful algae* (B. Reguera, J. Blanco, M.L. Fernandez and T. Wyatt eds.), pp. 80-83. Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO 1998: Spain.

Steidinger K.A. 1997. Dinoflagellates. In: Identifying marine phytoplankton (C. R. Thomas ed.), pp. 387-585. Academic Press: United States of America.

Turki S., El Abed A. 2001a. On the presence of potentially toxic algae in the lagoons of Tunisia. *Harmful Algae News,* **22**: 10.

Turki, S., El Abed A. 2001b. Détection du phytoplankton marin toxique dans les zones de production des mollusques bivalves du Nord de la Tunisie. In: *Actes des 8èmes Journées Nationales sur les résultats de la Recherche Agronomique*, pp. 360-367. Institut de la Recherche et de l'Enseignement Scientifique Agricole : Tunis.

Turquet J., Quod J. P., Couté A., Faust M.A. 1998. Assemblage of benthic dinoflagellates and monitoring of harmful species in Reunion island, SW Indian Ocean, 1993-1996: Harmful algae. In: *Proceedings of the Eighth International Conference on harmful algae* (B. Reguera, J. Blanco, M.L. Fernandez and T. Wyatt eds.), pp. 44-47. Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO 1998: Spain.

Yasumoto T., Uday R., Bagnis R. 1984. Seafood poisonings in tropical regions. Laboratory of Food Hygiene, Faculty of Agriculture, Tohoku University, 74 pp.

Yasumoto T., Satake M. 1998. New toxins and their toxicological evaluations: Harmful algae. In: *Proceedings of the Eighth International Conference on harmful algae* (B. Reguera, J. Blanco, M.L. Fernandez and T. Wyatt eds.), pp. 461-464. Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO 1998: Spain.