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— SHORT COMMUNICATION —

Potentially harmful microalgae from lagoons of the NW Ionian Sea, Greece

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Lagoons, including estuaries and wetlands, are important biotopes of the coastal wetland ecosystem of the Ionian Sea (NW Greece) that harbour a variety of marine species including phytoplankton. While investigating phytoplankton strains for aquaculture applications, the diversity of potentially harmful microalgae in six coastal lagoons of the Epirus region (Ionian Sea) was appraised. Results show that there is a great variety of potentially harmful species, mainly of the class Dinophyceae (*Prorocentrum*, *Dinophysis, Protoperidinium*, *Amphidinium*, *Alexandrium*, *Scrippsiella*, *Gyrodinium* and *Gymnodinium*) and the harmful *Prymnesium parvum* of the class Prymnesiophyceae. These results stress the need for maintenance of the ecological balance of these biotopes and for their protection from eutrophication which can cause extensive blooming of harmful species.

Key words: harmful algae, phytoplankton, lagoons, Ionian Sea.

INTRODUCTION

Lagoons, estuaries and wetlands are important biotopes of the coastal wetland ecosystems. These biotopes act as "reservoirs" and natural nurseries for a variety of marine species including phytoplankton, and are therefore physically and biologically exploited by fisheries and aquaculture worldwide (Costanza *et al*., 1997; Beck *et al*., 2001; Gillanders *et al*., 2003; Marques *et al*., 2004; Able, 2005; de Groot *et al*., 2006). Phytoplankton species in lagoons, which are potentially harmful due to their capability to bloom or produce toxins, pose a threat not only to fisheries and aquaculture but also to other marine species that are part of the ecological web (Luttenberg *et al*., 2000; Anderson *et al*., 2001; GEOHAB, 2001). Potentially harmful microalgae in Greek coastal waters have been reported in several areas of the Aegean Sea and only in the closed embayment of the Amvrakikos Gulf in the Ionian Sea. Most frequently, species that cause harmful incidents are those of the genera *Dinophysis*, *Gymnodinium*, *Alexandrium* and *Prorocentrum* (Moncheva *et al*., 2001; Nikolaidis *et al*., 2005).

While investigating phytoplankton strains for aquaculture applications, the diversity of potentially harmful microalgae that could pose problems in six coastal lagoons of the Ionian Sea in Epirus region (NW Greece) was appraised. These lagoons are exploited by local fisheries and are considered as sites of ecological significance by the Natura 2000 Network and the Ramsar Convention on Wetlands.

MATERIALS AND METHODS

The investigated lagoons were Alykes (39°36′ N 20°11′ E), Vodas (39°34′ N 20°09′ E), Kalaga (39°31′ N 20°10′ E), Vatatsa (39°31′ N 20°11′ E), Rhodia (39°05′ N 20°49′ E) and Tsoukalio (39°04′ N 20°50′ E). Alykes, Vodas, Kalaga and Vatatsa are semi-closed coastal lagoons of the Kalamas river delta system on the Ionian Sea, whereas the closed lagoons Rhodia and Tsoukalio are part of the Louros river delta in

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the Amvrakikos Gulf (Ionian Sea) and are interconnected with a narrow opening about 4 m deep.

Monthly water sampling was carried out from October 2005 until December 2006 using a Hydro-Bios 1L Ruttner type flask (Kiel, Germany) with water temperature, salinity, dissolved oxygen (DO) and pH also being measured using a YSI 550 instrument (Yellow Springs, Ohio). The biotic samples taken at a 1 m depth were fixed with Lugol solution and transported to the lab for phytoplankton identification and imaging using a Zeiss (Göttingen, Germany) optical microscope (at 100-1000×) and a Sony CCD Hyper Had camera (Minato, Japan). Cell densities were estimated using 25 ml Kolkwitz settling chambers and a Zeiss inverted microscope (at 400×). Identification of harmful phytoplankton species was based on works by Tomas (1997), Zingone *et al*. (1998) and Hallegraeff *et al*. (2003).

RESULTS

Identification of phytoplankton species

Sampling revealed a variety of potentially harmful dinoflagellates belonging to different orders of Dinophyceae (Gymnodiniales, Prorocentrales, Peridiniales, Gonyaulacales). The species *Prymnesium parvum* Carter of the Prymnesiophyceae (identified via cell shape, golden-yellow colour and the 2.5-3.0 μm long haptonema being on average 0.3-0.4 times the cell length) was also present in the lagoons (Fig. 1). The frequently occurring taxa are listed in Table 1. From the data it appears that there are differences concerning the occurrence of taxa between lagoons of the different systems. In the two closed and interconnected coastal lagoons of the Amvrakikos Gulf (Rhodia, Tsoukalio) four to five potentially harmful species appear at detectable densities for long periods of time (4-10 months) while the other harmful species appear in an erratic manner with a total number of 11 for Rhodia and 15 for Tsoukalio. These two lagoons exhibited the lowest average salinity and temperature values with the highest seasonal variation (Table 2) and the observed persistent taxa were *Gymnodinium*, *Karenia*, *Karlodinium* and *Prorocentrum*. In the four semi-closed lagoons the occurrence of harmful species tended to be erratic with one or two species persisting in Vodas, Kalaga and Alykes and three species persisting in Vatatsa. The total number of harmful species occurring in these lagoons at detectable densities was 16 and 21 for Kalaga and Vatatsa (Vatatsa embayment) and 12 and 15 for Alykes and Vodas (near the Kalamas river mouth), respectively. The most persistent taxa in these four lagoons were *Prorocentrum*, *Scrippsiella* and *Protoperidinium*. Overall, the seasonal fluctuations of microalgal diversity and species persistence were observed to be related to salinity and temperature changes within the lagoon waters (annual mean values of abiotic water conditions are listed in Table 2).

Populations of these potentially harmful microalgae were observed to reach low density levels (< 100 cells l^{-1}), except for the abundant planktonic *Prorocentrum* species and especially *P. minimum* (Pavillard) Schiller which was observed to reach *ca* 1950 cells l^{-1} during early summer in Rhodia and Tsoukalio.

DISCUSSION

Most of the potentially harmful dinoflagellates found in the investigated lagoons are considered cosmopolitan species and are found in the Mediterranean basin (Gómez, 2003). Accordingly, many of these potentially harmful and/or toxic species have been previously reported in Greek coastal waters (Ignatiades, 1976; Pagou & Ignatiades, 1990; Moncheva *et al*., 2001; Gómez, 2003; Koukaras & Nikolaidis, 2004; Nikolaidis *et al*., 2005; Ignatiades *et al*., 2007). Likewise, the presence of the dinoflagellate species listed in Table 1 coincides with reports from Italian lagoons (Alimini, Grande-Ionian Sea, see Vadrucci *et al*., 2004; Venice, Adriatic Sea, see Tolomio & Moschin, 1995 and Aubry & Acri, 2004; Fusaro, Tyrrhenian Sea, see Sarno *et al*., 1993 and Zingone *et al*., 1998; Varano, Adriatic Sea, see Caroppo, 2001). These observations demonstrate that many of these potentially harmful species find a favourable environment to thrive within these unique coastal ecosystems. The marked seasonal fluctuations of salinity and temperature values in the tested lagoons were accompanied by respective fluctuations in microalgal diversity and species persistence, in agreement with observations in other similar ecosystems (e.g. Fusaro lagoon in the Tyrrhenian Sea, Sarno *et al*., 1993).

The toxic haptophyte *P. parvum* has a wide distribution from temperate to subtropical coastal and brackish waters (Tomas, 1997; Edvardsen & Imai, 2006) and there are reports on its presence in brackish waters of the Mediterranean, including a lagoon in the Ebro Delta, Spain (Edvardsen & Paasche, 1998). In Greece, its presence has been documented by a toxic event that caused massive deaths of birds and fish in the brackish (*ca* 5‰) Lake Koronia of N. Greece

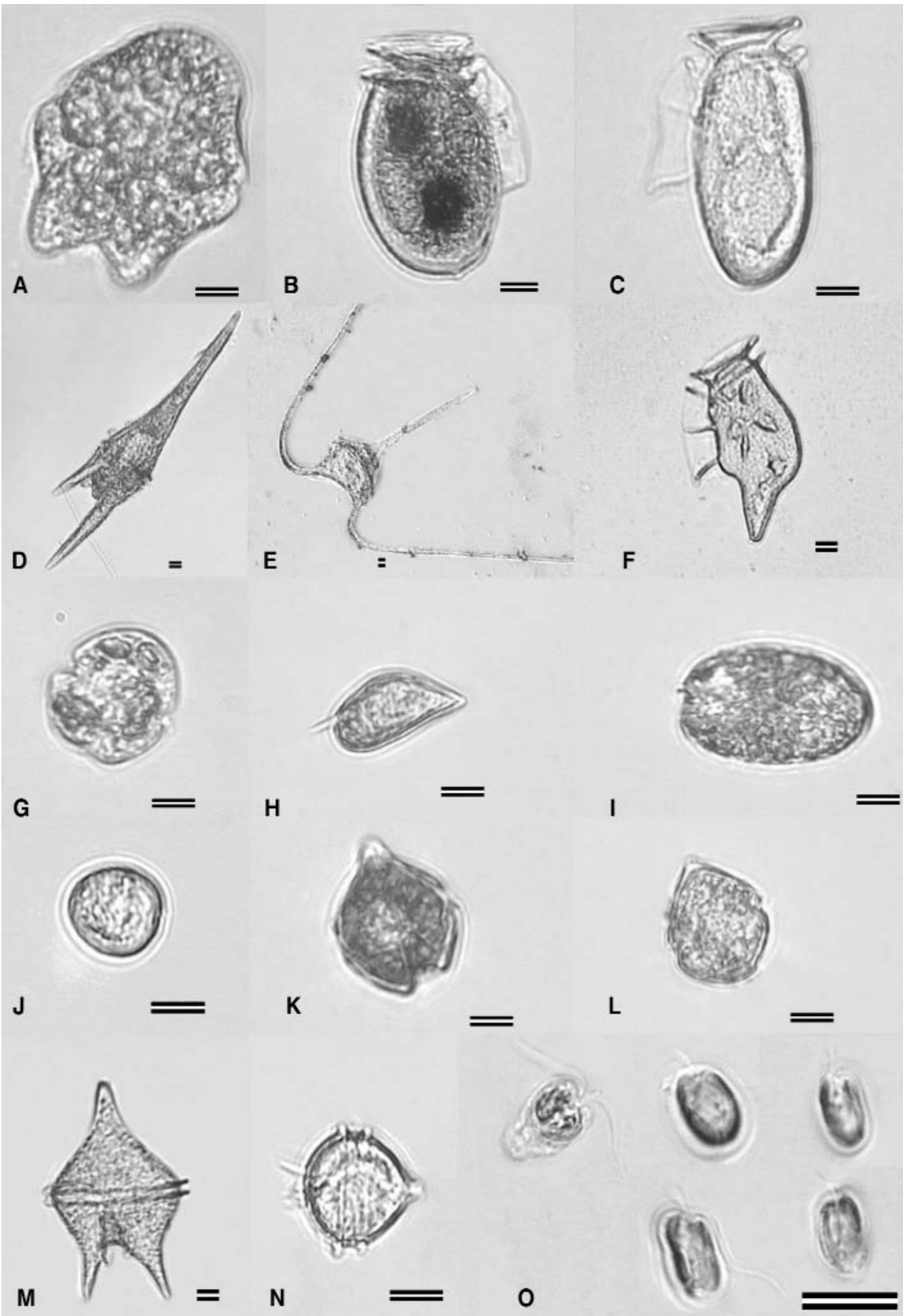

FIG. 1. Microscopic images of potentially harmful microalgal species observed in samples collected monthly during the year 2006 in six lagoons of Epirus (Ionian Sea, NW Greece). (A) *Akashiwo sanguinea* (Hirasaka) G. Hansen et Moestrup, (B) *Dinophysis acuminata* Claparède et Lachmann, (C) *Dinophysis sacculus* Stein, (D) *Ceratium furca* (Ehrenberg) Claparède et Lachmann, (E) *Ceratium massiliense* (Gourret) Jørgensen, (F) *Dinophysis cuadata* Saville-Kent, (G) *Karenia* sp., (H) *Prorocentrum gracile* Schütt, (I) *Prorocentrum rhathymum* Loeblich, Sherley et Schmidt, (J) *Prorocentrum minimum* (Pavillard) Schiller, (K) *Scrippsiella spinifera* Honsell et Cabrini, (L) *Scrippsiella trochoidea* (Stein) Loeblich III, (M) *Protoperidinium oblongum* (Aurivillius) Parke et Dodge, (N) *Protoperidinium pellucidum* Bergh, (O) Five cells of *Prymnesium parvum* Carter. Scale bars = 10 µm.

TABLE 1. Frequently occurring harmful microalgae observed during monthly samplings of the year 2006 in six lagoons of Epirus (Ionian Sea, NW Greece)

Species	Alykes	Kalaga	Vatatsa	Vodas	Rhodia	Tsoukalio
DINOPHYCEAE						
Akashiwo sanguinea (Hirasaka) G. Hansen et Moestrup	–	–	Sep	Oct	–	Sep, Oct
Alexandrium spp. Halim	Feb, Nov	May, Nov	–	Oct	Apr	–
Oxytoxum sp. Stein	–	–	May, Sep	Feb	–	–
Amphidinium carterae Hulburt	–	–	Apr, Nov	–	–	Jun, Oct
Amphidinium spp. Claparède et Lachmann	–	Dec	Sep, Dec	–	–	Dec
Ceratium spp. Schrank	–	–	Jun, Dec	Dec	–	–
Dinophysis acuminata Claparède et Lachmann	–	Oct, Dec	Aug, Dec	Oct	Oct	Sep, Dec
Dinophysis caudata Saville-Kent	–	–	Jul-Sep	Oct	–	–
Dinophysis sacculus Stein	Apr	Apr, Aug, Sep	Apr, Aug-Oct	Oct	Sep, Oct	Sep, Oct
Gonyaulax spinifera (Claparède et Lachmann) Diesing	–	–	Jun	–	–	–
Gymnodinium spp. Stein	Jan, Apr-Jun	Oct-Dec	Apr-Aug, Dec	Nov, Dec	Apr-Oct	Jan-Oct
Gyrodinium sp. Kofoid et Swezy	–	Nov	–	–	Jul, Aug, Nov	Jul, Aug, Nov
Karenia sp. G. Hansen & Moestrup	Mar-Apr	Dec	Mar-Jul, Dec	–	Mar-Aug	Mar-Aug
Karlodinium sp. J. Larsen	Mar-May	Mar	May-Aug	–	Jul-Oct	Mar, Jul-Oct
Prorocentrum gracile Schütt	Feb	Oct, Dec	Feb, Sep-Dec	Dec	–	Oct, Dec
Prorocentrum minimum (Pavillard) Schiller	Apr, Jun	Jun	Apr, Jun, Jul	Jun	May-Dec	Apr- Dec
Prorocentrum nanum Schiller	Feb	Apr	Jun	–	–	–
Prorocentrum rhathymum Loeblich Sherley et Schmidt	–	Dec	Dec	–	–	–
Prorocentrum triestinum Schiller	Jun, Oct	Jun	Jun	Aug	–	–
Prorocentrum spp. Ehrenberg (benthic & planktonic)	Feb-Apr, Sep-Dec	–	Apr-Jun, Sep-Dec	Oct, Dec	Aug, Oct, Dec	Mar-Jul, Oct-Dec
Protoperidinium spp. Bergh	Apr-Jun	Apr, Jun, Oct	Mar-Nov	Sep-Dec	–	Sep-Oct
Scrippsiella spp. Balech ex Loeblich III	Feb-Jul	Feb-Jul, Dec	Apr-Jun, Dec	Jun, Jul, Nov	Aug	Jul
PRYMNESIOPHYCEAE						
Prymnesium parvum Carter	–	Nov, Dec	Jan-Mar	Mar	Mar	Apr, Dec, Feb

TABLE 2. Mean values and standard deviations (s.d.) of water temperature (T), salinity (Sal.), dissolved oxygen (DO) and pH (at 1 m depth) during the period of study (Oct 2005 - Dec 2006) in the sampling stations of the six investigated coastal lagoons of the Epirus region

	Rodia		Tsoukalio		Alykes		Vodas		Kalaga		Vatatsa	
	mean	*s.d.*	mean	*s.d.*	mean	*s.d.*	mean	*s.d.*	mean	*s.d.*	mean	*s.d.*
T (ºC)	19.3	*7.3*	19.4	*7.2*	20.3	*5.3*	20.3	*5.4*	20.2	*5.5*	20.2	*5.5*
Sal. (‰)	14.1	*8.4*	14.9	*7.7*	36.3	*2.7*	35.4	*3.4*	36.3	*3.6*	36.6	*2.6*
DO (mg l^{-1})	8.3	*3.1*	9.4	*6.8*	9.1	*3.6*	8.2	*1.3*	8.2	*1.4*	8.2	*1.7*
pH	7.8	*0.3*	7.7	*0.7*	7.9	*0.4*	7.9	*0.3*	8.0	*0.3*	7.9	*0.3*

(Moustaka-Gouni *et al*., 2004).

The potentially harmful microalgal populations of this study were found at low density levels during sampling; however, certain toxic species such as *Prorocentrum minimum* (Pavillard) Schiller were present in high numbers along with other *Prorocentrum* species. The negative impact that *Prorocentrum* species can have on fisheries and aquaculture has already been demonstrated (Hallegraeff *et al*., 2003; Wikfors, 2005; Ajuzie, 2008). Furthermore, potentially harmful microalgal species are generally prone to blooming and/or production of toxins under favourable conditions, especially eutrophication (Hallegraeff *et al*., 2003; Zingone & Wyatt, 2004; Glibert & Burkholder, 2006; Granéli & Flynn, 2006), which results in an increase of bloom incidents and associated impacts. Eutrophication in the investigated lagoons from intensive aquaculture, industrial, sewage and land-farming runoff has already been reported and increasingly observed (Kormas *et al*., 2001; Sylaios & Theocharis, 2002; Lekka *et al*., 2004). The ecological balance of these biotopes has apparently already been disturbed due to toxic algal blooms and water pollution, especially in the Amvrakikos Gulf, with new impacts on the local fishery and aquaculture activities. Ultimately such environmental degradation also affects human health and the tourism industry as well.

Our preliminary data and other scientific investigations imply the need for environmental protection of the broader lagoon areas of the Epirus region already referred to as “sites of ecological significance” by the Natura 2000 Network (European Union’s Council Decision 2006/613/EC pursuant to Council Directive 92/43/EEC) and the Ramsar Convention on Wetlands (Frazier, 1999). The maintenance of the ecological balance of these biotopes is imperative in order to maintain a healthy phytoplankton population structure and avoid the establishment of harmful, adaptive and dominant microalgal species (Hallegraeff, 1993; Legrand *et al*., 2003; Zingone & Wyatt, 2004; Granéli & Hansen, 2006; Turner, 2006).

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