I - EXECUTIVE SUMMARY

This synthesis, initiated during the meeting, was consolidated thereafter by inputs received from all participants. Special thanks to Urania Christaki, Patricia Mozetič, Fernando Gómez and Catherine Legrand who acted as leaders of four distinct writing groups. Tim Wyatt did provide special editorial assistance on the overall volume to Frédéric Briand, the Monograph Series Editor, while Valérie Gollino took care of the physical production process.

1. INTRODUCTION

The workshop took place from 7 to 10 October 2009 at the INSTM Headquarters in Salammbô. On behalf of the sixteen scientists invited by CIESM, its Director General, Dr Frederic Briand, warmly thanked Dr Ridha M'Rabet, Director of INSTM, for his hospitality and invited him to present an overall survey of the research carried out nation-wide by his Institute. This was followed by a brief introduction to the subject of the workshop by Dr Urania Christaki, Chair of CIESM Committee on Marine Microbiology.

2. SCALES OF VARIABILITY OF PHYTOPLANKTON IN THE MEDITERRANEAN SEA - OR WHY IT IS SO DIFFICULT TO DETECT TRENDS

The response time of phytoplankton to changes in the environment, for example an episodic input of nutrients, is rapid, generally a few days to a week. Paradoxically, due to this capacity for rapid response, long term changes in phytoplankton populations, such as those expected from the global change, are neither easy to detect nor to quantify, due to the high background variability and to sampling limitations. Nonetheless, shifts in phytoplankton community structure determine the structure of higher trophic levels that rely on phytoplankton as food, as well as biogeochemical cycles of many elements. In order to capture phytoplankton responses that will occur on decadal time scales following global changes, the ranges and scales of variability in the Mediterranean Sea need to be determined.

Spatial variability - Besides the well known longitudinal gradient of increasing oligotrophy from the western to the eastern basins with higher phytoplankton biomass and production in the west, hydrodynamic mesoscale activity (eddies, fronts) are known to control phytoplankton dynamics. For example, in the western Mediterranean, the Almeria-Oran front is an area of high primary production compared to surrounding waters, while the Ierapetra eddy in the eastern MS is a zone of very low phytoplankton production.

Another important source of variability in phytoplankton is linked with the distance from shore. Spatial variability is more pronounced in coastal areas. Coastal ecosystems are influenced by natural and anthropogenic inputs and of course local hydrodynamics. Examples of the complex interactions between such inputs and local hydrodynamics include the plumes of the Rhone River in the west and the Black Sea waters in the east.

Finally phytoplankton distribution and composition vary vertically. In the Mediterranean, the base of the euphotic zone (1% incident light) lies at more than 100 m in the eastern basin. As a result, low productivity due to oligotrophy is somewhat compensated by a deeper euphotic zone. It is noteworthy that this deep light penetration is also observed in the center of anticyclonic eddies in the western part. From this point of view, vertical species distributions in the water column, given that the characteristics of the water column change with depth, should be considered when looking for alterations due to global changes.

Phytoplankton biomass and species composition vary on all time scales, daily, weekly, seasonally, annually, and on longer term scales, as well as reacting to exceptional events (drought, flood, storms, tsunamis, oil spills, etc.). Currently, we cannot clearly relate observations on phytoplankton with global change in the Mediterranean. The question is, what sampling strategies should be considered in order to capture phytoplankton responses to environmental changes on decadal time scales? A weekly scale variability can be considered as minimal in monitoring phytoplankton dynamics and their relation to environmental variables. For example, this is the frequency that should be considered to verify hypotheses concerning shifts in the timing of bloom occurrence with global change. In some cases, we might compensate the lack of high frequency sampling with length in time. For example, changes such as increase or decrease in phytoplankton biomass, production, and community composition can be examined using monthly and seasonal variability over decadal scale observations. For practical and financial reasons, high frequency sampling in open waters, and integration of data at mesoscales, can only be achieved using sampling from ships of opportunity using CPR (Continuous Plankton Recorder) or ferry boxes. This approach has provided valuable results in the North Sea and Baltic Sea, but has not been yet been put into practice in the Mediterranean.

As well as the changes due to natural variations in the environment, human activities can influence marine ecosystems. Low turbulence and flushing rates, and sediments where benthic life-history stages can remain undispersed during dormancy - conditions promoted by weak tidal regimes - appear to favour the appearance of some well known dinoflagellates in the northwest Mediterranean, such as *Alexandrium* species (Vila *et al.*, 2001; Garcés *et al.*, 2001). The conditions listed are further enhanced by harbour constructions such as yacht marinas and other coastal engineering developments; undispersed dormant stages can provide more concentrated inocula locally when excystment occurs, and dense planktonic populations in semi-enclosed areas can in turn inoculate adjacent coastal waters.

Furthermore, such constructions, by increasing the extent of surfaces appropriate for colonization by macroalgae, may also provide new habitats for potentially harmful epiphytic microalgae such as *Ostreopsis* and *Prorocentrum*. These kinds of trends noted on the Catalan coast of Spain are probably not attributable to either eutrophication or recent climatic trends.

The construction of dams can have profound impacts on marine systems. For example, the construction of the Aswan Dam fundamentally altered the hydrodynamic and nutrient regime of the Eastern Mediterranean, as did the Iron Gates Dam (on the Danube) in the Black Sea. The Nile formerly provided the largest freshwater input to the eastern basin of the Mediterranean, but as a result of hydraulic engineering has ceded that role to the rivers along the Turkish Mediterranean coast. Indeed, following the reduction in the discharge of the Nile River by almost 90% in the 1960s, Turkish rivers, especially the Seyhan and Ceyhan Rivers, now seem to be main source of freshwater and nutrients for the Levantine Basin (Özsoy and Sözer, 2006).

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Finally, global change scenarios may vary, since the same cause - increase of temperature - may have different or even contrasting effects in different areas. For example, following the increase of greenhouse gases in the atmosphere, an increase in sea surface temperature is expected in some regions. In and of itself, this may lead in a change of stratification parameters and less input of nutrients to the surface waters where light allows phytoplankton to develop. However, such a scenario of decreased nutrient inputs could easily be wrong if storms and accompanying wind-mixing events increase with sea surface temperatures. Therefore, phytoplankton biomass and production might decrease or increase on a global scale. Similarly, river inputs may increase or decrease, depending on changes in precipitation in different watersheds.

3. Long-term series and phytoplankton community responses in the Mediterranean

Global changes, through direct and indirect impacts, can alter the abundance, composition and phenology of phytoplankton. These three parameters have been shown to be important for ecosystem functioning: from structuring food webs and ultimately impacting fish stocks to having feedbacks on the climate through biogeochemical cycling (Platt *et al.*, 2003; Hays *et al.*, 2005). Time series observations are important for detecting changes in the phytoplankton community in relation to different environmental changes. In the last two decades, the number of studies on climate-induced changes has increased, but their number and coverage of different environments is still very low. This is particularly true for the Mediterranean, where there is not much published information from time series of at least 10-year length, and almost all these are from coastal waters. There are nevertheless data bases whose results have not been published or time series that started only recently. Of great value are also those from the past that ended decades ago.

The time series so far analysed in the Mediterranean show a general decrement in phytoplankton biomass in recent years, as judged by higher concentrations of chlorophyll *a* from more than two decades ago in different coastal areas: Kaštela Bay (Ninčević Gladan *et al.*, 2010), northern Adriatic (Mozetič *et al.*, 2009), Gulf of Naples (Ribera d'Alcalà *et al.*, 2004; Zingone *et al.*, 2009) and Bay of Calvi (Goffart *et al.*, 2002). In some cases, the response of phytoplankton is more clearly related to inter-annual variations of meteorological parameters, confirming that phytoplankton is susceptible to climate variations. It is also possible that the large-scale changes observed in the offshore Mediterranean circulation in the 1980s (Roether *et al.*, 1996; Brankart and Pinardi, 2001) have triggered changes in coastal phytoplankton. Responses of phytoplankton to large-scale events provoked by different meteorological situations have also been observed in some coastal embayments outside the Mediterranean (e.g. San Francisco Bay, Cloern *et al.*, 2007; Narragansett Bay, Borkman and Smayda, 2009). In other shallow coastal areas (e.g. northern Adriatic), phytoplankton signals of meteorological origin are more difficult to extract. There, reduced eutrophication of northern Adriatic coastal waters is proposed as a more plausible reason (Mozetič *et al.*, 2009) for a decrement in phytoplankton biomass.

To our knowledge, the only time series relatively unbiased by terrestrial impacts comes from the DYFAMED station in the NW Mediterranean (Marty *et al.*, 2002). Here, the increase of mainly pico- and nanoplankton biomass during the 1990s has been interpreted as a specific response to lengthening of the summer stratification period.

Changes in biomass have in some cases been accompanied by changes in primary production (Marasović *et al.*, 1995) and in community structure. The latter are expressed as changes in the ratio of microplankton groups (dinoflagellates *vs.* diatoms) (Marasović *et al.*, 2005), or as changes in the size spectrum (Marty *et al.*, 2002; Ribera d'Alcalà *et al.*, 2004; Bel Hassen *et al.*, 2009; Mozetič and Francé, this volume). A shift towards smaller-sized forms and a more regeneration-dominated community can have consequences for higher trophic levels and overall carbon cycling (Legendre and Fevre, 1995).

4. Methodological issues

Many time series originate from monitoring programmes. As a result they have a variety of methodological advantages (continuous and long-lasting sampling at fixed stations with presumably unchanging sampling design and methodology), as well as drawbacks (low sampling frequency, insufficient depths of sampling, insufficient level of identification); the latter mainly reflect limitations of human and economic resources. In general, the ecological issues that can be addressed are strongly constrained by sampling frequency. For example, at least weekly sampling is needed to follow seasonal peaks and their inter-annual variability. The lower sampling frequencies in open waters compared with coastal waters can be compensated by integrating field work with observations from oceanographic buoys and remote sensing.

Besides, current observation systems often do not cover enough parameters with which to track community changes. For instance, chlorophyll *a* measurements are used as a proxy for phytoplankton biomass, and due to their simplicity and relative objectiveness in comparison with e.g., biovolume calculations, are one of the most frequently measured parameters. However, this parameter gives no information about community structure and its possible changes, nor about the sudden appearance, increase or decrease of selected species that could have an impact on the whole planktonic system.

5. DATA AVAILABILITY

The easiest way to get information on time series (i.e., metadata) is from data websites; this should be encouraged in both directions - by sending and retrieving information under clear data policy rules. A further step in the accessibility of data offers projects - networks, which provide ready access to historical and newly collected raw data to the project consortium and to the public, as is the case of SESAME integrated EU project (data website: <http://isramar.ocean.org.il/sesamemeta/>)

Other sources of data and information can be found in international networks such us:
ILTER: International Long Term Ecological Research consists of networks of scientists engaged in long-term, site-based ecological and socioeconomic research (<http://www.ilternet.edu/>)
LTER-Europe: European Long-Term Ecosystem Research Network, a member network of ILTER (<http://www.lter-europe.net/>)

6. RANGE AND FLUCTUATIONS OF INDICATOR SPECIES

The Mediterranean Sea is rich in biodiversity. The basin is landlocked except for the Straits of Gibraltar and the Suez Canal. Despite these routes for the entrance of (sub)tropical phytoplankton species, numerous distinctive tropical species are lacking. Among the dinoflagellates, the Dinophysiales are especially diverse in open tropical waters. Large and highly ornamented species of *Histioneis* or species of *Amphisolenia* with ramified antapical extremes (e.g., *A. thrinax*) are absent from the Mediterranean basin. Distinctive species such as *Dinophysis miles* or *Pyrodinium bahamense* var. *schilleri* are ubiquitous in the Red Sea and Indo-Pacific tropical regions. However, these species have not been reported from the Mediterranean. This may indicate that, despite the thousands of ships containing ballast waters which pass through the Suez Canal each year, it continues to be a barrier for the northward expansion of tropical Indo-Pacific species into the Mediterranean. However, the paucity of phytoplankton studies in the region makes it difficult to track the origin of recently established tropical species in the Mediterranean. Among the diatoms, large tropical species belonging to the genus *Ethmodiscu*s and other common tropical taxa such as *Pseudo-eunotia doliolus* and some species of *Asteromphalus* are absent. The records of some distinctive diatoms with symbiotic coccoid cyanobacteria such as *Neostreptotheca* or *Climacodium* have been very scarce. The apparent lack of distinctive tropical dinoflagellate and diatom species precludes considering the phytoplankton flora in the Mediterranean as fully tropical.

The distinctive dinoflagellate *Citharistes regius*, known from surface waters of warm oceans, seems to have increased its distribution in the last year (Gómez, this volume), even in cooler regions like the Gulf of Lions, and is a possible indicator of warming of the Mediterranean.

Citharistes and other tropical dinophysiaceans have modified morphologies to harbour symbiotic diazotrophic cyanobacteria. Under oligotrophic conditions, such as those associated with warming-induced stratification, it can be expected that organisms able to fix nitrogen will have a competitive advantage and expand their distributions. Beyond the picoplanktonic size fractions, blooms of the filamentous cyanobacterium *Trichodesmium* are a common feature in tropical seas. In the Eastern Mediterranean basin, Hamza and Ben Maiz (1990) reported a "red tide" of *Trichodesmium erythraeum* in summer in the Gulf of Gabes. However, there is no record of this phenomenon in other Mediterranean regions. Several centric diatoms such as *Rhizosolenia clevei* and *Hemiaulus hauckii* are known to harbour the diazotrophic cyanobacterium *Richelia intracellularis*. These consortia have been reported for the first time in the Gulf of Marseille, an area with a long history of phytoplankton studies. Although there is no quantitative evidence to affirm that consortia of diazotrophic cyanobacteria with diatoms and dinoflagellates are spreading in the Mediterranean, it can be suggested that this phenomenon is increasing in recent years.

The heterogeneous sampling efforts in the Mediterranean make it difficult to differentiate between genuine introductions and marginal dispersal. The dinoflagellate, *Gymnodinium catenatum*, has a strong interannual variability in abundance that may be confused with a recent introduction. The sudden occurrence of a species should not be confused with a recent introduction. This situation can be extrapolated to other "opportunist" organisms. For example, *Alexandrium catenella* is a neritic species that requires stratification and eutrophic conditions, and is favoured by modification of the coastal environment due to the human activities (Vila *et al.*, 2001).

Little is known about the life cycle of most phytoplankton species. Changes of size and body shape can be expected in response to warming-induced stratification. A progressive warming of the Mediterranean can be expected to lead to an increase of the smaller fractions of the phytoplankton and reduction of the relative abundance of larger diatoms and dinoflagellates. Some dinoflagellates may increase their size with the development of ornamentation and body extensions under highly stratified conditions, as is usual in tropical species. The first record of the distinctive dinoflagellate *Asterodinium* in the western Mediterranean coincided with a heat wave, in September 1999. As well as *Asterodinium*, other similar genera with large extensions, and several species of *Microceratium*, were observed for the first time in the Mediterranean at that time. Morphological studies showed similarities between *Brachidinium*, *Asterodinium*, *Microceratium*, and *Karenia*. The last is considered the coastal form of the first three taxa (Gómez *et al.*, 2005b). Consequently, in a future warming scenario, it is expected that species may develop "tropical morphotypes". These will resemble species found in tropical waters, but should not be confused with species of tropical origin (Gómez, this volume).

7. Harmful Algal Blooms response to environmental changes

7.1 Diversity and complexity of the phenomenon

Harmful Algal Blooms are a recurrent phenomenon caused by a small fraction of phytoplankton taxa causing a range of negative physiological, environmental and economical effects. Harmful Algal Blooms (HAB) in the Mediterranean are a widespread problem; all kinds of HAB related events have been reported, with fewer cases in the south and southeastern parts where little information is available Sournia, 1972a; Zingone, this volume; Aligizaki, this volume). Two main types of HAB phenomena can be distinguished: intense accumulations of microalgae in surface waters causing water discoloration or the formation of unpleasant mucilage (or foam), and the proliferation of toxic microalgae. In coastal waters, mucilage often originates from extracellular phytoplankton secretions, and occasionally from benthic algae. In the Mediterranean, discolorations have been reported from nearly all coastal areas, while mucilage events have been reported mostly from the northern part. Mucilage events are recurrent in the Adriatic Sea (Fonda Umani, 1989; Zingone, this volume) while they occur mainly during the warm period in Greek waters (Nikolaidis *et al.,* 2008). These events are mostly detrimental for tourism. Coastal zones with the highest occurrence of these types of non toxic events seem to be at higher economical risks compared with areas exposed to other types of blooms (e.g. toxic).

Some Mediterranean HAB species, mainly dinoflagellates and diatoms, produce potent toxins that can accumulate through the food web and induce a number of human syndromes, such as paralytic (PSP) Diarrhetic (DSP) and Amnesic (ASP) Shellfish Poisoning (Hallegraeff, 1993). Intoxications through aerosols in recreational sites have been reported from Spanish coasts, although the causative organism was not always identified (Vila *et al.*, 2008). In the Tyrrhenian Sea, Adriatic and NW Mediterranean, the dinoflagellate *Ostreopsis ovata* has been associated with human respiratory distress and skin irritations (Brescianini *et al.*, 2006; Durando *et al.*, 2007; Ciminiello *et al.*, 2008). Some other HAB groups (mainly dinoflagellates, raphidophytes and prymnesiophytes) produce ichthyotoxins which are responsible for massive shellfish or fish kills. Algal toxins are monitored mainly in exploited shellfish areas. Fish are rarely analyzed for specific phycotoxins, although pilchards can contain ASP toxins (Costa and Carido, 2004) and Pacific sardines can contain other neurotoxins (palytoxins) (Onuma *et al.*, 1999). While Ciguatera Food Poisoning (CFP) is common in circumtropical areas (Lehane and Lewis, 2000), it has not been reported in the Mediterranean apart from some questionable cases (Aligizaki, this volume and references therein), although the causative organism (*Gambierdiscus*) has been reported in Greek waters (Aligizaki and Nikolaidis, 2008). Brevetoxins, which affect humans mainly through inhaling aerosols, have not been reported yet in the Mediterranean.

7.2 Observed changes in Mediterranean HABs

During recent decades, there has been an apparent increase of HAB events (including discolorations, mucilage aggregations and toxic episodes) in the Mediterranean (Zingone; Aligizaki; Fernández-Tejedor *et al.*; Legrand and Casotti, this volume). Whether this increase is a response to environmental changes and/or better detection, increased awareness, or more reports from the scientific community and the media, is unknown. But in some areas with long term established monitoring programmes, it is clear that HABs have increased both in intensity and frequency (Vila *et al.*, 2001). Mucilage events seem to have increased in recent decades in the Mediterranean, as in other parts of the world. Other long-term trends have also been identified, such as the decrease of blooms of the toxic dinoflagellate *Alexandrium minutum* in Egyptian coastal waters (Ismael and Halim, 2001) and the shift in the timing of occurrence of *Karlodinium* spp. blooms in Spanish coastal waters (Fernández-Tejedor *et al.*, this volume).

Although benthic dinoflagellates have been recorded in the Mediterranean for more than a century (e.g. *Prorocentrum lima,* Gulf of Sorrento, Italy, Ehrenberg, 1860), an apparent increase in records and abundance levels has been observed during the last decade (Aligizaki, this volume; Shears and Ross, 2009; Zingone, this volume). *Ostreopsis* blooms have become a recurrent phenomenon along Greek, Italian and Spanish coasts (Vila *et al.*, 2001; Aligizaki and Nikolaidis, 2006; Zingone, this volume and references therein), while the tropical ciguatera causing *Gambierdiscus* has been recorded in the Mediterranean since 2003 (Aligizaki and Nikolaidis, 2008). Without neglecting the fact that research is more intense in northern Mediterranean countries, the occurrence of *Ostreopsis* blooms is probably higher on the rocky northern shores, where brown and red algae, i.e. branching algae with large surface areas, are more abundant than on the sandy shores of North Africa. The fact that many Mediterranean benthic dinoflagellates (*Ostreopsis* spp., *Prorocentrum* spp.) are toxic (Riobó *et al*., 2006; Aligizaki *et al*., 2008a; 2009b; Ciminiello *et al.*, 2008) indicates the necessity to include these taxa and their toxicity in monitoring programmes. Ciguatera fish poisoning is one of the most important food related diseases in subtropical and tropical regions, and an epidemiological approach could be useful to predict the integrated impact of HABs on marine resources. This also calls for exploring these unusual toxins that are not part of routine monitoring.

7.3 Possible reasons for observed changes and trends

As HABs are complex phenomena involving different species and processes, environmental changes may affect their expansion and impact in several ways. For example, changes in temperature could lead to changes in the biogeography of harmful species, while other environmental changes might lead to the extension of the window of opportunity of certain HAB species in time or in space (Moore *et al*., 2008). The expression of toxicity can also vary under certain conditions (Granéli and Flynn, 2006) or among strains (Tillmann *et al*., 2009), which can react differently to changing environmental conditions. The complexity of HAB responses to environmental changes will also be reflected in their impact on marine resources (Legrand and Casotti, this volume). While the lack of long-term monitoring data all over the Mediterranean does not allow predictions, it is likely that the impact of HAB on the marine ecosystem, fisheries and aquaculture will increase with the increased need to exploit marine resources. Although HAB responses to environmental changes have not received much attention in the Mediterranean. In a limited number of cases, increases in HABs during the last three decades have been related to the impact of anthropogenic activities in coastal zones (Vila *et al.*, 2001), mostly with elevated nutrient discharge (Hamza *et al*.; Sakka Hlaili *et al*., this volume), or to the increase in stratification nearshore (Drira *et al*., 2008). Construction work (new harbours, marinas) or dredging may affect coastal hydrodynamics and favour the accumulation nearshore of harmful phytoplankton or the proliferation of benthic species. The spreading of mucilage in the Mediterranean in the last 3-4 decades has been linked to sea surface warming (Danovaro *et al.*, 2009).

The workshop highlighted both the high quality monitoring of HABs in north Mediterranean countries and the scarce coordination among countries throughout the region, except for a few coordinated projects e.g. HANA (North African countries), ICES (France-Spain) and REDIBAL (Spain and Portugal <http://www.redibal.org/>). There are international initiatives set up to organize/coordinate the available data on HABs (see Table 1). The scientific community around the Mediterranean is encouraged to contribute their data to these programmes in close collaboration with CIESM. Many studies of phytoplankton provide valuable information on HABs, especially from the eastern and southern Mediterranean, but it does not reach the international scientific community for various reasons. We encourage public authorities and stakeholders (Fisheries, Food Safety, Maritime and Tourism industry) to maintain/develop and facilitate monitoring of HAB in collaboration with scientists.

Table 1. International initiatives on HAB available to the scientific community and public.

HAEDAT (ICES/IOC)	Harmful Algae Event Database	<http://www.iode.org/haedat/>
HAB MAP (IOC/ISSHA)	Biogeography of harmful algal species	in preparation
IOC Taxonomic Reference List of Harmful Microalgae (IOC/UNESCO)	Identification, toxicity and references for a particular HAB species	<http://www.marinespecies.org/hab/index.php>
ASFA HAB-BIB	Bibliographic database on harmful algae	<http://ioc.unesco.org/RIS/RISWEB.ISA>
ISSHA	International Society of Studies on Harmful Algae	<http://issha.org>

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3. Long-term series and phytoplankton community responses in the Mediterranean

Global changes, through direct and indirect impacts, can alter the abundance, composition and phenology of phytoplankton. These three parameters have been shown to be important for ecosystem functioning: from structuring food webs and ultimately impacting fish stocks to having feedbacks on the climate through biogeochemical cycling (Platt *et al.*, 2003; Hays *et al.*, 2005). Time series observations are important for detecting changes in the phytoplankton community in relation to different environmental changes. In the last two decades, the number of studies on climate-induced changes has increased, but their number and coverage of different environments is still very low. This is particularly true for the Mediterranean, where there is not much published information from time series of at least 10-year length, and almost all these are from coastal waters. There are nevertheless data bases whose results have not been published or time series that started only recently. Of great value are also those from the past that ended decades ago.

The time series so far analysed in the Mediterranean show a general decrement in phytoplankton biomass in recent years, as judged by higher concentrations of chlorophyll *a* from more than two decades ago in different coastal areas: Kaštela Bay (Ninčević Gladan *et al.*, 2010), northern Adriatic (Mozetič *et al.*, 2009), Gulf of Naples (Ribera d'Alcalà *et al.*, 2004; Zingone *et al.*, 2009) and Bay of Calvi (Goffart *et al.*, 2002). In some cases, the response of phytoplankton is more clearly related to inter-annual variations of meteorological parameters, confirming that phytoplankton is susceptible to climate variations. It is also possible that the large-scale changes observed in the offshore Mediterranean circulation in the 1980s (Roether *et al.*, 1996; Brankart and Pinardi, 2001) have triggered changes in coastal phytoplankton. Responses of phytoplankton to large-scale events provoked by different meteorological situations have also been observed in some coastal embayments outside the Mediterranean (e.g. San Francisco Bay, Cloern *et al.*, 2007; Narragansett Bay, Borkman and Smayda, 2009). In other shallow coastal areas (e.g. northern Adriatic), phytoplankton signals of meteorological origin are more difficult to extract. There, reduced eutrophication of northern Adriatic coastal waters is proposed as a more plausible reason (Mozetič *et al.*, 2009) for a decrement in phytoplankton biomass.

To our knowledge, the only time series relatively unbiased by terrestrial impacts comes from the DYFAMED station in the NW Mediterranean (Marty *et al.*, 2002). Here, the increase of mainly pico- and nanoplankton biomass during the 1990s has been interpreted as a specific response to lengthening of the summer stratification period.

Changes in biomass have in some cases been accompanied by changes in primary production (Marasović *et al.*, 1995) and in community structure. The latter are expressed as changes in the ratio of microplankton groups (dinoflagellates *vs.* diatoms) (Marasović *et al.*, 2005), or as changes in the size spectrum (Marty *et al.*, 2002; Ribera d'Alcalà *et al.*, 2004; Bel Hassen *et al.*, 2009; Mozetič and Francé, this volume). A shift towards smaller-sized forms and a more regeneration-dominated community can have consequences for higher trophic levels and overall carbon cycling (Legendre and Fevre, 1995).

4. Methodological issues

Many time series originate from monitoring programmes. As a result they have a variety of methodological advantages (continuous and long-lasting sampling at fixed stations with presumably unchanging sampling design and methodology), as well as drawbacks (low sampling frequency, insufficient depths of sampling, insufficient level of identification); the latter mainly reflect limitations of human and economic resources. In general, the ecological issues that can be addressed are strongly constrained by sampling frequency. For example, at least weekly sampling is needed to follow seasonal peaks and their inter-annual variability. The lower sampling frequencies in open waters compared with coastal waters can be compensated by integrating field work with observations from oceanographic buoys and remote sensing.

Besides, current observation systems often do not cover enough parameters with which to track community changes. For instance, chlorophyll *a* measurements are used as a proxy for phytoplankton biomass, and due to their simplicity and relative objectiveness in comparison with e.g., biovolume calculations, are one of the most frequently measured parameters. However, this parameter gives no information about community structure and its possible changes, nor about the sudden appearance, increase or decrease of selected species that could have an impact on the whole planktonic system.

5. Data availability

The easiest way to get information on time series (i.e., metadata) is from data websites; this should be encouraged in both directions - by sending and retrieving information under clear data policy rules. A further step in the accessibility of data offers projects - networks, which provide ready access to historical and newly collected raw data to the project consortium and to the public, as is the case of SESAME integrated EU project (data website: <http://isramar.ocean.org.il/sesamemeta/>)

Other sources of data and information can be found in international networks such us:
ILTER: International Long Term Ecological Research consists of networks of scientists engaged in long-term, site-based ecological and socioeconomic research (<http://www.ilternet.edu/>)
LTER-Europe: European Long-Term Ecosystem Research Network, a member network of ILTER (<http://www.lter-europe.net/>)

6. Range and fluctuations of indicator species

The Mediterranean Sea is rich in biodiversity. The basin is landlocked except for the Straits of Gibraltar and the Suez Canal. Despite these routes for the entrance of (sub)tropical phytoplankton species, numerous distinctive tropical species are lacking. Among the dinoflagellates, the Dinophysiales are especially diverse in open tropical waters. Large and highly ornamented species of *Histioneis* or species of *Amphisolenia* with ramified antapical extremes (e.g., *A. thrinax*) are absent from the Mediterranean basin. Distinctive species such as *Dinophysis miles* or *Pyrodinium bahamense* var. *schilleri* are ubiquitous in the Red Sea and Indo-Pacific tropical regions. However, these species have not been reported from the Mediterranean. This may indicate that, despite the thousands of ships containing ballast waters which pass through the Suez Canal each year, it continues to be a barrier for the northward expansion of tropical Indo-Pacific species into the Mediterranean. However, the paucity of phytoplankton studies in the region makes it difficult to track the origin of recently established tropical species in the Mediterranean. Among the diatoms, large tropical species belonging to the genus *Ethmodiscu*s and other common tropical taxa such as *Pseudo-eunotia doliolus* and some species of *Asteromphalus* are absent. The records of some distinctive diatoms with symbiotic coccoid cyanobacteria such as *Neostreptotheca* or *Climacodium* have been very scarce. The apparent lack of distinctive tropical dinoflagellate and diatom species precludes considering the phytoplankton flora in the Mediterranean as fully tropical.

The distinctive dinoflagellate *Citharistes regius*, known from surface waters of warm oceans, seems to have increased its distribution in the last year (Gómez, this volume), even in cooler regions like the Gulf of Lions, and is a possible indicator of warming of the Mediterranean.

Citharistes and other tropical dinophysiaceans have modified morphologies to harbour symbiotic diazotrophic cyanobacteria. Under oligotrophic conditions, such as those associated with warming-induced stratification, it can be expected that organisms able to fix nitrogen will have a competitive advantage and expand their distributions. Beyond the picoplanktonic size fractions, blooms of the filamentous cyanobacterium *Trichodesmium* are a common feature in tropical seas. In the Eastern Mediterranean basin, Hamza and Ben Maiz (1990) reported a “red tide” of *Trichodesmium erythraeum* in summer in the Gulf of Gabes. However, there is no record of this phenomenon in other Mediterranean regions. Several centric diatoms such as *Rhizosolenia clevei* and *Hemiaulus hauckii* are known to harbour the diazotrophic cyanobacterium *Richelia intracellularis*. These consortia have been reported for the first time in the Gulf of Marseille, an area with a long history of phytoplankton studies. Although there is no quantitative evidence to affirm that consortia of diazotrophic cyanobacteria with diatoms and dinoflagellates are spreading in the Mediterranean, it can be suggested that this phenomenon is increasing in recent years.

The heterogeneous sampling efforts in the Mediterranean make it difficult to differentiate between genuine introductions and marginal dispersal. The dinoflagellate, *Gymnodinium catenatum*, has a strong interannual variability in abundance that may be confused with a recent introduction. The sudden occurrence of a species should not be confused with a recent introduction. This situation can be extrapolated to other "opportunist" organisms. For example, *Alexandrium catenella* is a neritic species that requires stratification and eutrophic conditions, and is favoured by modification of the coastal environment due to the human activities (Vila *et al.*, 2001).

Little is known about the life cycle of most phytoplankton species. Changes of size and body shape can be expected in response to warming-induced stratification. A progressive warming of the Mediterranean can be expected to lead to an increase of the smaller fractions of the phytoplankton and reduction of the relative abundance of larger diatoms and dinoflagellates. Some dinoflagellates may increase their size with the development of ornamentation and body extensions under highly stratified conditions, as is usual in tropical species. The first record of the distinctive dinoflagellate *Asterodinium* in the western Mediterranean coincided with a heat wave, in September 1999. As well as *Asterodinium*, other similar genera with large extensions, and several species of *Microceratium*, were observed for the first time in the Mediterranean at that time. Morphological studies showed similarities between *Brachidinium*, *Asterodinium*, *Microceratium*, and *Karenia*. The last is considered the coastal form of the first three taxa (Gómez *et al.*, 2005b). Consequently, in a future warming scenario, it is expected that species may develop "tropical morphotypes". These will resemble species found in tropical waters, but should not be confused with species of tropical origin (Gómez, this volume).

7. Harmful Algal Blooms response to environmental changes

7.1 Diversity and complexity of the phenomenon

Harmful Algal Blooms are a recurrent phenomenon caused by a small fraction of phytoplankton taxa causing a range of negative physiological, environmental and economical effects. Harmful Algal Blooms (HAB) in the Mediterranean are a widespread problem; all kinds of HAB related events have been reported, with fewer cases in the south and southeastern parts where little information is available Sournia, 1972a; Zingone, this volume; Aligizaki, this volume). Two main types of HAB phenomena can be distinguished: intense accumulations of microalgae in surface waters causing water discoloration or the formation of unpleasant mucilage (or foam), and the proliferation of toxic microalgae. In coastal waters, mucilage often originates from extracellular phytoplankton secretions, and occasionally from benthic algae. In the Mediterranean, discolorations have been reported from nearly all coastal areas, while mucilage events have been reported mostly from the northern part. Mucilage events are recurrent in the Adriatic Sea (Fonda Umani, 1989; Zingone, this volume) while they occur mainly during the warm period in Greek waters (Nikolaidis *et al.,* 2008). These events are mostly detrimental for tourism. Coastal zones with the highest occurrence of these types of non toxic events seem to be at higher economical risks compared with areas exposed to other types of blooms (e.g. toxic).

Some Mediterranean HAB species, mainly dinoflagellates and diatoms, produce potent toxins that can accumulate through the food web and induce a number of human syndromes, such as paralytic (PSP) Diarrhetic (DSP) and Amnesic (ASP) Shellfish Poisoning (Hallegraeff, 1993). Intoxications through aerosols in recreational sites have been reported from Spanish coasts, although the causative organism was not always identified (Vila *et al.*, 2008). In the Tyrrhenian Sea, Adriatic and NW Mediterranean, the dinoflagellate *Ostreopsis ovata* has been associated with human respiratory distress and skin irritations (Brescianini *et al.*, 2006; Durando *et al.*, 2007; Ciminiello *et al.*, 2008). Some other HAB groups (mainly dinoflagellates, raphidophytes and prymnesiophytes) produce ichthyotoxins which are responsible for massive shellfish or fish kills. Algal toxins are monitored mainly in exploited shellfish areas. Fish are rarely analyzed for specific phycotoxins, although pilchards can contain ASP toxins (Costa and Carido, 2004) and Pacific sardines can contain other neurotoxins (palytoxins) (Onuma *et al.*, 1999). While Ciguatera Food Poisoning (CFP) is common in circumtropical areas (Lehane and Lewis, 2000), it has not been reported in the Mediterranean apart from some questionable cases (Aligizaki, this volume and references therein), although the causative organism (*Gambierdiscus*) has been reported in Greek waters (Aligizaki and Nikolaidis, 2008). Brevetoxins, which affect humans mainly through inhaling aerosols, have not been reported yet in the Mediterranean.

7.2 Observed changes in Mediterranean HABs

During recent decades, there has been an apparent increase of HAB events (including discolorations, mucilage aggregations and toxic episodes) in the Mediterranean (Zingone; Aligizaki; Fernández-Tejedor *et al.*; Legrand and Casotti, this volume). Whether this increase is a response to environmental changes and/or better detection, increased awareness, or more reports from the scientific community and the media, is unknown. But in some areas with long term established monitoring programmes, it is clear that HABs have increased both in intensity and frequency (Vila *et al.*, 2001). Mucilage events seem to have increased in recent decades in the Mediterranean, as in other parts of the world. Other long-term trends have also been identified, such as the decrease of blooms of the toxic dinoflagellate *Alexandrium minutum* in Egyptian coastal waters (Ismael and Halim, 2001) and the shift in the timing of occurrence of *Karlodinium* spp. blooms in Spanish coastal waters (Fernández-Tejedor *et al.*, this volume).

Although benthic dinoflagellates have been recorded in the Mediterranean for more than a century (e.g. *Prorocentrum lima,* Gulf of Sorrento, Italy, Ehrenberg, 1860), an apparent increase in records and abundance levels has been observed during the last decade (Aligizaki, this volume; Shears and Ross, 2009; Zingone, this volume). *Ostreopsis* blooms have become a recurrent phenomenon along Greek, Italian and Spanish coasts (Vila *et al.*, 2001; Aligizaki and Nikolaidis, 2006; Zingone, this volume and references therein), while the tropical ciguatera causing *Gambierdiscus* has been recorded in the Mediterranean since 2003 (Aligizaki and Nikolaidis, 2008). Without neglecting the fact that research is more intense in northern Mediterranean countries, the occurrence of *Ostreopsis* blooms is probably higher on the rocky northern shores, where brown and red algae, i.e. branching algae with large surface areas, are more abundant than on the sandy shores of North Africa. The fact that many Mediterranean benthic dinoflagellates (*Ostreopsis* spp., *Prorocentrum* spp.) are toxic (Riobó *et al*., 2006; Aligizaki *et al*., 2008a; 2009b; Ciminiello *et al.*, 2008) indicates the necessity to include these taxa and their toxicity in monitoring programmes. Ciguatera fish poisoning is one of the most important food related diseases in subtropical and tropical regions, and an epidemiological approach could be useful to predict the integrated impact of HABs on marine resources. This also calls for exploring these unusual toxins that are not part of routine monitoring.

7.3 Possible reasons for observed changes and trends

As HABs are complex phenomena involving different species and processes, environmental changes may affect their expansion and impact in several ways. For example, changes in temperature could lead to changes in the biogeography of harmful species, while other environmental changes might lead to the extension of the window of opportunity of certain HAB species in time or in space (Moore *et al*., 2008). The expression of toxicity can also vary under certain conditions (Granéli and Flynn, 2006) or among strains (Tillmann *et al*., 2009), which can react differently to changing environmental conditions. The complexity of HAB responses to environmental changes will also be reflected in their impact on marine resources (Legrand and Casotti, this volume). While the lack of long-term monitoring data all over the Mediterranean does not allow predictions, it is likely that the impact of HAB on the marine ecosystem, fisheries and aquaculture will increase with the increased need to exploit marine resources. Although HAB responses to environmental changes have not received much attention in the Mediterranean. In a limited number of cases, increases in HABs during the last three decades have been related to the impact of anthropogenic activities in coastal zones (Vila *et al.*, 2001), mostly with elevated nutrient discharge (Hamza *et al*.; Sakka Hlaili *et al*., this volume), or to the increase in stratification nearshore (Drira *et al*., 2008). Construction work (new harbours, marinas) or dredging may affect coastal hydrodynamics and favour the accumulation nearshore of harmful phytoplankton or the proliferation of benthic species. The spreading of mucilage in the Mediterranean in the last 3-4 decades has been linked to sea surface warming (Danovaro *et al.*, 2009).

The workshop highlighted both the high quality monitoring of HABs in north Mediterranean countries and the scarce coordination among countries throughout the region, except for a few coordinated projects e.g. HANA (North African countries), ICES (France-Spain) and REDIBAL (Spain and Portugal <http://www.redibal.org/>). There are international initiatives set up to organize/coordinate the available data on HABs (see Table 1). The scientific community around the Mediterranean is encouraged to contribute their data to these programmes in close collaboration with CIESM. Many studies of phytoplankton provide valuable information on HABs, especially from the eastern and southern Mediterranean, but it does not reach the international scientific community for various reasons. We encourage public authorities and stakeholders (Fisheries, Food Safety, Maritime and Tourism industry) to maintain/develop and facilitate monitoring of HAB in collaboration with scientists.

Table 1. International initiatives on HAB available to the scientific community and public.

HAEDAT (ICES/IOC)	Harmful Algae Event Database	<http://www.iode.org/haedat/>
HAB MAP (IOC/ISSHA)	Biogeography of harmful algal species	in preparation
IOC Taxonomic Reference List of Harmful Microalgae (IOC/UNESCO)	Identification, toxicity and references for a particular HAB species	<http://www.marinespecies.org/hab/index.php>
ASFA HAB-BIB	Bibliographic database on harmful algae	<http://ioc.unesco.org/RIS/RISWEB.ISA>
ISSHA	International Society of Studies on Harmful Algae	<http://issha.org>

Can we detect meaningful changes in Mediterranean phytoplankton?

Tim Wyatt

Instituto de Investigaciones Marinas, Vigo, Spain

INTRODUCTION

The initial theme of this workshop, given as "Phytoplankton response to Mediterranean environmental change", called for some qualifications. Firstly, *responses*, plural. Each species responds in its own way to environmental changes and stresses, and to co-occurring species of phytoplankton and other organisms. Secondly, *changes*, also plural: and we need to be clear about the scales, spatial and temporal, over which changes of interest occur. A great many changes had already occurred in the Mediterranean basin before complex human societies emerged - read Messinian Salinity Crisis (see CIESM, 2008a), or Ice Ages for two extreme examples.

The concerns at this workshop are with natural climate fluctuations on decadal to secular time scales about which there is no doubt, man-made climate changes, about which there are doubts, about the local and regional effects of very diversified economic activities, and about more widespread changes which may be affecting the whole Mediterranean basin and beyond, such as ocean acidity (see CIESM, 2003), microplastics (Barnes *et al.*, 2009; Gregory, 2009), the coming *Age of Jellies* (see CIESM, 2001), or "info-disruption", the disturbance of intraspecific, trophic, and symbiotic interactions in food webs by pesticides, surfactants, or heavy metals (Lürling and Scheffer, 2007). With respect to the timescales, there may not be that many decades left if we believe the doomsayers! "[E]cosystems are being degraded and biodiversity is being lost at rates not seen in human history" (EASAC Policy Report, 2009) say terrestrial experts, yet some marine phytoplankton experts are having serious trouble detecting changes at all.

DETECTION OF CHANGES

A recent issue of the *Journal of Sea Research* (61: 1-124, January 2009) contains 14 papers which examine time series of inshore phytoplankton or chlorophyll in relation to environmental data, with particular attention to two issues, the putative impacts of anthropogenic eutrophication and climate variability on phytoplankton community dynamics. The collection is based on an ICES sponsored meeting held in Tisvildeleje, Denmark, 11-14 September 2006. The lengths of the time series range from about one to four decades; some are based on sampling at a single station, others on more extensive collections. One contribution examines the same problems using sedimentary records, and opens windows on secular and millenial scales. Most are based on microplankton sampling at weekly to monthly intervals, in a few cases more frequently; a few look at chlorophyll records.

The initial hope that these data sets *do* contain significant signals, and that they can be identified and described, is not realized. An overall conclusion is that the variability and complexity of these data sets muddy the recognition of unambiguous signals. For example, cell concentrations of individual species vary between stations and years by as much as six orders of magnitude, and variability increases continuously with time, even in 40-year time series. These characteristics are also found in terrestrial time series. It was noted more than twenty years ago (before some of our phytoplankton series were initiated), for a variety of terrestrial groups, that population variability increases as the number of years included in their calculation rises; this was true over most of the time intervals available for study, some of them longer than 40 years (Pimm and Redfearn, 1988). Since some definitions of ecological *stability* are based on this variability, this result raises difficult questions about what we mean by change in the first instance.

Thus, one message of the Tisvildeleje discussions might appear to be that, despite indisputable and often major changes in coastal waters caused by human activities, no clear signals are detectable in phytoplankton composition and dynamics, nor any which are distinguishable from changes in areas relatively unaffected by such impacts. Nor are there any obvious signals which can be clearly attributed to climate variability. Furthermore, reference conditions are difficult (impossible?) to establish. “Each species is … unique, and parameters important to growth and sustainability of one organism do not apply to another” (Martin *et al.*, 2009).

The only possibly introduced species recorded here are an ichthyotoxic *Chattonella* sp. (now *Verrucophora farcimen*) which first appeared in 1998, and has been abundant seasonally since, and *Gymnodinium chlorophorum* which formed a dense bloom in 1999, both these in Danish waters (Henriksen, 2009). Given the density of maritime traffic in northern European waters, and the high profile which ballast water studies have had in the last decade, two is a remarkably small number. Some reasons why introduced phytoplankton species can escape detection despite intensive sampling are reviewed by Wyatt and Carlton (2002).

Some difficulties of time-series analysis are summarized in CIESM (2003), and are worth repeating here. A somewhat idealized distinction is made in that report between deterministic series in which there is a trend in the mean but the variance remains constant, and stochastic series in which the mean is constant but the variance increases. There is no doubt that time-series of interest in the present context exhibit features of both categories. To this difficulty must be added the fact that none of the time-series of phytoplankton so far available from the Mediterranean basin match the temporal scales of change in the supposed forcings (climate fluctuations, anthropogenic influences, etc.) so that there are too few degrees of freedom to examine *issues of significant complexity*.

Factors which potentially regulate phytoplankton dynamics - temperature, wind speed, water column stability, turbulence, nutrients, flushing rates, runoff, climatic oscillations, competition, grazing, and so on - are relatively well understood, at least with respect to *bulk* features like primary productivity. But this is not in general true for individual species of phytoplankton. It is widely recognized that changes in light availability can alter chlorophyll levels while stocks remain unchanged, that high winter temperatures might lead to earlier grazing pressure in spring, hence lower stocks, and so on, but top down regulation of phytoplankton dynamics has not been explored in such detail. Other factors which can impact phytoplankton communities, such as fishing, aquaculture, introduced species, seabed mining, engineering projects (harbour development, irrigation schemes, urbanization, etc.) are often not taken into account. Top down control of phytoplankton can be severe as two contrasting examples make clear: over-exploitation of oysters in the Chesapeake system combined with destruction of its settlement habitats has led to persistent algal problems for nearly a century there (Rothschild *et al.*, 1994), and the accidental introduction of an Asian clam into San Francisco Bay reduced summer phytoplankton stocks tenfold in two years (Cloern, 1996).

The *agricultural hypothesis* which attributes a dominant rôle to nutrient inputs, that the keystone rogue is eutrophication, seems to have generated a kind of intellectual inertia in some quarters. There seems to be a consensus that coastal waters were on the whole ‘pristine’ as recently as the 1950s, despite the enormous reductions of biomass from virgin levels known already to have taken place, sometimes long before that time, in other components of the food web (elasmobranchs, fish, birds, mammals) in most areas accessible to their exploitation - the top down effects of these reductions, sometimes extinctions, on regional communities have hardly been explored.

Collos and colleagues (2009) conclude that “HABs are not related to eutrophication of the Mediterranean zone”, and Lancelot *et al.* (2009) states that “the sustained pressure of anthropogenic nutrients has not modified substantially the structure and function of the ecosystem” in the southern North Sea since the 1950s.

It would appear then that some of the best time series data in the world are not adequate to detect the impact of eutrophication on phytoplankton dynamics, that neither the intensity nor species of harmful or high biomass blooms are linked to eutrophication or to altered nutrient ratios, and that data sets are still too short to evaluate the effects of climate change on phytoplankton dynamics. It can be concluded that the long-standing agricultural hypothesis which basically maintains that increased nutrient inputs result in higher phytoplankton stocks should be given less prominence; whatever rôle eutrophication plays in phytoplankton dynamics, it is not necessarily either simple or dominant. If analyses of 20 and 40 year time series compiled by conventional procedures are not suitable to address the questions we wish to answer, then it might be that we need new sampling protocols. Sampling should be designed to answer questions, not just to compile time series! New analytical procedures could also lead to advances; reliance on standard statistical tools is as ingrained as the agricultural hypothesis. We need to explore different kinds of models and hypotheses to improve our understanding of phytoplankton dynamics. This view emerged very clearly at a recent GEOHAB meeting (Galway, June 2009; special issue of *Journal of Marine Systems*, in prep.).

Rather different traditions have reached different conclusions. The “Russell cycle” (Cushing and Dickson, 1976; Southward, 1980), and analyses of the Continuous Plankton Recorder survey (CPR, established in 1931) data show that major trends *can* be identified (Richardson and Schoeman, 2004; Edwards and Richardson, 2004), and linked with varying degrees of persuasiveness to climatic phenomena. But even relatively short term local monitoring can lead to the identification of changes, as for example in some northern Adriatic lagoons (Sfriso and Facca, 2007; Specchiulli *et al.*, 2008; Giordani *et al.*, 2009). So phytoplankton *can* provide information on ecosystem status, especially when supplemented by or combined with other kinds of information such as zooplankton counts, fisheries yields, macrophyte surveys in shallow waters, etc (e.g., Facca and Sfriso, 2009).

These conclusions reinforce the value of approaches to the same questions based on sediment cores, in which, although only the preservable components of phytoplankton communities are accessible for analysis, clear-cut signals attributable to anthropogenic impacts and to climate variations *are* detectable. The integration by sedimentary processes of the variability and complexity mentioned earlier is clearly advantageous when searching for patterns in very noisy ecological data.

Common objectives of ordination in ecology are to help generate hypotheses about relationships between species and environmental axes so as to dissect community patterns. The patterns discovered in this way depend on the data and on the axes chosen. Almost all routine phytoplankton data to date concern the vegetative phase of the life cycle, and temporal changes in other life history stages *on the same time scales* are poorly known. The resolution of sedimentary records, in which dormant life stages such as dinoflagellate cysts are frequently prominent, is coarse relative to time series based on plankton sampling, and cannot normally substitute for detailed surveys of living systems. But it can certainly reveal decadal trends, as the Po River delta example mentioned below indicates. So, one drawback of standard phytoplankton sampling protocols is the lack of concern with life stages other than the vegetative.

The characteristic time scales of *micro*phytoplankton blooms are of the order of a few days to two or three weeks, although longer lasting blooms do occur. So, a second drawback concerns sampling frequency. Weekly sampling can miss short-lived blooms entirely, and monthly sampling can miss several consecutive blooms. But daily sampling is usually logistically impossible, even if spatial coverage is sacrificed. If the mechanisms we are trying to understand take place on shorter time scales than our sampling, we will always be blind to them. On the other hand, a shift of focus to the less dynamic phases of a life history would clearly lower the severity of sampling demands.

MEDITERRANEAN PHYTOPLANKTON CHANGES

Three main *phytoplanktonic* problems have been recorded to date in the Mediterranean, none of them specific to the region. These are i) *mare sporco*, or mucilage events, sometimes associated with anoxia, ii) algal hyperproduction leading to seasonal anoxia independent of mucilage events, and iii) outbreaks of toxic species of particular concern to the shellfish industry and health authorities. Introduced species might constitute a fourth category, and are discussed elsewhere (see papers by Aligizaki and by Gómez in this volume); the topic is contentious (CIESM, 2002). At least one species, *Alexandrium catanella*, formerly considered an introduction to the Mediterranean from Asian waters, has now been shown not to be (Genovesi *et al.*, 2009).

Mare sporco, the massive occurrence of mucilage, is characteristic of the northern Adriatic but reported with increasing frequency elsewhere in the region (e.g., Tyrrhenian Sea, Sea of Marmara). It has been recorded in the Adriatic for centuries; the species which produce the mucilage are possibly diatoms, and it has been linked by some authors to high nutrient inputs, by others to increased temperatures, but general agreement on the mechanisms by which it is formed has not been reached. Nor is it clear why it accumulates in some years more than others. Earlier views that it is linked to eutrophication have not been strongly sustained. There are weak indications of decadal scale fluctuations in the extent of Adriatic mucilage events, possibly related to Po River discharge rates (Wyatt, 2003). Two special issues of *Science of the Total Environment* have been devoted to this topic, volumes 165 (1995) and 353 (2005).

Local shallow water anoxia in the Mediterranean seems to be a new phenomenon; it is favoured by the high winter temperatures and microtidal regimes, which make this sea more susceptible to the effects of local nutrient enrichment than more northerly coastal waters or the open Atlantic. A palynological analysis of an upper Adriatic core representing the last 150 years (Sangiorgi and Donders, 2004) indicates that in the area off the Po delta, a eutrophic signal in phytoplankton reached a peak in the 1970s, and has since weakened. In addition to the upper Adriatic, the areas most severely affected by eutrophication are Thermaikos Gulf and the northwestern Black Sea. Nutrient enrichment has in fact been reversed in several Mediterranean coastal areas, though not all (see papers by Polat, Sakka Hlaili *et al.* and Abboud-Abi Saab in this volume).

There is no firm evidence of a general increase in the frequency or intensity of toxic algal blooms in the Mediterranean in recent decades. What has changed is the intensity with which man interacts with the coastal zone; aquaculture in particular is sensitive to toxic algae, and has expanded greatly in recent decades. Phytoplankton itself may soon face problems in coping with some of the changes occurring such as increasing oceanic acidity, especially species with carbonate shells.

The range of axes or *dimensions* which can be chosen for ordination is very wide. Each selection reveals a different view of the data. There has been a persistent focus on the roles played by nutrients and turbulence in biological growth and mortality rates, etc.) by population ecologists. Systems ecologists are now providing new views based on network analysis. All are important, but our mental limitations prevent us from keeping more than two or three dimensions in mind simultaneously.

Harmful Algal Blooms in the Mediterranean: an historical overview

Adriana Zingone

Stazione Zoologica Anton Dohrn di Napoli, Italy

INTRODUCTION

The Mediterranean Sea has long been renowned for its enticing coasts and blue waters which have long attracted crowds of tourists from all over the world. Nonetheless, seawater discolourations, cases of toxicity and fish kills caused by marine microalgae have occurred in the area with increasing frequency, in parallel with the increase recorded in the world' oceans over the last couple of decades. In addition, in the years straddling the turn of the century, a novel and unique menace has suddenly emerged in several Mediterranean localities in the form of blooms of the epiphytic dinoflagellate *Ostreopsis* spp.

The development of harmful algal blooms (HABs) in certain areas has four main requirements, which relate to the geographic range of microalgae, their bloom dynamics, their harmfulness and, principally, to the human interest in marine resources (Zingone and Wyatt, 2005). First, the harmful species must be part of the local microflora, a non-trivial issue due to taxonomic complexities and sampling limitations. Second, the species must reach some critical concentrations. This process is under the control of several different biological and physical processes, including life strategies, grazing, and mortality from other causes, physical advection and dispersal. A third, important element of HAB development is the expression of harmfulness, which also is under the control of both exogenous and endogenous factors. Toxicity, for example, depends on both genetic and environmental factors, and may vary among strains and natural populations of a given species. Also the impairment of the aesthetic value of coastal waters may depend on biological factors (e.g. excessive polysaccharide production, phototactic responses) as well as on physical advection and high residence time. Finally, to have a harmful bloom, a target resource must be hit, which reminds us that HABs are a purely operational, anthropocentric concept. Indeed, toxic algae are not dangerous in the absence of tasty biological vectors (e.g. mussels). Similarly, discolourations are not an issue in wintertime or when they occur far from recreational sites.

Here, harmful events that have been recorded in the Mediterranean are reviewed, addressing the distribution of toxic species and the occurrence of negative effects imputable to marine microalgae, such as fish kills and discolourations. The current range of harmful events in the Mediterranean is evaluated based on the recent literature and on a comparison with previous reviews of the phenomena (Jacques and Sournia, 1978-79; Honsell *et al.*, 1995).

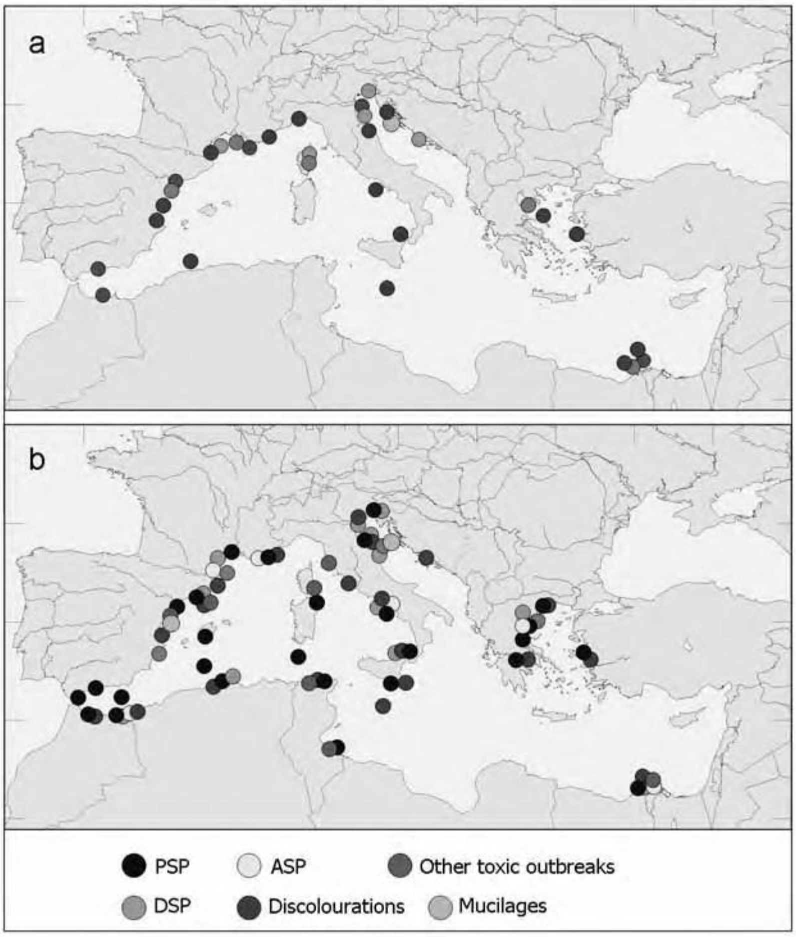

Figure 1. Distribution of harmful species and events along Mediterranean coasts based on a) reviews and reports until 1985 (Jacques and Sournia, 1976; Honsell *et al.*, 1995); and b) up-to-date findings.

DISCOLORATIONS AND MUCILAGE

A number of phytoplankton species, including heterotrophic ones, occasionally or regularly reach very high concentrations (10^3-10^5 cells ml^{-1}), thus changing the color of seawater and impairing the aesthetic quality and the recreational use of coastal areas. Biomass consumption processes may subtract high amounts of oxygen, leading to hypoxia and anoxia in some confined coastal areas. A negative impact is also exerted by mucilage of algal origin, and this can also interfere with fishing activity. The species that produce discolourations and mucilage vary geographically, are additional to the truly toxic ones, and significantly increase the number of potentially harmful species. Some species are typically a problem in specific areas, whereas others (e.g. some raphidophytes, some planktonic dinoflagellates such as *Prorocentrum*, *Noctiluca* and *Alexandrium*, and the colonial prymnesiophyte *Phaeocystis*) are common to several areas, including Mediterranean coasts. Here the impact on human economics is particularly relevant, considering that tourism is a major activity in many places.

The formation of large, often monospecific blooms which confer unnatural colours to seawater was noticed early in the last century, in both lagoons and coastal sites of the Mediterranean, where expressions like *purga de mar* and *punti verdi* preceded the term *red tides* later used for such events. Among the older records are a bloom of *Alexandrium minutum* in 1957 the Alexandria harbour (Halim, 1960b), and a conspicuous discolouration caused by *Prorocentrum minimum* in the Gulf of Naples in September 1962 (Yamazi, 1964). Causative species of these events include several different dinoflagellates (including heterotrophic species), raphidophyceans (*Chattonella* *subsalsa*, *Fibrocapsa japonica*) and prasinophytes (*Tetraselmis wettsteinii*, *Pyramimonas* spp. and *Halosphaera viridis*). In some cases these 'red tides' have been associated with fish kills and massive death of marine invertebrates (Piccinetti and Manfrin, 1969; Arzul, 1994; Halim and Labib, 1996; Garces *et al.*, 1999).

It is interesting that discolourations have been either sporadic, or have occurred for several consecutive years, after which they did not occur again. This is the case for blooms of *Alexandrium minutum*, which have not been recorded since 1994 in Egyptian waters (Ismael and Halim, 2001). Blooms of *A. catenella* occurred along the Catalan coast from 1996 to 1998 (Vila *et al.*, 2001b,d), but have been rare since (Solé *et al.*, 2006). Jacques and Sournia (1976) noticed that widespread blooms of the ciliate *Myrionecta rubra*, which hosts a cryptophyte as symbiont, were apparently absent from the Mediterranean. In recent years, *M. rubra* blooms have been recorded in both the Tyrrhenian Sea (Siano *et al.*, 2006) and in the Adriatic (Sorokin *et al.*, 1999). Periodic behaviour is also shown by the spectacular blooms of the heterotrophic dinoflagellate *Noctiluca scintillans*, which have been recorded in the Adriatic (Fonda Umani *et al.*, 2004), and on Moroccan (Tahri Joutei *et al.*, 2002), Catalan (Lopez and Arte, 1971) and French coasts (M.-O. Soyer in Jacques and Sournia, 1976). The reasons for the interannual variability of these blooms are unclear. In the Adriatic Sea, no relationship was found between their occurrence and eutrophic conditions (Fonda Umani *et al.*, 2004).

There is a wide literature about mucilage formation in the Adriatic, and a still unresolved debate on it causes. Algal polysaccharides might not be degraded efficiently and, due to peculiar hydrographic conditions, would then accumulate along Adriatic coasts (Fonda Umani *et al.*, 2007). *Mare sporco* (dirty sea) has been known for more than a century (Forti, 1906), and different species have been considered as potential producers of the mucilaginous substances. Initially diatoms were blamed; they are usually found in association with *mare sporco* (Forti, 1906), and are known to produce large amounts of mucopolysaccharides, especially when nutrient-limited. In recent years a dinoflagellate, *Gonyaulax fragilis*, which produces large amount of mucus, has been associated with mucilage both in the Adriatic (Pompei *et al.*, 2003) and along Catalan coasts (Sampedro *et al.*, 2007). Recently, based on biochemical characterization, prymnesiophytes have become suspect, especially the colony forming *Phaeocystis* (Flander-Putrle and Malej, 2008), which is responsible for conspicuous foam accumulations along North Sea coasts. It is noteworthy that mucilage events in the North Adriatic follow a fluctuating pattern over the long term: they went unrecorded for about 50 years, then showed up again at the end of the 1980s, with a peak in 2000 (Fonda Umani *et al.*, 2007). This irregular pattern hardly relates to known eutrophication trends, but points rather to a climatic/hydrographic mechanism behind the phenomenon, and suggests that *mare sporco* is caused by a combination of different mechanisms.

TOXIC MICROALGAE

About 100 eukaryotic microalgal species are known which produce toxins that can harm other organisms, including humans, either by direct contact or through accumulation in vector species. Of these, more than a half inhabit Mediterranean coastal waters. There are remarkable absences from the list, including some *Pseudo-nitzschia* and several of the most toxic *Alexandrium* and *Dinophysis*, especially those typical of cold temperate waters. Some tropical/subtropical species like the PSP producer *Pyrodinium bahamense* and the benthic *Gambierdiscus toxicus*, which is responsible for ciguatera, do not belong to the typical Mediterranean microflora either. The latter species however has recently been reported from Greek waters off Crete (Algizaki and Nikolaidis, 2008), confirming that species ranges can change over time. In the Mediterranean, relevant changes in geographic range have been noticed for two other toxic species, *Alexandrium catenella* (Luglié *et al.*, 2003) and *G. catenatum* (Gomez, 2003b); neither of these is apparently related to the climatic changes observed in the Mediterranean.

Since the first scientifically documented reports of toxic HABs date back to the 1960s, it seems that this problem has appeared quite recently in the Mediterranean. Risks for human health were first posed in the 1980s in the Gulf of Lyon (Belin *et al.*, 1993) and in the Adriatic (Boni *et al.*, 1993) by the presence of several species of Diarrhoeic Shellfish Poisoning (DSP)-producing species of the genus *Dinophysis*. Contamination by Paralytic Shellfish Poisoning (PSP) toxins produced by *G. catenatum* was first reported in Southern Spain in 1987 (Bravo *et al.*, 1990). The worst and apparently unique fatal episode of intoxication in the whole Mediterranean is that due to a bloom of *G. catenatum*, which caused four deaths and the hospitalization of 23 people in Morocco (Tagamouti-Talha *et al.*, 1996), where other toxic algae have also frequently been recorded (Tahri Joutei *et al.*, 2003). Since then, reports of PSP and DSP toxins have become quite widespread, whereas cases of domoic acid in mussels in the Mediterranean sea are still infrequent (Amzil *et al.*, 2001; Kaniou-Grigoriadou *et al.*, 2005).

THE CASE OF *OSTREOPSIS*

In 2002 more than 200 people reported fever, red eyes and respiratory problems after having spent time on the beach of Genoa (Ligurian Sea). That case received much attention from the newspapers, also because it took some days to unequivocally attribute it to a bloom of the benthic, epiphytic dinoflagellate *Ostreopsis ovata*. A few years earlier, the species had been associated with a series of different problems, including fish and invertebrate kills, along the coasts of Tuscany (North Tyrrhenian Sea) (Sansoni, 2003; Simoni 2003; 2004). Until then, problems related to benthic microalgae had only been reported from tropical areas, where they are responsible for a distinct syndrome, ciguatera fish poisoning (CFP).

The earliest Mediterranean report of *Ostreopsis* (identified as *O. siamensis*, the only species known at that time) dates back to 1972, when the species produced a bloom on macroalgae in Villefranche-sur-mer (Taylor, pers. comm.). *O. ovata* was first reported from Lebanese coastal waters in 1980 (Abboud-Abi, 1989), and from mid-Tyrrhenian waters in 1986 (Zingone *in* Tognetto *et al.*, 1995). After these casual findings, it was not until around 2000 that the wide geographic range of *Ostreopsis* species on rocky Mediterranean shores became clear (Fig. 2). In those years, problems to human health like those recorded in Genoa, in addition to dermatitis, were reported from other Italian localities, from Apulian, Sicilian and Marche coasts. Two different species are found in the Mediterranean, *O. ovata* and *O.* cf. *siamensis*, whose taxonomic identities are not fully assessed (Penna *et al.*, 2005).

Figure 2. Current distribution of *Ostreopsis* species in the Mediterranean, with dates of first record at each place. The species in the inset is *Ostreopsis* cf. *ovata.*

The apparent range expansion and increasing impact of *Ostreopsis* is in line with an increasing trend of species of the same genus in New Zealand and some other temperate areas around the world (Shears and Ross, 2009). However, problems related to an aerosol have only been reported for *O. ovata* in the Mediterranean. The effects on the food web are controversial and particularly relevant for their sanitary implications. Sea urchins have been reported to be damaged in some cases both in the Mediterranean (Sansoni *et al.*, 2003; Simoni *et al.*, 2003) and elsewhere (Shears and Ross, 2009). But healthy benthic micro- and macrofauna are commonly observed e.g. along the Campania coasts (unpublished results), where both sea-urchins and mussels can accumulate fairly large amount of palytoxin (Fattorusso and Soprano, pers. comm.). An interesting aspect of the annual dynamics of *Ostreopsis* species is that they exhibit rather repetitive seasonal patterns that vary from place to place (Table 1) and are scarcely related to temperature or to other obvious environmental parameters.

Table 1. Occurrence and seasonality of *Ostreopsis* spp. in some Mediterranean areas.

	First record	Occurrence	Peak	Toxicity	
Ligurian Sea (Villefranche-sur-mer)	1972	Summer	_	No	F.J.R. Taylor *in* Zingone *et al.*, 2006
Ligurian Sea (Genoa)	2005	Jul-Sept	End of July	Yes	Mangialajo *et al.*, 2008
North Tyrrhenian Sea	1998	Jul-Sept	Jul-Aug	Yes	Simoni *et al.*, 2004
Mid Tyrrhenian Sea (Latium)	1989	Summer		No	Zingone *in* Tognetto *et al.*, 1995
Mid Tyrrhenian Sea (Latium)	1994	Aug-Oct	End of Aug	No	Tognetto *et al.*, 1995
Mid Tyrrhenian Sea (Naples)	2004	June-Oct	Mid-July and Sept	No	Zingone *et al.*, 2006 and Zingone (unpubl. data)
South Tyrrhenian (Palermo)	2005	Jul-Aug	_	Yes	Barone and Prisinzano, 2006
Catalan coasts	1997	Mar-Sept	July	Yes	Vila *et al.*, 2001
North Adriatic (Ancona)	2006	Sept-Nov	Sept	Yes	Totti *et al.*, 2006
North Adriatic (Trieste)	2006	Late Sept-Oct	_	No	Monti *et al.*, 2007
South Adriatic (Lecce)	2001	July-Aug	_	Yes	Di Turi *et al.*, 2003.
North Aegean Sea	2003	Jul-Oct	Aug-Sept	No	Algizaki *et al.*, 2006
Egyptian waters	2005	June	_	No	Ismael and Halim, 2006
Tunisian waters	2001	Aug-Oct	Aug-Sept	No	Turki, 2005
Lebanese waters	1979-1980	_	_	No	Abboud-Abi Saab, 1989

CONCLUSIONS

In recent years, reports of both toxic algal species and corresponding toxicity in seafood have greatly increased along Mediterranean coasts. The discontinuous spatial distribution of HABs, along with their seasonal and interannual variations, reflect the variety and complexity of these events. Specific requirements, e.g. rocky shores in the case of *Ostreopsis*, may be the reason for notable spatial discontinuities in HABs, but the vicinity of research laboratories and aquaculture plants, which work as sentinels of HABs, contributes largely to the observed distribution. The latter explains, for example, the rarefaction of HAB records along the southern and eastern coasts of the Mediterranean basin already noted by Jacques and Sournia (1976). The ecological characteristics of the affected areas are rather diverse, and apparently there are no specific typologies that favour the occurrence of HABs. The temporal variability of Mediterranean HABs depends on the seasonal pattern of causative species, but also reflects the anthropocentric concept of HABs, the most severe cases being those which occur in summer, at the peak of the tourist season and of human attention to coastal waters.

A remarkable increase of harmful events has been recorded over the last 40 years in the Mediterranean. Little more than a handful were known until the 1970s (Jacques and Sournia, 1976) but, along with the growth of knowledge on toxic algae, toxin-producing organisms have been found in many Mediterranean localities. In recent years, the presence of toxins in seafood has also been recorded more frequently. In many cases it is easy to recognize in this trend the effects of more intense monitoring and deeper knowledge of the phenomenon. On the other hand, interannual and long term variations in harmful events are also evident. These include both decreasing trends, e.g.

in the blooms of *A. minutum* in Egypt and in those of *A. catenella* in the Catalan Sea, and increasing trends, as in the case of *Ostreopsis*. As harmful algal blooms are complex and diverse processes, caused by totally distinct organisms, reasons for the trends should be sought by analyzing the unique features (i.e. biogeography, ecology, toxicology and social aspects) of each individual case. In the case of *Ostreopsis*, several issues require further investigation. The apparent range expansion and bloom intensification of the species should be analysed in the context of its physiological characteristics and of a changing environment, in terms of climate and of human-caused changes in coastal circulation. This issue also requires a definitive assessment of the taxonomic identity of the two species recognized in the area. The range expansion of macroalgae on which *O. ovata* grows also needs to be monitored, e.g. in the case of *Asparagopsis taxiformis*, which seems to have invaded the Mediterranean in the same period as *O. ovata*. As for bloom dynamics, there are many questions, from the apparent dual modality of *Ostreopsis* blooms, with and without the production of a thick mucous carpet covering the macroalgae, to the differing seasonality of the species across the basin. The evolution of blooms, and the conditions in which they sporadically lead to intoxication through high concentrations of toxins in the seawater and in the aerosol also deserve attention.

For several years, increasing coastal eutrophication has been identified as the main cause for the expanding occurrence of HABs around the world. As in the Mediterranean, this explanation is only valid for a limited number of cases. In general, it is clear that the impacts of benthic and planktonic harmful algae are enhanced when retentive circulation patterns prevail over dispersal, allowing development of blooms and their accumulation nearshore, close to the most relevant marine resources (seafood, recreational sites). Modelling exercises support this conclusion, indicating that physical factors can play a greater role than interspecific relationships in promoting bloom development (Solé *et al.*, 2006). Therefore, it is predictable that changes in coastal circulation caused by climate variations - and by human engineering of the coastline - may significantly affect the impact of harmful algae on human activities. The development of models of coastal circulation under different climatic scenarios can be of great help to predict the areas at highest risk.

General trends in phytoplankton production and nutrient availability in the Mediterranean: an overview of previous French trans-Mediterranean cruises

Thierry Moutin

Université de la Méditerranée, Laboratoire d'Océanographie Physique et Biogéochimique,
OSU/Centre d'Océanologie de Marseille, France

INTRODUCTION

Phytoplankton production in the ocean may be strongly influenced by climate change. Despite the high complexity of the planktonic foodweb in the pelagic ecosystem, it is obvious that the ultimate constraints for phytoplankton production are abiotic factors, light and inorganic nutrients availability (De Baar, 1994). Unquestionably, variation of light is a dominant factor controlling phytoplankton production. Light condition at the sea surface can be approximated with simple calculations (Kirk, 1994; Moutin *et al.*, 1999), and light conditions with depth can be seen as a consequence of biological activity in the open ocean. In addition, nutrient (including iron) availability is a key factor to understand and eventually predict phytoplankton responses in the upper photic zone (Moutin and Raimbault, 2002). Phytoplankton production may be supported by new nutrients coming into the photic zone (new production) and by internal recycling of organic matter (regenerated production). Only new production (not linked with recycling of nutrients) can create an increase (or decrease) in biomass, and is a fundamental criterion of fertility (Minas, 1988), which must be considered to forecast phytoplankton biomass in response to climate change. The central question to study future phytoplankton production change is then "how will new nutrient inputs to the photic zone be altered following climate change?"

The response is not obvious; it depends largely on how these new nutrients enter the photic zone, and their dependence on space and time. Let us consider the general features of the Mediterranean.

GENERAL FEATURES OF THE MEDITERRANEAN

The general circulation of the Mediterranean is largely influenced by the existence of straits with sills (Lacombe, 1988), and by large seasonal variability of hydrological structures due to its mid latitude location. There is deep mixing of the water column in winter and strong stratification in summer. During winter, deep nutrient rich waters reach the surface allowing maximum phytoplankton production (spring algal blooms) to occur. Mediterranean production is thus characterized by two extreme annual nutritive limits. The first corresponds to winter mixing which determines the initial stock of nutrients. The quantities of nutrients brought to the photic zone will mainly depend on the depths reached by cooling waters. The second limit corresponds to the maximum summer stratification, and to the lower availability of new nutrients (mostly input by diffusion from deeper layer). Atmospheric inputs (including nitrogen fixation) may then provide significant new nutrients input during that period (Sandroni *et al.*, 2005). For coastal areas, additional new nutrient inputs by rivers should also be considered; the Mediterranean is a semi-enclosed area where nutrient inputs by rivers are a significant source (Bethoux *et al.*, 1998; Ludwig *et al.*, 2009). As an example, around 50% of the primary production in the Gulf of Lions in the western Mediterranean is attributed to terrigenous input (Coste, 1974; Morel *et al.*, 1990).

AN OVERVIEW OF PREVIOUS FRENCH TRANS-MEDITERRANEAN CRUISES

The distribution of primary production, particulate carbon export from the photic zone to deeper layers, and nutrient concentrations, were investigated in the first trans-Mediterranean cruise during May-June 1996 (MINOS cruise: Mediterranean INvestigation of Oligotrophic Systems: <http://www.ifremer.fr/sismer/program/mater/cruise_f/caCRNO=96020030>). A decrease in integrated primary production, particulate carbon export and nutrient availability towards the eastern side of the Mediterranean was observed, while integrated chlorophyll *a* remained constant. Integrated primary production ranged from 350 to 450 mgC m^{-2} day^{-1} in the west, decreasing toward the east to a value of about 150 mgC m^{-2} day^{-1}. The latter value appears to be a lower limit for primary production rates under strong oligotrophic conditions (Figure 1). Particulate carbon export represents 4.0 + 2.9% of integrated primary production. Up to 90-95% of primary production is then sustained by internal recycling of organic matter. Evidence of a limitation of production by phosphate availability was obtained from differences between the depths of the nitracline and phosphacline, and by enrichment experiments (Moutin and Raimbault, 2002). The Mediterranean can be considered one of the most P-depleted marine areas in the world (Moutin *et al.*, 2008).

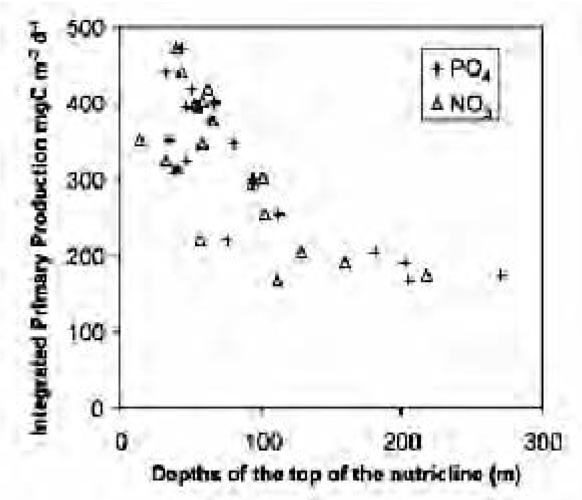

Figure 1. Integrated Primary Production (mgC m^{-2} day^{-1}) against depths of the top of the nutricline (m). Minos cruise (May-June 1996), from Moutin *et al.* (2002).

It has long been suspected that primary production is limited by phosphate availability in the Mediterranean. Bioassays have shown that phosphate enrichments stimulate photosynthesis (Berland *et al.*, 1980; Diaz *et al.*, 2001). However, not only phytoplancton production, but also bacterial production may be controlled by phosphate availability. During the PROSOPE (PROductivité des Systèmes Océaniques Pelagiques, <http://www.obs-vlfr.fr/cd_rom_dmtt/pr_main.htm>) cruise in September 1999, it was demonstrated that phosphate limitation on bacterial production, already observed in several locations (Thingstad *et al.*, 1998), was a general feature of the western and eastern Mediterranean (Van Wambeke *et al.*, 2002). Dissolved inorganic phosphate concentrations in the upper photic zone were shown to decrease from west to east, reaching levels well below 1 nM (Moutin *et al.*, 2002). *Synechococcus* spp., the most abundant phytoplankton in Mediterranean surface waters during summer (Vaulot *et al.*, 1996), were shown to have specific advantages concerning dissolved inorganic phosphate uptake that may explain their abundance in P-depleted environments (Moutin *et al.*, 2002).

$\delta^{15}N$ data from fossilised chlorophyll (MINOS cruise) provides geochemical evidence for extensive nitrogen fixation in the eastern Mediterranean (Sachs and Repeta, 1999). Thus, it becomes of great interest to describe and quantify the nitrogen input by nitrogen fixation, as well as understand the organisms responsible for this biogeochemical function.

As the quantity of dissolved atmospheric nitrogen is inexhaustible, it is important to understand the control of N_2-fixation. It seems that phosphate or iron availability are key factors in controlling these fluxes on a global ocean scale (Falkowski, 1997; Karl *et al.*, 2002). If nitrogen availability by nitrogen fixation is important in the Mediterranean, where low phosphate availability is thought to be the key factor controlling this flux, then the control of new production, initially defined as the fraction of production associated with new nutrients (generally nitrate), should be defined starting from new phosphate (Dugdale and Goering, 1967). It is important to further our understanding of the phosphate cycle in surface waters, which is inadequately studied (Benitez-Nelson, 2000; Karl, ASLO meeting 2006), to improve our understanding of phytoplankton production in the Mediterranean. The chemical element phosphorous ^{31}P only exists in the water in the form of phosphate, organic or mineral, particulate or dissolved, and is not reducible under natural conditions. Thus the many complex reactions of oxydoreduction found in the nitrogen cycles are not found in the phosphate cycle (Moutin, 2000), so it is possible to envisage coupling with production and the establishment of a budget from a different angle. Although there is a consensus that the major control of production in the Mediterranean is exerted by phosphate availability, nitrogen is scarce as well, and the availability of silicic acid may play a central role during spring (Leblanc *et al.*, 2003). Biological diversity may reflect multiple kinds of organic production limitation, so that a multi-element approach is necessary to increase our understanding of phytoplankton production and marine food webs.

Nutrient dynamics and its role in the variability of the stoechiometry of organic matter pools was the central aspect of the BOUM experiment (Biogeochemistry from the Oligotrophic to the Ultraoligotrophic Mediterranean : <http://www.com.univ-mrs.fr/BOUM/>) scheduled in June-July 2008 (Figure 2).

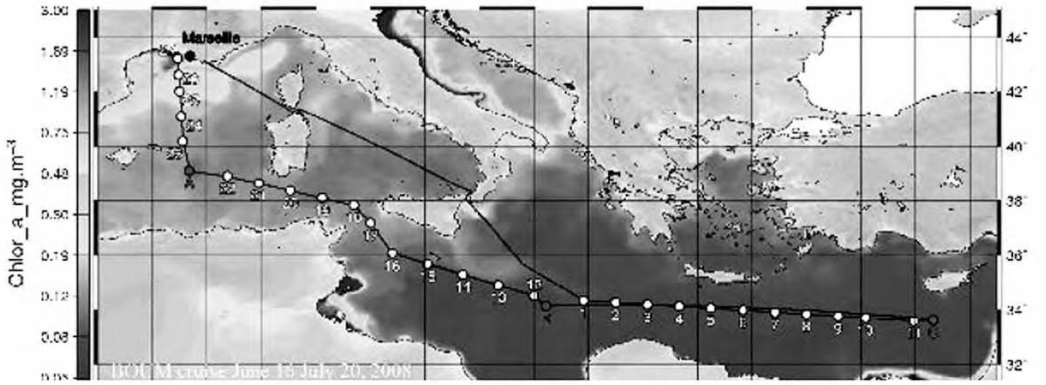

Figure 2. Transect of the BOUM cruise superimposed on a SeaWiFS composite image of Chl-a concentration in the upper layer of the Mediterranean between June 16 and July 20, 2008. The two types of station, short duration and long duration, are indicated. The three long duration stations investigated for periods longer than four days are indicated in red, and are located in the centres of anticyclonic eddies (courtesy E. Bosc).

The BOUM experiment has one overall goal: to obtain a better representation of the interactions between planktonic organisms and the cycles of biogenic elements, considering scales from the microscale to the whole Mediterranean. It was organized around three main objectives:

1) To give a longitudinal description of the biogeochemistry and biological diversity of the Mediterranean during the most strongly stratified period.

2) To study in three contrasting oligotrophic environments, the production and fate of organic matter, with particular emphasis on the processes which drive the divergence of the stoichiometric

ratios of the biogenic elements in the organic matter found, in the surrounding water, and in the exported materials.
3) To obtain a satisfactory representation of the main biogeochemical fluxes (C, N, P, Si) and the dynamics of the planktonic trophic network, both *in situ* and by using microcosm experiments.

The results are under scrutiny at the present time. Preliminary results confirm the general trends in phytoplankton production and biomass already observed during previous cruises. Furthermore, we highlight the importance of mesoscale activity on biogeochemical budgets. The numerous and large (around 100 km) anticyclonic gyres in the Mediterreanean are likely to largely increase phytoplancton production by allowing deeper winter mixed layer depths and subsequent higher new nutrient input to the photic layer during winter. Such areas are marked by deeper nutrient exhaustion during the stratified period (Figure 3).

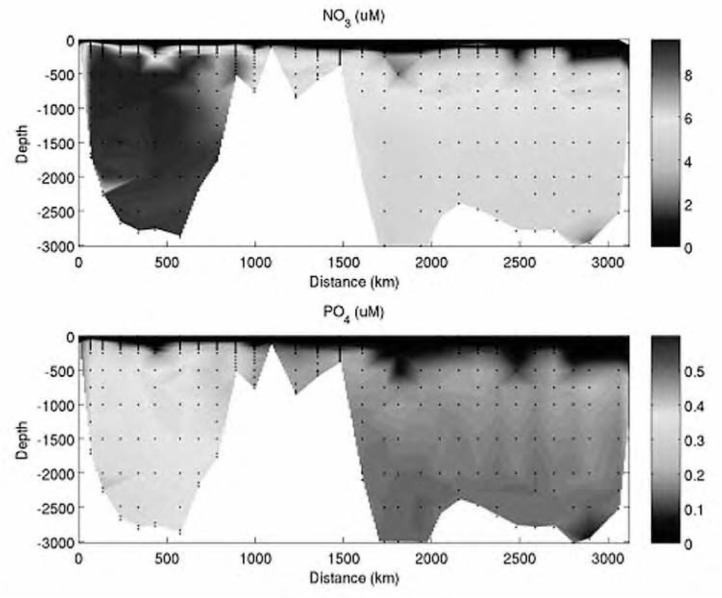

Figure 3. Nitrate and phosphate concentrations vs depth during the BOUM cruise from the River Rhone mouth to a station south of Cyprus (courtesy M. Pujo-Pay and L. Oriol).

EXPECTED CHANGES IN NEW NUTRIENT INPUTS TO THE PHOTIC ZONE

The different processes which may affect new nutrient availabity to the photic layer following climate change, and therefore ecosystem responses, are alterations of external sources (rivers and groundwater discharges, atmospheric deposition, and exchanges through the Straits), or internal ones (upwelling, convective overturning, mixing). It is then obvious that different kinds of changes may be expected from local to basin scales. The main expected change at a basin scale will follow the expected temperature increase and the corresponding increase in stratification, that will reduce the internal sources of nutrients for the photic layer (decrease of vertical transport). This process may have altered Mediterranean ecosystems already on a decadal time scale (Somot *et al.*, 2007; Herrmann, 2007). Therefore, a global decrease in nutrient availability is expected to be followed by a global decrease in phytoplankton production on the scale of the whole Mediterranean. At a local scale, the increase of stratification can create an increase in phytoplankton production. As an example, nutrient input by a river may have a stronger local impact because mixing is reduced, so that nutrients remain a longer time in the photic layer and allow higher phytoplankton production. Put simply, the most probable trends in phytoplankton production following climate change would be an oligotrophication of the open Mediterranean and eutrophication of coastal areas. Nevertheless, the large variability in time and space scales of the main processes affecting phytoplankton production might drive specific systems in opposite directions, as shown by the following two examples: during winter 2008, an unexpectedly large volume of dense water formed in the western Mediterranean (Fuda *et al.*, 2009), and a shift towards oligotrophication has been observed since 2000 in the Gulf of Trieste, northern Adriatic (Mozetic and France, this volume).

Abundance and distribution patterns of phytoplankton in the coastal waters of northeastern Mediterranean

Sevim Polat

University of Cukurova, Faculty of Fisheries, Balcali, Adana, Turkey

ABSTRACT

The Levantine Basin, located in the easternmost part of the Mediterranean is the most oligotrophic basin of that sea. However, in recent years eutrophication and harmful algal blooms have occurred in Levantine coastal waters. Studies on phytoplankton communities are scarce in the Levantine Basin compared with other Mediterranean sub-basins. This paper focuses on the abundance and distribution patterns of phytoplankton in the northeastern Mediterranean coastal waters of Turkey. The response of phytoplankton to environmental perturbations is evaluated. Coastal waters of the northeastern Mediterranean show great variability in the abundance patterns of phytoplankton; moreover in some bays there are also eutrophication signals. Due to scarcity of long term studies, it is difficult to evaluate phytoplankton data from past to present. It is concluded that long term and large-scale studies are necessary to determine the response of phytoplankton to environmental changes in the region.

INTRODUCTION

The Mediterranean is a unique body of water due to its climatic, hydrographic and biological characteristics. This sea is largely oligotrophic due to the lack of significant upwelling areas and small amount of discharge from land (Azov, 1991). The oligotrophy of the Mediterranean increases from the west to the east basin (Krom *et al.*, 1991). The abundance of species is also low in the eastern Mediterranean compared to the western basin. In recent years, the Mediterranean biota has witnessed biological invasions as well as pollution and climate change. As a result of warming, warm-water phytoplankton species may increase in abundance and expand into the northwestern Mediterranean (Gomez and Claustre, 2003; Tunin-Ley *et al.*, 2009). In this respect, phytoplankton is one of the best indicators to assess the status of the ecosystem and the impacts of environmental perturbations.

GENERAL PROPERTIES OF THE LEVANTINE BASIN

The Levantine Basin, located in the easternmost part of the Mediterranean is the second largest basin of the eastern Mediterranean, and its most oligotrophic region (Azov, 1991). River discharge to the eastern Mediterranean is low and upwelling areas are scarce. Since the operation of the Aswan dam on the Nile, Turkish rivers, mainly the Seyhan and Ceyhan rivers have become the major contributors of freshwater to the Levantine Basin (Bingel *et al.*, 1993). Nutrient distributions in the Levantine Basin are strongly associated with the hydrographic features. The supply of nutrients from deep waters by winter mixing is more efficient in cyclonic areas where the nutricline is relatively shallower than in anticyclonic regions (Yilmaz *et al.*, 1993). Thus, in cyclonic Rhodos Basin, where nutrient rich deep waters are upwelled to the euphotic zone, phytoplankton abundance is relatively high compared to anticyclonic areas in the region. In general in the northeastern Mediterranean, increased phytoplankton biomass occurs in late winter due to vertical mixing of the water column (Ediger and Yilmaz, 1996). In addition, local and temporarily high biomass can be found due to nutrient enrichment of coastal waters by human activities.

Construction of the Suez Canal in 1869 between the Red Sea and the Mediterranean and the completion of the Aswan Dam on the Nile River in 1965 (Kimor, 1983) mostly impacted the biodiversity and community structure of the Levantine Basin compared to other basins. The Canal opened the way for migration of species in both directions, but due to the currents in the region this migration is primarily from south to north for most of the year (Kimor, 1983). The Aswan Dam diminished the flow of nutrient-rich freshwater and caused alterations in phytoplankton composition and abundance in the southeastern Mediterranean (Dowidar, 1984).

PHYTOPLANKTON ABUNDANCE AND DISTRIBUTION IN NORTHERN LEVANTINE COASTAL WATERS

The Turkish Mediterranean coast borders most of the northern Levantine Basin continental shelf. Apart from the Bay of Iskenderun, this shelf is narrow. Phytoplankton biomass and production along this coast have eastern Mediterranean characteristics except in areas of high land-based inputs. However, in recent years, nutrient enrichment of these coastal waters has caused eutrophic conditions and local phytoplankton blooms. The coastline between Iskenderun and Mersin provinces is also intensively industrialized, with petroleum pipelines, iron-steel, fertilizer and paper products industries. Thus, the coastal bays such as Iskenderun Bay, Mersin Bay and Antalya Bay are under the risk of pollution (Figure 1). There are many local phytoplankton studies of these coastal bays, but data from the open waters are scarce. Although Turkey has one of the longest coastlines in the Mediterranean, there were few phytoplankton studies until the 1990s.

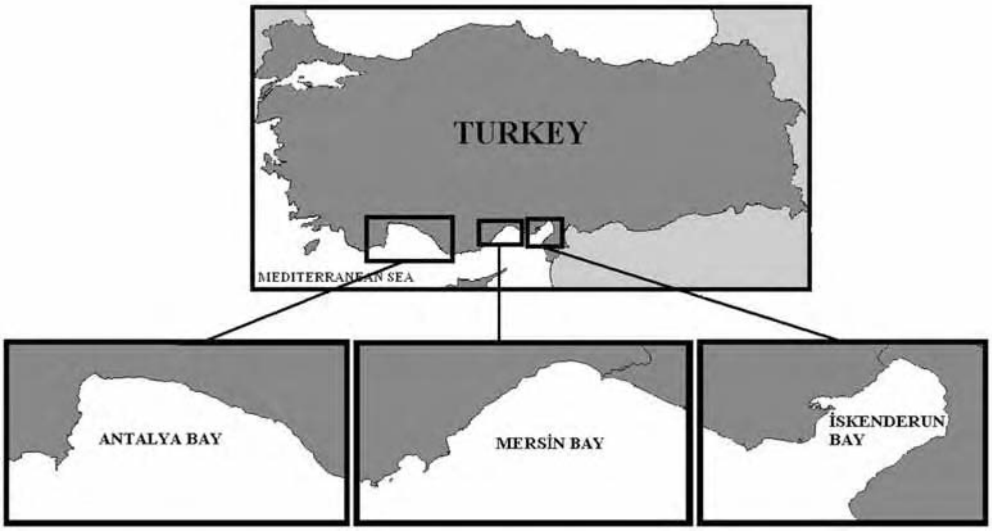

Figure 1. The coastal bays on the northeastern Mediterranean coast of Turkey.

From the 1990s onwards, phytoplankton distribution, abundance and biomass have been investigated in Iskenderun Bay, Mersin Bay and their surroundings (Table 1). Then, a detailed checklist of phytoplankton for Turkish coastal waters including Levantine coasts was prepared by Koray (2001). The range of abundance of phytoplankton found in different studies in Iskenderun Bay and surroundings are shown in Table 2. In this bay, phytoplankton abundance reached its maximum in spring (Polat and Piner, 2002a; Polat, 2006). However, the periodicity of phytoplankton peaks here does not completely conform with the classical bimodal scheme. Indeed, local and sudden increases of phytoplankton are seen as a result of nutrient enrichment of coastal waters by river discharge and anthropogenic activities. Maximum abundance in summer is typical for many coastal areas, as in Iskenderun Bay and its surroundings (Polat and Isik, 2002; Polat, 2007a). Size fractionation of water samples in Iskenderun Bay revealed that the picoplankton fraction dominates phytoplankton biomass, contributing 31-75% and 48-74% of the total chlorophyll a respectively (Polat, 2006; Polat and Uysal, 2009). This indicates that the area has eastern Mediterranean characteristics in terms of phytoplankton size distribution. The studies in Iskenderun Bay show that diatoms and dinoflagellates are similar in terms of species numbers (Polat *et al.*, 2000; Polat and Isik, 2002). Moreover, diatoms are dominant in the net plankton, contributing about 90% of total abundance.

Table 1. Some studies on phytoplankton of the northeastern Mediterranean coast of Turkey.

Region	**Sampling intervals**	**Reference**
Iskenderun Bay	Monthly	Avsar *et al.*, 1998
Iskenderun Bay	Monthly	Polat *et al.*, 2000
Karatas (West of Iskenderun Bay)	Seasonal	Polat and Isik, 2002
Iskenderun Bay	Monthly	Polat and Piner, 2002a
Iskenderun Bay	Seasonal	Polat, 2006
Iskenderun Bay	Monthly	Polat, 2007a
Iskenderun Bay	Seasonal	Polat, 2007b
Mersin Bay	Monthly	Kideys *et al.*, 1989
Mersin Bay	Weekly	Eker and Kideys, 2000
Babadillimani Bight (West of Mersin Bay)	Seasonal	Polat and Piner, 2002b
Mersin Bay	Weekly	Uysal *et al.*, 2003
Mersin Bay	Biweekly	Eker-Develi *et al.*, 2006
Iskenderun Bay, Mersin Bay and their surroundings	Monthly, Seasonal	Polat and Koray, 2007

Table 2. Range of abundance of phytoplankton in different studies in northeastern Mediterranean.

Region	**Phytoplankton abundance**		**Reference**
	Min.	**Max**	
Iskenderun Bay	2.64×10^2	1.27×10^4	Polat and Piner, 2002a
Iskenderun Bay	1.30×10^3	3.04×10^4	Polat, 2006
Iskenderun Bay	0.30×10^3	4.30×10^5	Polat, 2007a
Iskenderun Bay	0.70×10^3	4.20×10^4	Polat, 2007b
Mersin Bay	4.32×10^3	5.63×10^6	Uysal *et al.*, 2003
Babadillimani Bight (West of Mersin Bay)	3.36×10^2	5.72×10^4	Polat and Piner, 2002b

Inter-annual variations of phytoplankton abundance at a coastal station in Iskenderun Bay were analyzed by Polat (2007b) (Figure 2). She found that phytoplankton peaks occurred in spring (in April), and that these peaks increased in magnitude from 2001 to 2005, decreased in 2006 and 2007, and increased again in 2008 to 66 x 10^3 cells L^{-1} (Polat, unpub. data). Thus there may be no trend in phytoplankton abundance in the years studied (2001-2008). It was also found that a phytoplankton maximum in 2008 occurred in winter.

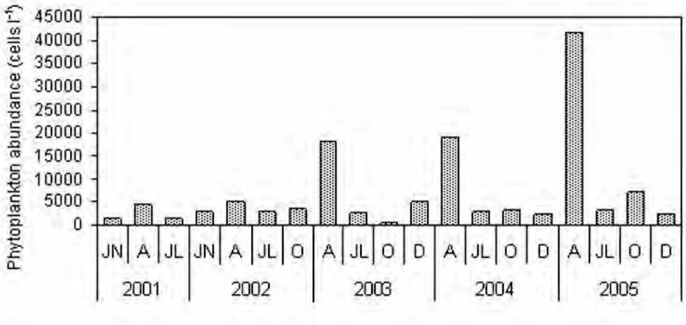

Figure 2. Interannual variations of phytoplankton at the coastal station in Iskenderun Bay (JN: January, A: April, JL: July, O: October, D: December (Polat, 2007b).

Despite the pollution of Iskenderun Bay, no red tides or eutrophication have been observed. The water column in the bay is well mixed due to the dynamic structure caused by stormy local winds and the circulation (Yilmaz *et al.*,1992). The bay is well flushed by open sea water. These processes transport pollutants to the open sea and provide renewal of the bay water.

Pollution level is relatively low in the Iskenderun Bay compared with Mersin Bay (Tugrul *et al.*, 2007). The coastal water of Mersin Bay is characterized by high levels of domestic and industrial pollutants and river run-off. These phenomena have contributed to coastal eutrophication in Mersin Bay in recent years. In previous studies by Kideys *et al.* (1989), Uysal *et al.* (2003) and Eker-Develi *et al.* (2006), diatoms were dominant in terms of species number and abundance. Diatom dominance was pronounced at coastal stations with highest cell numbers reached in early summer while coccolithophores (mainly *Emiliania huxleyi*) were numerically dominant at the open station (Eker-Develi *et al.*, 2006). Recently, Uysal *et al.* (2008) studied eutrophication and found maximum phytoplankton abundance (2.3 x 10^6 cells L^{-1}) at a near coastal station in Mersin Bay. This situation was explained by the weak interaction between coastal and open sea water in the area, and the east-west current regime which carries the nutrient-rich river discharges into the Bay.

Antalya Bay is the westernmost large bay on the Mediterranean coast of Turkey. This region is the most touristic area of Turkey and under the threat of pollution originating from tourism activities. Although pollution signals are reported here (Tugrul-Icemer *et al.*, 2007), there is no detailed study of the phytoplankton communities in Antalya Bay. However, the area between Mersin Bay and Antalya Bay is less polluted due to limited industrial and touristic activities. Polat and Piner (2002b) found the highest phytoplankton abundance as 5.72 x 10^4 cells L^{-1} in March 2000 in the Babadillimani Bight, west of Mersin Bay (between Silifke and Anamur province) which has a low pollution level. The authors concluded that the area has an oligotrophic character in terms of phytoplankton abundance and nutrient concentrations.

Most studies of phytoplankton have been conducted in the neritic waters of the Mediterranean coast of Turkey, and data from the open sea are scarce. In the neritic waters of the region, high levels of phytoplankton abundance generally occur in near surface layers. The distribution of phytoplankton in the offshore water is closely associated with the hydrographic and chemical properties of the environment (Ediger and Yilmaz, 1996). The Deep Chlorophyll Maxima (DCM) which is a result of increased phytoplankton biomass, is a permanent feature of the Mediterranean including the northeastern region (Yilmaz *et al.*, 1993; Ediger and Yilmaz, 1996): the DCM varies seasonally between 50 and 120 m depth in this region (Yilmaz *et al.*, 1993).

CONCLUSION

Despite generally oligotrophic conditions in the Levantine Sea, some coastal areas suffer from environmental problems. A significant part of the river discharges into the Levantine Basin originate from Turkey. Iskenderun and Mersin Bay, on the Mediterranean coast of Turkey, are areas which receive high levels of land-based pollutants. Of these, Iskenderun Bay is well flushed, and so far has shown no signs of eutrophication. Phytoplankton abundance in the bay is relatively high in comparison with the open sea, but not as high as in eutrophic areas. In contrast, eutrophication in Mersin Bay has led to higher phytoplankton abundance. In these areas, the timing of phytoplankton peaks does not show a regular periodicity, but local and sudden increases are observed. Phytoplankton peaks in adjacent offshore waters occur in late winter and early spring due to strong mixing of water column. But the lack of long term studies, and discontinuity between those which have been conducted, does not permit a detailed evaluation of phytoplankton dynamics in the area from past to present. Long-term large scale surveys are necessary to describe the phytoplankton dynamics and to evaluate their responses to environmental perturbations.

Seasonal patterns of phytoplankton abundance, hydrology, and climatic conditions in Gabes Gulf (southern Tunisia)

Asma Hamza, Wafa Feki and Malika Belhassen

INSTM - Centre de Sfax, Laboratoire Environnement Ressources, Tunisie

ABSTRACT

In this study, long-term (1995-2007) phytoplankton, oceanographic and climatic data from the Gulf of Gabes (southern Tunisia) were compiled from a shellfish monitoring programme. The aim was to define seasonal succession patterns of phytoplankton in six different areas in the gulf, characterised by different salinity, climate and tidal regimes, to identify a set of phytoplankton community indicators, and to describe the trophic status of this coastal area. Cluster analysis of the combined data set resulted in six phytoplankton community types, common for all locations, and characterised by different taxonomic composition representing different stages of seasonal succession. A hierarchy of explanatory variables that best predicted the communities, dominated by diatoms, cyanophytes, and dinoflagellates, was revealed.

INTRODUCTION

The Gulf of Gabes situated in southeastern Tunisia is characterized by semi-arid climatic conditions (Brandhorst, 1977). Temperature are high, the number of sunny days reaches 70%, and rainfall is scarce but most often torrential (Bousnina, 1997). Tides are very important in this area, and can greatly influence the migration and dispersal of phytoplankton.

Harmful Algae bloom studies in the Gulf of Gabes began twenty years ago (Hamza and El Abed, 1994) and have continued with improved intensity and spatial coverage since a phytoplankton monitoring programme was launched in 1995.

Consistent observations of plankton variability, environmental parameters and climatic conditions, such as water temperature, salinity, and tidal movements, have been collected since early 1997. Recurrent red tides, the spatio-temporal distribution of toxic phytoplankton, and dystrophic problems are archived in the data base, but the forcing mechanisms controlling the variability are still not understood.

The present study describes the seasonal variability of phytoplankton abundance and characteristic species of the main oceanographic and climatic regimes in the Gulf of Gabes. The results of 10 years of monitoring of water-column properties and plankton variability are analysed, and we compare seasonal and inter-annual variability in the phytoplankton in order to identify possible relationships between hydrological characteristics and biological features. Our focus is mainly on toxic species.

MATERIAL AND METHODS

The phytoplankton monitoring programme in the Gulf of Gabes includes the coasts from “Kapoudia” (35° 01’ 05”) to the Tunisian-Libyan border. Sampling was conducted in 14 areas with a total of 25 control stations (Figure 1).

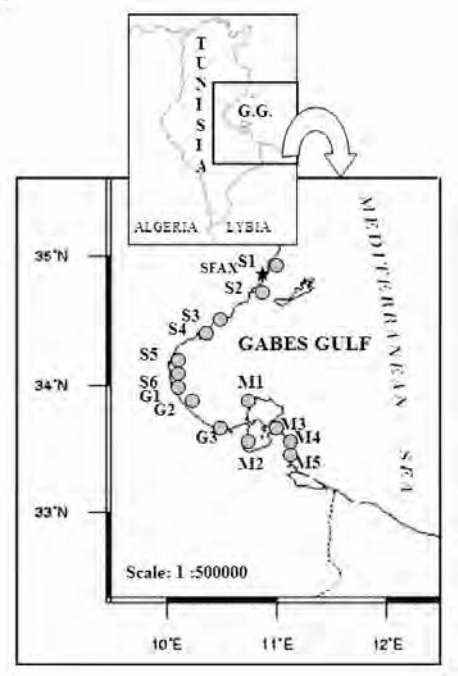

Figure 1. Locations of phytoplankton monitoring stations in Gulf of Gabes.

Water samples were collected at approximately weekly intervals, in the period of shellfish harvesting (from October to May) and monthly when the extraction of clams is stopped.

Samples for the determination of phytoplankton abundance were taken with a 1 L Niskin sampler, and preserved in 2 per cent (final concentration) neutralized formaldehyde solution. Phytoplankton abundance (cells per litre) was determined using the inverted microscope method. The entire phytoplankton community was identified to species or genus according to recent keys, as described elsewhere.

Profiles of temperature, pH and salinity were obtained *in situ* by probes (WTW).

The time series of phytoplankton abundance in this study are compiled from the INSTM data base. To produce monthly or annual values, data from all stations were averaged.

Analysis of variance (ANOVA) (significance level 0.05) was used to identify significant abiotic factors linked to the concentrations of toxic species.

A cluster analysis was used to describe different phytoplankton communities. Samples were classified according to the relative abundance of the main taxonomic groups of phytoplankton: Cyanophyceae, Bacillariophyceae, Dinophyceae and Chlorophyceae, and one cumulative group containing the rest of the phytoplankton species.

RESULTS

The data were derived from 5240 phytoplankton samples; approximately 30 to 35 samples for each location per year were analysed.

A total of 357 different taxa were identified in this period (1996-2007). Most taxa (about 60%) were dinoflagellate species. The most representative genera are *Protoperidinium* and *Gymnodinium*.

Increased bloom activity, due particularly to the dinoflagellate *Karenia selliformis*, was noted during two distinct periods, in 1996 and in 2006 (Figure 2). The year 2002 was characterized by the highest temperatures (Daoued *et al.*, 2007) and a resurgence of phytoplankton abundance, while 2006 was a relatively wet year marked by blooms of many species, especially toxic species.

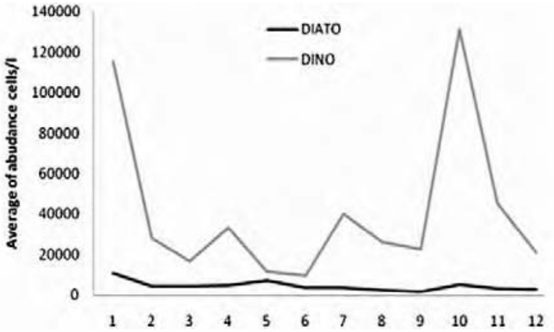

Figure 2. Monthly variability of phytoplankton in monitoring area in Gulf of Gabes.

Seasonal differences are much larger. Dinoflagellates at all stations (Figure 3) were more abundant (> 100 000 cells l) for a short time during winter and during a longer period in summer. In spring, peak bloom numbers reached around 40 000 cells l.

The relative abundance of diatoms was low in all seasons. During spring, decreased dinoflagellate abundance is balanced by increased diatom numbers. The relative pattern of diatom abundance is similar in different years.

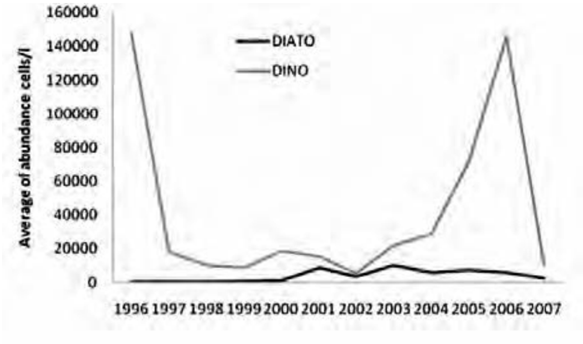

Figure 3. Annual variability of phytoplankton in monitoring area in Gulf of Gabes.

For toxic species, we noted a species-specific pattern of temporal occurrence of three dinoflagellates (*Karenia selliformis, Alexandrium minutum* and *Coolia monotis*). *Karenia selliformis* maintained a relatively stable inter-annual dynamics: concentrations are characterized by three maxima, in January, April and September-November. The autumn peak is more pronounced and we often observed concentrations higher than 10^6 cells l). Fluctuations of *Alexandrium minutum* and *Coolia monotis* show no regular annual variability. *Coolia monotis* affected some monitoring areas (M3), essentially in winter (Figure 4A), and showed different concentration ranges. *Karenia selliformis* is characterized by a typical late spring-early summer station (M2) (Figure 4B), but for *Alexandrium minutum*, we observed a tendency for occupation in the same areas (M2 &M3). Variance analysis (ANOVA) revealed no influence of temperature for any of these species. The influence of salinity on the abundance of *Karenia selliformis*, and the influence of pH on *Alexandrium minutum* and *Coolia monotis* are noted (Table 1).

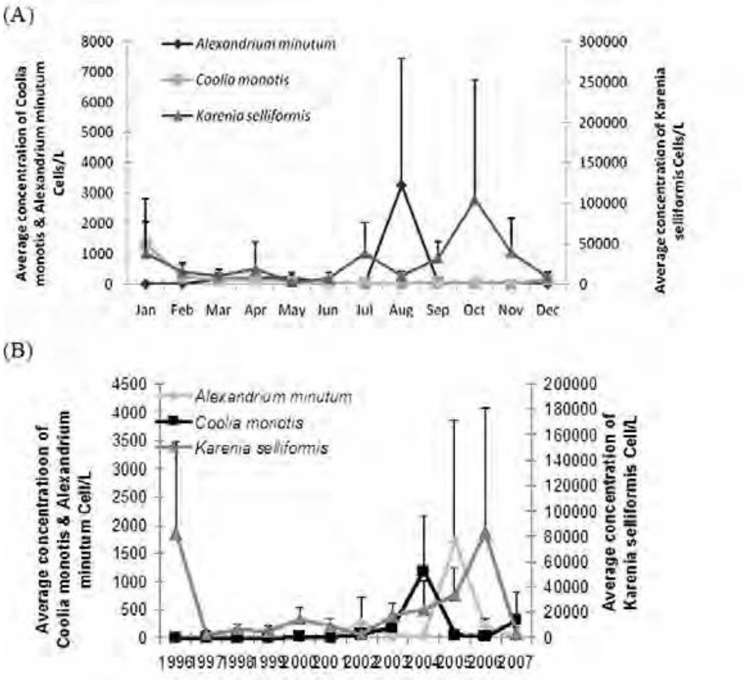

Figure 4. (**A**) Seasonnal; (**B**) Interannual variabilities of toxic phytoplankton species *(Karenia selliformis, Alexandrium minutum* and *Coolia monotis)* in the Gulf of Gabes.

Table 1. ANOVA results (P<0.05). A significant effect is indicated by S and no significant effect by NS.

Parameters	*Dependent variable*	*Signification*
PH	*Alexandrium minutum*	*S*
	Coolia monotis	*S*
	Karenia selliformis	*NS*
TEMPERATURE	*Alexandrium minutum*	*NS*
	Coolia monotis	*NS*
	Karenia selliformis	*NS*
SALINITY	*Alexandrium minutum*	*NS*
	Coolia monotis	*NS*
	Karenia selliformis	*S*

Cluster analyses of the relative abundance of the main taxonomic groups of phytoplankton at all stations reveal a relative contribution of 30% per individual group. According to the prevailing taxonomic group, five to six types of clusters were derived at each sampling location, dominated respectively by dinophytes (I), diatoms (II), cyanophytes (III), cryptophytes (IV), and a pool of other species (V).

The dominance of dinoflagellates (cluster type I) in summer and late autumn is characteristic for the phytoplankton succession on the south coast of the gulf, characterized by high salinity (>45psu). The dominance of diatoms (cluster type II) coincides with the biomass peak in April along the coasts of the central gulf. Species of diatom are mainly planktonic oceanic forms that can be carried with offshore currents. This type of analysis has shown that climatic conditions alone do not determine the appearance of species, but environmental characteristics may intervene intensely in this variability.

Changes in the Mediterranean phytoplankton community related to climate warming

Fernando Gómez

Marine Microbial Ecology Group, Laboratoire d'Océanographie, Station Zoologique,
Villefranche-sur-mer, France

ABSTRACT

Little information is available on phytoplankton composition changes related to climate warming in the Mediterranean. This study presents three examples of plankton as biological indicators of warming. The tropical dinoflagellate *Citharistes regius* is found for the first time in the Gulf of Lions, NW Mediterranean, and is reported for the first time in the open waters of the Ionian and Levantine basins. The tropical genus *Microceratium* is recorded for the first time in the Mediterranean; it is here considered to be a "tropical morphotype", a life stage of *Karenia* - *Brachidinium* - *Asterodinium* species complex that appears under warm and highly stratified conditions. *Microceratium* is a biological indicator of "tropicalization", an adaptation of a local species to the tropical environmental conditions that prevail in summer in the open Mediterranean Sea. The diazotrophic cyanobacterium *Richelia intracellularis*, an endosymbiont of the diatoms *Rhizosolenia clevei* and *Hemiaulus* spp., is common in summer-autumn in the Mediterranean and often dominant in oligotrophic open surface waters. The consortium *Richelia intracellularis*-*Rhizosolenia clevei* is reported for the first time in the Bay of Marseille.

Keywords: phytoplankton; climate change; long-term series; Mediterranean; biodiversity; biological indicator.

INTRODUCTION

Ocean temperatures have generally risen as the atmosphere warms (+0.3 ºC from 1950 to 2000, Levitus *et al.*, 2000). Surface warming increases the density vertical stratification of the ocean waters, leading to less mixing between the surface water layers, where phytoplankton live, and the deeper water layers, which contain the nutrients they need to flourish. The nutrient supply for phytoplankton will be reduced due to less upward mixing and a shallower "mixed layer", and stratification. These conditions are expected to favour the smaller phytoplankton fraction and motile groups such as flagellates and dinoflagellates versus diatoms, as observed in pigment signatures in the open NW Mediterranean (Marty *et al.*, 2002). Under a global warming scenario, we can expect geographical spreading of tropical species and species living in symbiotic associations with diazotrophic organisms, able to fix nitrogen. A number of studies show that the biodiversity of the Mediterranean is undergoing rapid alteration within the context of a globally changing climate. However, most of these studies correspond to macroscopic organisms (Francour *et al.*, 1994; Bianchi and Morri, 2000; Bianchi, 2007), and little is known about the changes of phytoplankton communities.

Projections for the Mediterranean basin for the 21st century indicate warmer and drier conditions, with a dramatic increase in the frequency and persistence of extreme events such as heat waves (Diffenbaugh *et al.*, 2007). These climatic anomalies provide a frame within which to investigate potential phytoplankton responses. In September 1999, a high mortality of sessile invertebrates was observed in the NW Mediterranean (Perez *et al.*, 2000). During this period, the dinoflagellate *Asterodinium* was observed for the first time in the western Mediterranean. *Asterodinium* was first described from tropical waters near Madagascar (Sournia, 1972b), and its occurrence was considered an indication of warming or "tropicalization" of the western Mediterranean (Gómez and Claustre, 2003). However, further studies suggested that *Asterodinium* may be a morphotype of a local species that is able to project body extensions under exceptional conditions. The tropical morphotype was described as a separate species from tropical waters (Gómez *et al.*, 2005). In summer 2003, the European region witnessed an exceptional heat wave, again associated with a mass mortality of sessile invertebrates (Garrabou *et al.*, 2009). In the NE English Channel, the 2003 summer heat wave was associated with an exceptional abundance of the dinoflagellates *Akashiwo sanguinea* and *Ceratium fusus*, and the first observations of some subtropical diatoms (Gómez and Souissi, 2007; 2008). To the best of my knowledge, no data exist on the response of the Mediterranean phytoplankton to the thermal anomaly of summer 2003.

The oligotrophic waters of the Mediterranean are characterized by a peculiarly high nitrate/phosphate ratio that differs from the Redfield ratio (N/P = 16) that predominates in the world ocean. It has been hypothesized that this might result from one or a combination of factors such as a high rate of biological nitrogen fixation (e.g. Béthoux *et al.*, 2002; Moutin, this volume). *Richelia intracellularis* as endosymbiont in the centric diatoms *Rhizosolenia clevei* and *Hemiaulus* spp. has been shown to have quantitatively substantial relevance in nitrogen fixation in Atlantic and Pacific warm waters (Carpenter *et al.*, 1999; Dore *et al.*, 2008). Oligotrophy associated with warming is expected to favour development of these symbiotic associations. However, very little is known about the distribution and tentative spreading of such symbiotic associations in the Mediterranean.

In comparison to the coasts of northern Europe, 19th century pioneers of plankton studies at the first marine stations founded along the coasts of Italy and France identified "quasi-tropical" conditions. For that reason, the Mediterranean is the locality where more species of dinoflagellates have been described (Gómez, 2003a; 2006a). Comparison of recent observations with historical bibliographical data will allow evaluation of changes in past decades. In this study, we report recent observations of phytoplankton composition from the Bay of Villefranche (1998-1999) (Gómez and Gorsky, 2003), the Bay of Marseille (2007-2008) and Banyuls-sur-mer (2008-2009), and open water research cruises PROSOPE (PROductivity of Oceanic PElagic Systems) in summer 1999 (Gómez and Claustre, 2003) and BOUM (Biogeochemistry from the Oligotrophic to the Ultra-oligotrophic Mediterranean) in summer 2009 (Figure 1). We combine recent data from sampling at monitoring sites with old bibliographic data in order to find examples of phytoplankton indicators of climate warming of the Mediterranean.

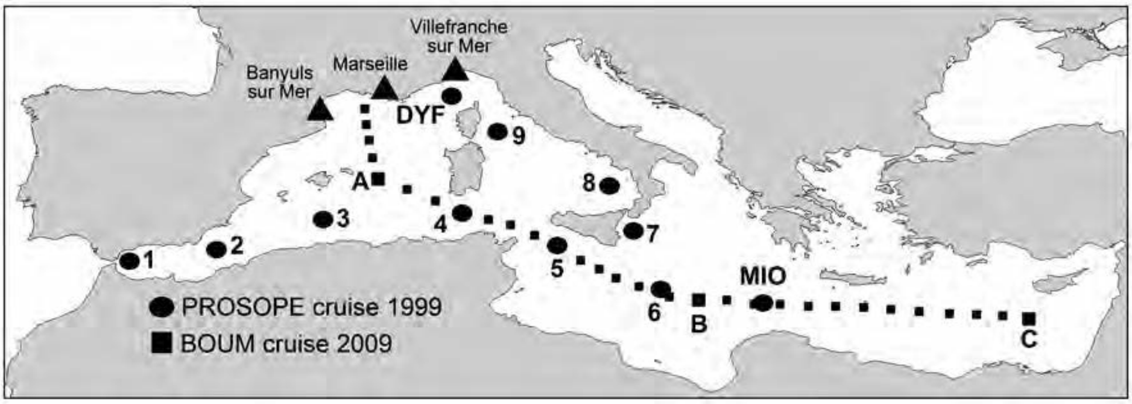

Figure 1. Map of sampling stations during the PROSOPE and BOUM cruises, and phytoplankton monitoring sites at Villefranche-sur-mer, Marseille and Banyuls-sur-mer.

OBSERVATIONS

1. Tropical dinoflagellates

Among the dinoflagellates, the order Dinophysiales has a special diversity in tropical waters. Most of the species are morphologically modified to harbour unicellular diazotrophic cyanobacteria as an adaptation for survival in oligotrophic waters. The genus *Histioneis*, that has clear tropical affinity, is well represented in the Mediterranean. However, distinctive tropical species such as *Histioneis highleyi*, *H. biremis* and the larger and highly ornamented species (i.e., *Histioneis megalocopa*-group) have not been recorded from the Mediterranean basin (Gómez, 2003a; 2007). Tropical species of the genus *Amphisolenia* characterized by ramified antapical extensions (i.e., *A. thrinax*) are absent in the Mediterranean.

The two species of the distinctive genus *Citharistes* were first described from the tropical Atlantic and Pacific Oceans. It seems that *Citharistes apsteini* was only cited from the open eastern Mediterranean (Kimor and Wood, 1975). In the Bay of Naples, there are historical citations of *Citharistes regius* by Schütt and Entz (*in* Schröder, 1906) and Schiller (1933) respectively. Later, it was reported in the Ligurian Sea (Halim, 1960a; Rampi and Bernhard, 1980) and the Tyrrhenian Sea (Magazzù and Andreoli, 1971). In the eastern Mediterranean, the first record from the Turkish coast is very recent (Polat, 2004). Historically, the Gulf of Lions is one of the best investigated regions in the world for phytoplankton. During this study, a live specimen of *Citharistes regius* was found off Banyuls-sur-mer in 2009 (Figure 2A). From the Lugol-fixed samples of the BOUM cruise, *Citharistes regius* appeared at five different stations of the Ionian and Levantine basin (Figures 2B-F). The tropical *Citharistes* is likely to have expanded its geographical range in recent years and can be considered an indicator of "tropicalization" in the Mediterranean.

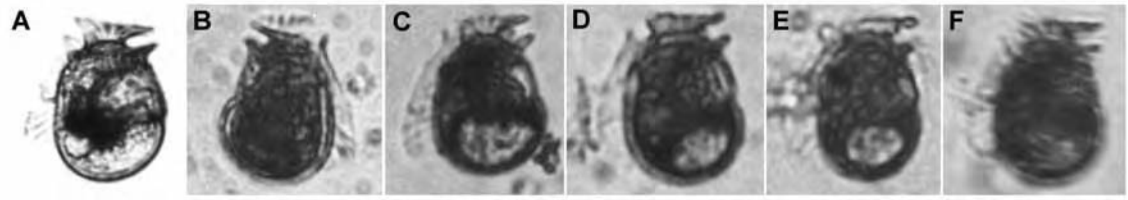

Figure 2. The tropical dinoflagellate *Citharistes regius*, found for the first time in the Gulf of Lions and in open waters of the Ionian and Levantine basins. **A.** Live specimen from Banyuls-sur-mer. **B-F.** Lugol-fixed specimens from the BOUM cruise.

2. Tropical morphotypes of local species

Dinoflagellates modify their morphologies to adapt to environmental conditions, and these morphotypes may be described as separate species. This phenomenon may be even more common in unarmoured dinoflagellates. During the thermal anomaly in September 1999, the genus *Asterodinium* appeared for the first time at several locations of the western Mediterranean (Gómez and Claustre, 2003). It was considered as a tropical species that spread its geographical range due to climate warming. However, further studies suggested that *Asterodinium* may be a life cycle stage of another species. Gómez *et al.* (2005), based on the observation of intermediate stages and detailed morphological studies by light and electron microscopy, suggested that *Asterodinium*, *Brachidinium*, *Microceratium* and *Karenia* may constitute a single species with high morphological versatility that is able to project body extensions as an adaptation to environmental conditions. Originally, *Microceratium* was only known from the tropical Indian and Pacific Oceans (Sournia, 1972b; Gómez, 2006b). During the BOUM cruise, records of *Asterodinium*, *Brachidinium* were numerous. The tropical *Microceratium* was found at five stations in the Levantine and Ionian Sea. This is the first record in the Mediterranean, and consequently it can be considered another biological indicator of "tropicalization" of the Mediterranean (Figures 3A-F). Here however, *Microceratium* is considered a life cycle stage of a local species, currently ascribed to *Karenia*, pooled in the past as *Gymnodinium* sp. This first occurrence of *Microceratium* in the Mediterranean should be considered as an indicator of climate warming. However, it should not be considered as a non-indigenous taxon. *Microceratium* is the "tropical morphotype" of a local species that appears in the Mediterranean when environmental conditions resemble those in tropical waters.

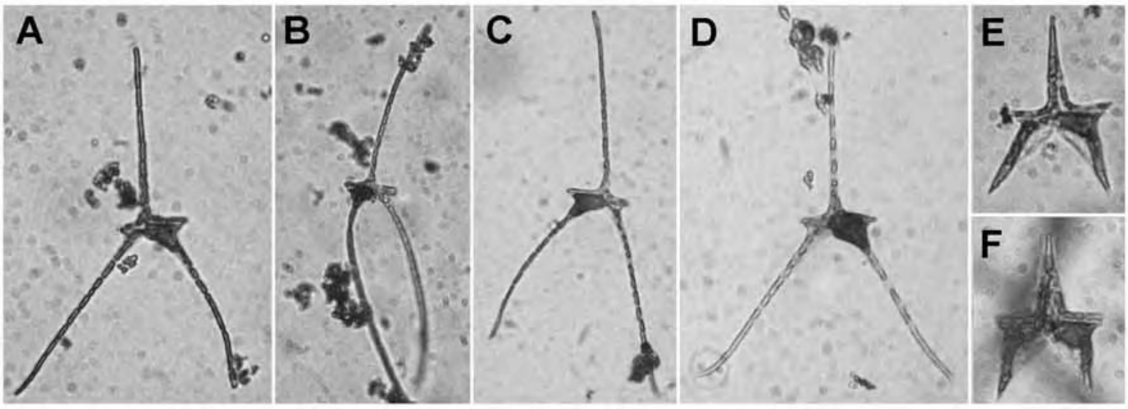

Figure 3. The dinoflagellate genus *Microceratium*, found for the first time in the Mediterranean. It is here considered to be a "tropical morphotype" of the local species, currently under the genus *Karenia*.

3. Symbiotic consortia of diatoms with diazotrophic cyanobacteria

The warming-induced oligotrophy of surface waters is expected to favour the development of diazotrophic organisms able to use atmospheric nitrogen as their sole nitrogen source. Surface blooms of the cyanobacterium *Trichodesmium* have been reported in some tropical waters (Dore *et al.*, 2008). *Trichodesmium* is commonly reported from the NW Mediterranean (Margalef, 1969, Figures 4A-B). In the Eastern Mediterranean, Hamza and Ben Maiz (1990) reported blooms of *Trichodesmium erythraeum* in summer in the Gulf of Gabes.

The diazotrophic cyanobacterium *Richelia intracellularis* is a symbiont which locates itself in the periplasmic space between the plasmalemma and silica cell wall in the diatoms *Rhizosolenia clevei*, *Hemiaulus* spp. and *Guinardia cylindrus* (Sundström, 1984), and more rarely as an epiphyte on *Chaetoceros* (Gómez *et al.*, 2005). The distribution of *Richelia*-diatom consortia have a clear tropical affinity, and were first described from the Red Sea. Analysis of a sediment core from the Levantine Basin reveals the historical occurrence of centric diatoms such as *Hemiaulus* and *Rhizosolenia* (Kemp *et al.*, 1999) that may harbour *Richelia intracellularis*. It is uncertain whether nitrogen fixation due to *Richelia intracellularis* is responsible for the peculiar high nitrate-phosphate ratio observed in Mediterranean deep waters. *Richelia intracellularis* is easily observable by light microscopy inside the frustule of *Rhizosolenia clevei*. However, the same endosymbiont in *Hemiaulus* spp. is hidden by the diatom chloroplasts, but visible by epifluorescent microscopy since the pigment composition of the cyanobacterium differs from that of the diatom (Figures 4E-H).

Historically, the phytoplankton composition of the Gulf of Lions has been intensively investigated. In the Bay of Marseille, Travers and Travers (1975, p. 253) remarked that they never found *Richelia intracellularis* in the frustules of *Rhizosolenia*. In this study, phytoplankton composition was examined from autumn 2007 to late summer 2008 in the Bay of Marseille. *Richelia intracellularis* as endosymbiont of *Rhizosolenia clevei* was observed in October 2007 and September 2008 (Figures 4C-D). In Banyuls-sur-mer, *Hemiaulus hauckii* was a common member of the summer phytoplankton assemblage, and all specimens examined revealed the presence of *Richelia intracellularis* under epifluorescent microscopy (Figures 4E-H). The *Hemiaulus-Richelia* consortia often appeared attached to the lorica of the tintinnid ciliate *Eutintinnus*. Oligotrophic conditions seem to favour the development of this peculiar association of three organisms, a cyanobacterium, a diatom and a ciliate (Figure 4I).

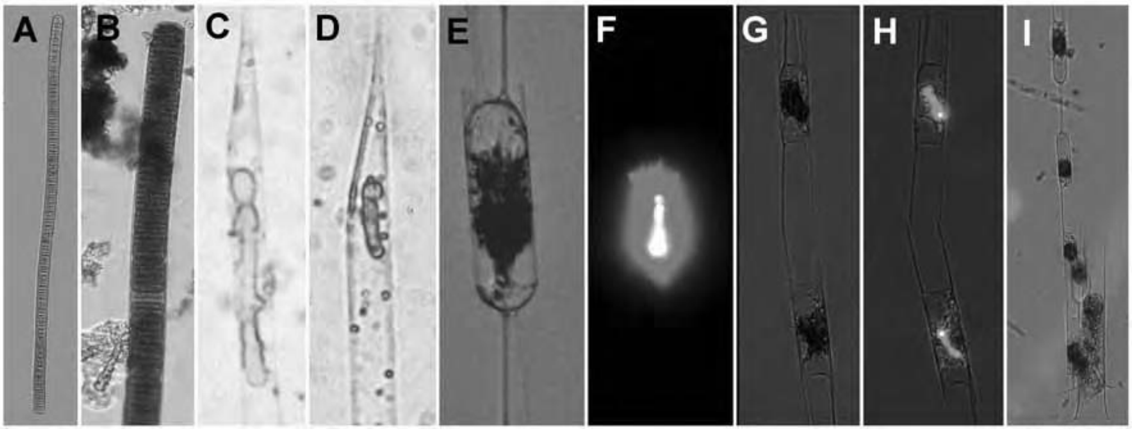

Figure 4. **A-B.** Tentatively, the cyanobacterium *Trichodesmium* from Banyuls-sur-mer. **C-D.** *Richelia intracellularis* endosymbiont in *Rhizosolenia clevei* from Endoume, Marseille and from the open Mediterranean during the BOUM cruise. **E-F.** *Richelia intracellularis* in *Hemiaulus hauckii.* **F-H.** Epifluorescent microscopy micrographs. **I.** Association between *Hemiaulus*, containing *Richelia*, and the ciliate *Eutintinnus*.

During the PROSOPE cruise in September 1999, *Hemiaulus hauckii* was the dominant microphytoplanktonic species in the surface layer during an oligotrophic period in the western and central Mediterranean (Table 1). The abundance was ~100 cells per litre. During the BOUM cruise in July-August 2009, *Hemiaulus hauckii* was present at most stations, especially in the surface waters of the eastern Mediterranean, with an average abundance of 20-50 cells per litre (Figure 5). During the PROSOPE or BOUM cruises, the presence of *Richelia* was not confirmed by epifluorescent microscopy because the samples were fixed with Lugol´s solution. During the BOUM cruise, the abundance of *Richelia* as endosymbiont of *Rhizosolenia clevei* was very low, with average values of 2 cells per litre (Figure 5). Although the abundance of the diazotroph-diatom consortium is low, they are important primary producers in oligotrophic surface waters. It is uncertain whether these organisms are playing a role in the high nitrate-phosphate ratio of the Mediterranean.

Table 1. *Hemiaulus* as percentage of total microphytoplankton in upper 50 m depth during the PROSOPE cruise in 1999. At stations 3 and 8, 5 m depth samples were not available.

Depth (m)	St2 %	St3 %	St4 %	St5 %	St6 %	MIO %	St7 %	St8 %	St9 %	DYF %
5	29		100	61	50	40	0		0	0
15						0				0
25		0	25	0						0
30	0.2				100	100	0	0	0	0
50	1.4	0		0		66		0		0

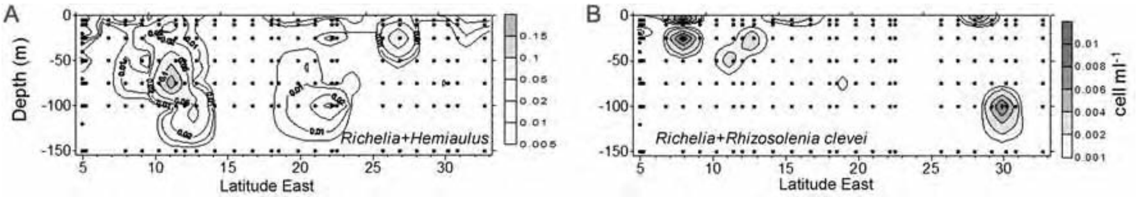

Figure 5. Distribution and abundance of *Richelia intracellularis* as endosymbiont in *Hemiaulus hauckii* (**A**) and *Rhizosolenia clevei* (**B**) during the BOUM cruise.

Our knowledge of the response of phytoplankton to climate change is restricted by the lack of long-term studies, especially those reporting species data, and by shortage of competent taxonomists. The lack of studies, especially in the southern and eastern Mediterranean basins, hinders differentiation between cryptic residents and new immigrant phytoplankton species.

Acknowledgements: I acknowledge financial support from the ANR Biodiversity program ‘Aquaparadox’ of the French CNRS. This is a contribution to the project DIVERPLAN-MED supported by a post-doctoral grant of the Ministerio Español de Educación y Ciencia #2007-0213. This is a contribution of the JGOFS-France PROSOPE and BOUM (Biogeochemistry from the Oligotrophic to the Ultraoligotrophic Mediterranean) project of the French national LEFE-CYBER program and of the European IP SESAME.

Phytoplankton in the Gulf of Trieste, 1984-2007: trends, community changes, and links to recent environmental changes

Patricija Mozetič and Janja Francé

Marine Biology Station Piran, National Institute of Biology, Slovenia

ABSTRACT

A negative trend in chlorophyll *a* concentrations, used as a proxy for phytoplankton biomass, has been observed all over the northern Adriatic during the last 10 years, in comparison with the previous 20-year period. This trend is more distinctive in certain areas (in the proximity of the Po River mouth) than in others, and in the Gulf of Trieste amounts to 0.09 µg Chl *a* L^{-1} year^{-1}. In contrast to biomass behaviour, there has been an increase of phytoplankton abundance due to an increase in small flagellates and the reduction of massive diatom blooms in the same period. Changes are also recorded in the pattern of succession of phytoplankton species; the once late spring-early summer community characterised by large dinoflagellate species was replaced by diatoms at the beginning of this century. Several suggestions have been proposed to account for this scenario. A substantial decline of freshwater discharges over the recent decade, having consequences on nutrient concentrations in seawater in an already phosphorus-limited environment, seems the most plausible. Other factors, such as an increase in temperature and salinity in certain seasons and changes in the grazer community, should not be ignored.

INTRODUCTION

A recent compilation of 84 data-sets from coastal and estuarine ecosystems worldwide (Cloern and Jasby, 2009) shows the complexity of patterns (seasonal and annual variability and exceptional events) and scales (months, years, events) of phytoplankton variability. While in the open ocean phytoplankton variability is generated mainly by the annual cycles of temperature and solar radiation (Sverdrup, 1953), in coastal ecosystems several processes acting through the connections between land, ocean, atmosphere and sediments are involved. In their comparative study, Cloern and Jasby (2009) identified sampling sites from the Gulf of Trieste (northern Adriatic; Figure 1) as ones where the seasonal pattern somewhat dominates over the annual, but where exceptional events are also likely to occur. Besides confirming our findings of the high variability of different parameters, presented below, the characterisation of waters in the Gulf of Trieste by Cloern and Jasby (2009) also points out the difficulty in discerning the difference between natural oscillations and induced changes, which, if long-lasting, can be detected as trends. The latter can be seen as a response to anthropogenic pressure (e.g. nutrient loads) or, recently, to climate changes.

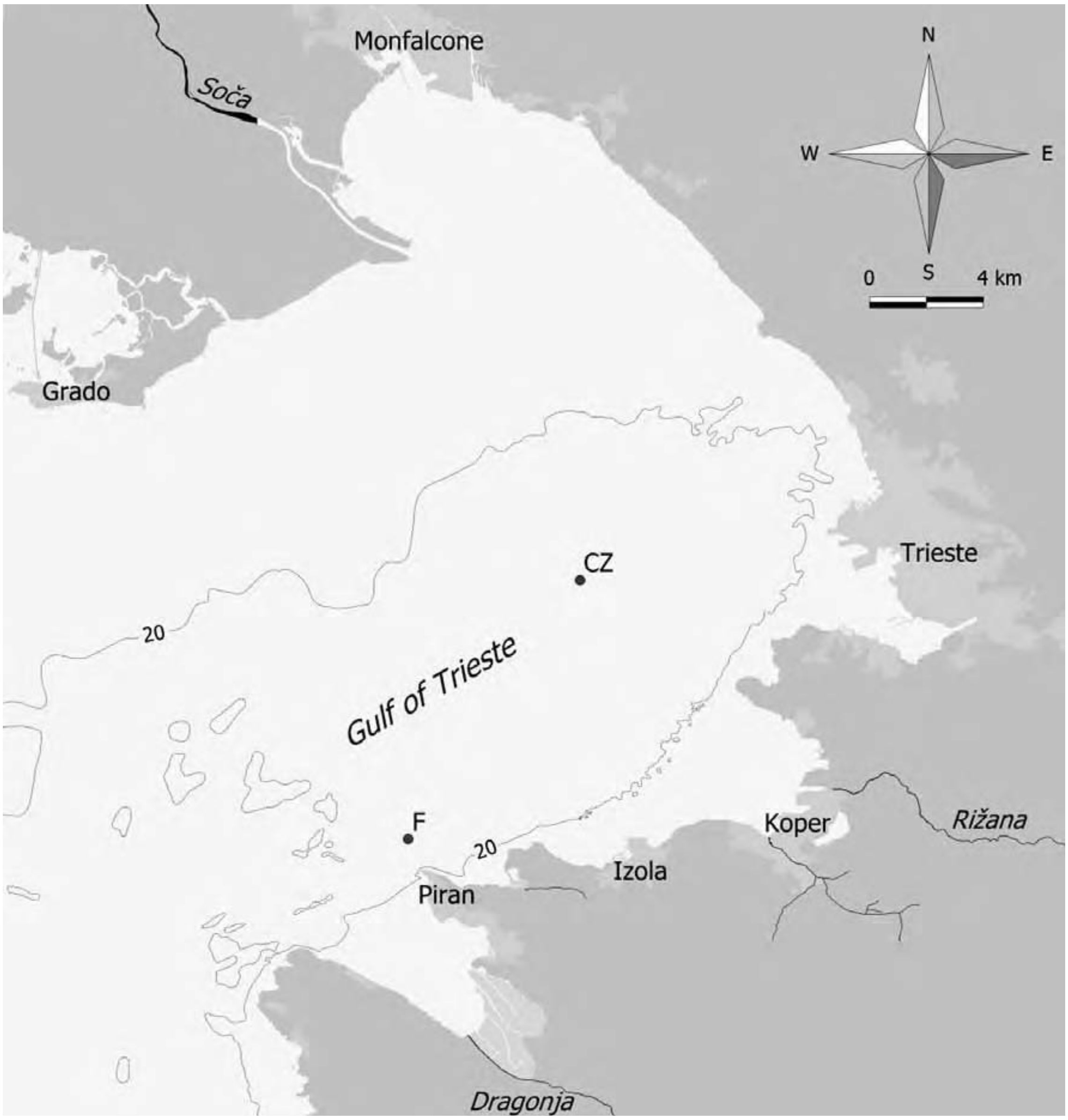

Figure 1. Study area and locations of sampling stations.

This contribution is an overview of different analyses of phytoplankton time series, published and not, and aims to explain the observed changes in the phytoplankton community in terms of environmental perturbations. Data were acquired through different monitoring programmes, and the longest and most continuous data sets from two sampling sites encompassing periods of 19 years (1989-2007; station CZ) and 24 years (1984-2007; station F) will be presented.

SEASONAL AND INTER-ANNUAL VARIATIONS OF PHYTOPLANKTON BIOMASS AND RECENT TRENDS

A previous studies (Mozetič *et al.*, 2005) has shown that concentrations of chlorophyll *a* (Chl *a*) exhibit large oscillations and that differences between seasons/months and sampling sites in the Gulf of Trieste can be high enough to be statistically significant. The deeper sampling site (24 m) was chosen to present the typical mean annual pattern of Chl *a* biomass for the whole area (Figure 2). By calculating mean monthly concentrations for the 1989-2007 time series, two annual maxima can be identified: a smaller one in the late winter-early spring period (Feb to Apr), and a larger one in autumn (Nov), both regularly limited to the upper 5-10 m layer. Summer is the period of the lowest surface concentrations of Chl *a*, although high values are measured in the bottom layer.

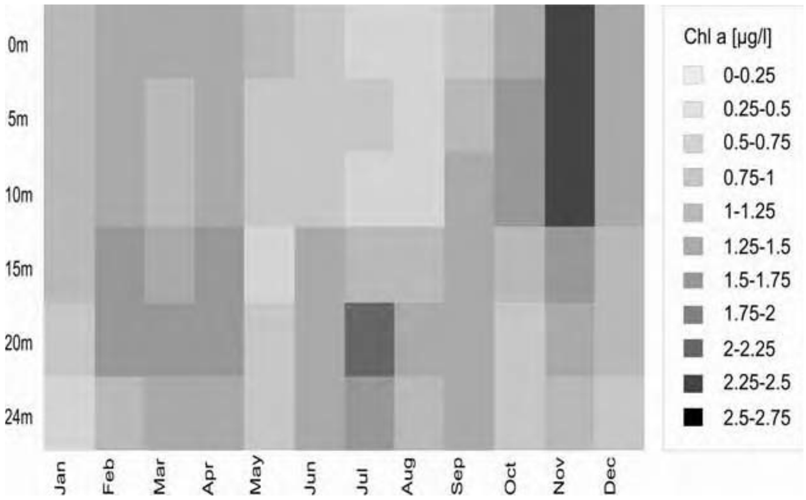

Figure 2. Mean annual pattern of Chl *a* concentrations at a selected station: vertical profiles of monthly geometric means of the time series 1989-2007.

Large inter-annual variations are also observed considering either raw time series data or yearly means (Figure 3); however, only by dividing the time series into shorter periods was it possible to detect trends. For both sampling sites, the coefficients of trend were negative and most significant only in the last decade (1998-2007): -0.09 and -0.05 µg Chl *a* L^{-1} $year^{-1}$ on stations CZ and F, respectively.

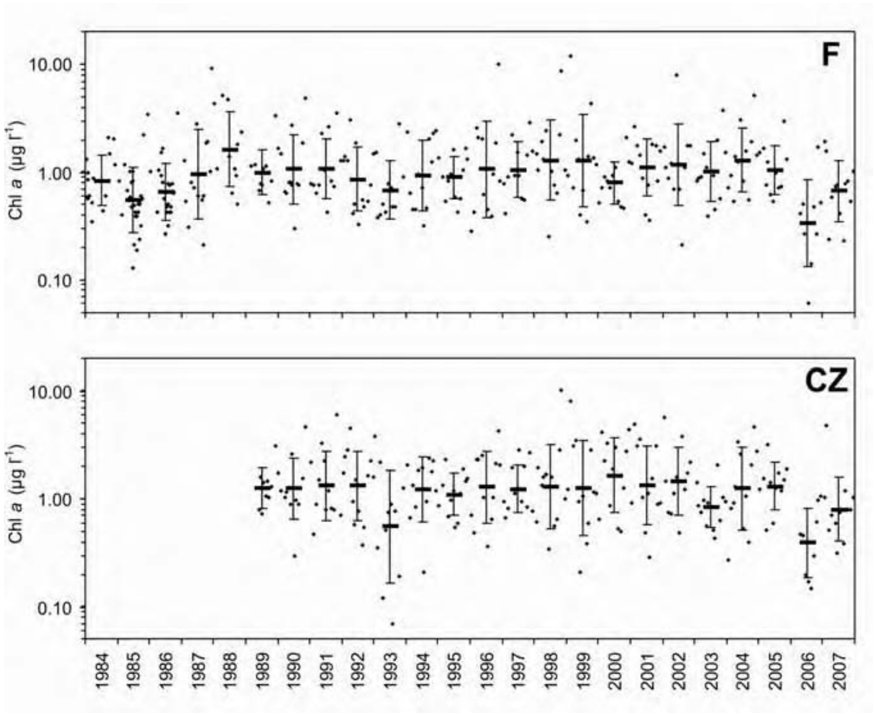

Figure 3. Time series of surface Chl *a* concentrations presented as raw data (dots) and as annual geometric means with standard deviations.

This is not an isolated case, since constantly decreasing concentrations of Chl *a* over the last 10 years in comparison with earlier decades of oscillating values were observed all over the northern Adriatic basin (Mozetič *et al.*, 2009) after applying the nonparametric Kendall-Tau-Sen test, either on field or satellite-derived data. Satellite time series of the last decade, which reproduced well field data and were unbiased by uneven sampling, showed a basin-average decrement of 0.11 µg Chl *a* L^{-1} $year^{-1}$, while zone-specific negative trends were greatest in the most eutrophic area directly affected by the Po River discharge (-0.27 µg L^{-1} $year^{-1}$), and lowest in the oligotrophic waters on the Istrian coast (-0.02 µg L^{-1} $year^{-1}$).

CHANGES IN PHYTOPLANKTON ABUNDANCE AND COMMUNITY STRUCTURE

As with biomass, phytoplankton abundance is also characterised by large variations. The mean seasonal abundance pattern, constructed from a 1989-2007 time series of surface abundances (Figure 4) slightly differs from the mean biomass pattern (see Figure 2): while both annual maxima in November overlap, the first annual peak of phytoplankton abundance extends from April to June in contrast to the Feb-Apr biomass peak. Nanoflagellates are the dominant phytoplankton group most of the time, and largely contribute to the first seasonal peak, whereas diatoms predominate only during exceptional events (outliers in Figure 4) and numerically dominate the second seasonal peak.

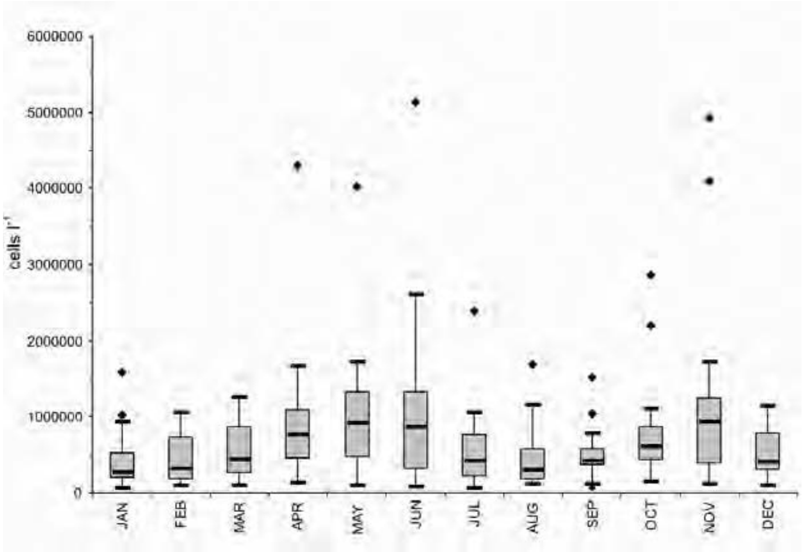

Figure 4. Mean annual pattern of total phytoplankton abundance in the surface layer at station F in the period 1989-2007.

The long-term dynamics of phytoplankton abundance and trend detected by the simple cumulative sums method (Figure 5a) show behaviour opposite to that of biomass. If the latter tends to decrease, abundance increases and moments of changes merely coincide (1998/1999). The time series can be divided into three periods that correspond to changes in the phytoplankton community. The first and longest period (1989-1998) is characterised by periodic massive diatom blooms with abundances over 2.0 x 10^6 cells L^{-1}, yet there was no positive or negative trend during this period (Figure 5b, period I). Such massive blooms were not recorded in the intermediate period (1999-2002) when diatom abundances showed a slight negative trend (Figure 5b, period II). From 2003 onwards the abundance of diatoms was higher again, without reaching the peak values of the first period. Concomitantly, a rise in nanoflagellate abundance was observed (Figure 5c, period III) causing an increase of total phytoplankton as well.

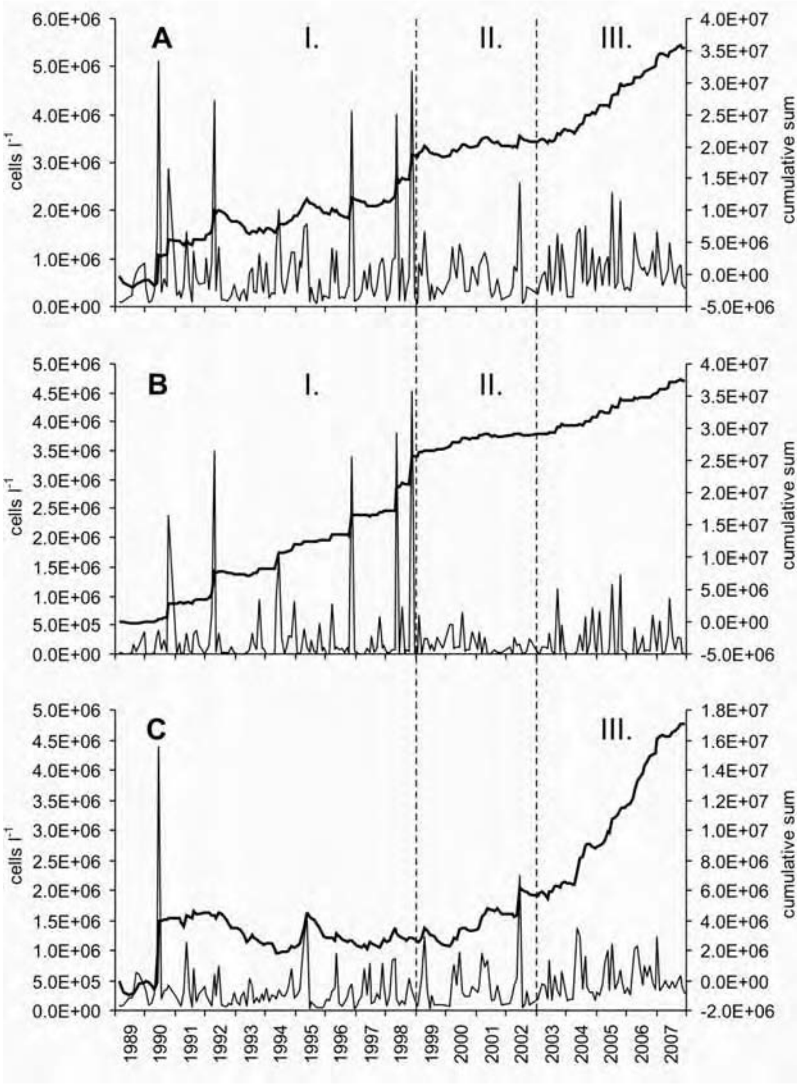

Figure 5. Time series of phytoplankton abundance (thin line) in the surface layer at station F and respective cumulative sums (thick line): (**A**) total phytoplankton, (**B**) diatoms and (**C**) nanoflagellates. Roman numerals indicate the three periods (see text for explanation).

Changes were also recorded in the occurrence pattern of some characteristic species of which the most evident is the replacement of a group of large dinoflagellates, which dominated the late spring-early summer community in the early '90s, by the typical summer assemblages of diatoms in the last few years. Similarly, the massive dinoflagellate blooms that often occurred during summer-early autumn in the '70s and '80s all over the northern Adriatic were no longer recorded from the late '80s onwards (*in* Sellner and Fonda Umani, 1999). Further evidence of change is the more frequent appearance of small-sized species of *Chaetoceros* and of *Cylindrotheca closterium* in the last three years.

LINKS TO ENVIRONMENTAL CHANGES

The recent decreasing trend in Chl *a* concentrations in the northern Adriatic has been proposed as one of the first documented cases of cultural oligotrophication (Mozetič *et al.*, 2009). This decrease parallels reductions in the outflows of the Po River (Zanchettin *et al.*, 2008) and the observed increase in nutrient limitation, especially phosphate and ammonia, in the coastal waters (Solidoro *et al.*, 2009), which is most probably due, in addition to reduced freshwater discharges, to the ban on phosphorus ordered by Italian law in the mid 1980s and the general improvement in sewage treatment.

Reductions in freshwater discharges from the beginning of this century onwards have also been observed for the Soca River (Comici and Bussani, 2007), which plays a similar role in the Gulf of Trieste to that of the Po River in the northern Adriatic. The negative trend has continued after 2005 when the study of Comici and Bussani (2007) stopped (Figure 6) and it is accompanied by increasing surface salinity. Approximately in the same period, i.e. the early 2000s, we also observed a decrease in nitrate concentrations and, to a lesser extent, of silicate, whereas those of phosphate remain notoriously limited in the area.

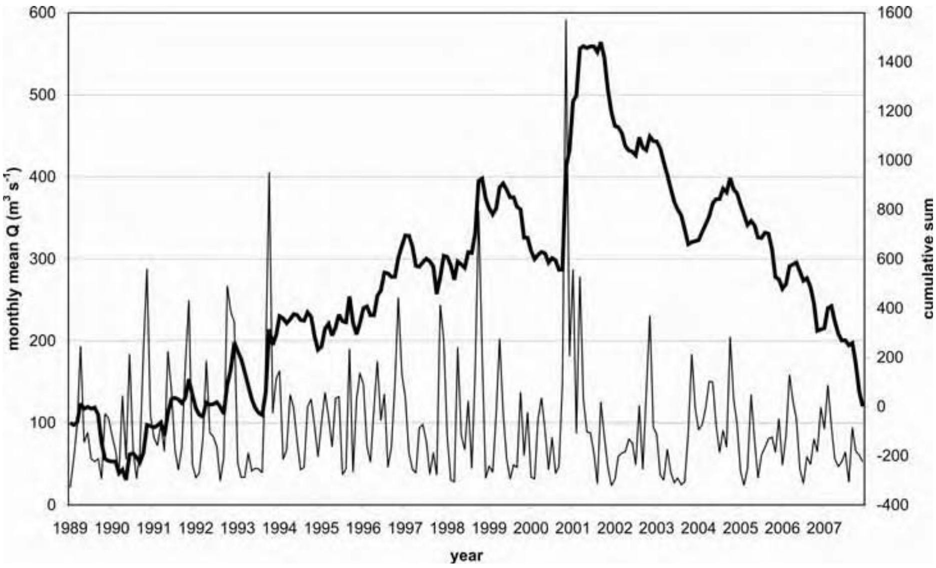

Figure 6. Monthly mean values (thin line) of Soca River flow at Solkan measuring station in the period 1989-2007 and respective cumulative sums (thick line).

The coupling of timing and magnitude of all these parameters and the subsequent cause-effect relationships among them is difficult to demonstrate due to the resilience of the system. We believe, however, that recent changes in the coastal environment and in the watershed have influenced phytoplankton: first by reducing its biomass due to limited nutrient resources and secondly, by inducing changes in the community structure through favouring smaller-sized motile organisms such as nanoflagellates of which several species are also mixotrophs. The above mentioned changes in the succession of species give additional support for the effect of decreased nutrients on the phytoplankton community.

The extent to which environmental factors other than nutrients could have influenced these changes is difficult to determine. Despite several studies, there is still no conclusive evidence of a significant warming of northern Adriatic waters over a longer period of time (Solidoro *et al.*, 2009), and indications of increasing temperature and salinity are limited either to shorter periods of time (about 10 years) or smaller areas. For instance, Malačič *et al.* (2006) have demonstrated a positive trend of sea temperature (especially in summer) and salinity in the Gulf of Trieste for the period 1991-2003, which in turn provoked a significant reduction of the vertical gradient of density in the upper 10 m. This is the reverse of the accepted understanding of the predominant role of temperature on phytoplankton, mainly in the open ocean (e.g. Falkowski and Oliver, 2007). There, the indirect role of temperature is observed through enhanced stratification and consequently reduced nutrient supply to the photic layer from below the pycnocline, possibly resulting in a decrease of phytoplankton abundance, at least of large cells (Richardson and Shoeman, 2004). Since stratification in a shallow, semi-enclosed basin such as the northern Adriatic can easily be disrupted, the importance of whether long-term sea surface warming has occurred or not becomes less relevant.

The recent increase of sea temperature (and salinity) should not, however, be ignored since its effects have been reported on higher trophic levels such as zooplankton (Conversi *et al.*, 2009) and fish (Dulčić *et al.*, 2007), which may result in a different top-down control of phytoplankton by grazers. Last but not least, a warm-water dinoflagellate species of Indo-Pacific origin, *Ceratoperidinium yeye*, whose first and only appearance was recorded in the Gulf of Trieste in the extremely hot and dry summer of 2003 (Francé and Mozetič, 2008), reappeared in August 2009 when temperatures were above 28°C. However, the extremely low abundance of the species (< 20 cells L^{-1}) do not imply, for the moment, a major role in the community.

Recent changes in the phytoplankton community of the Adriatic Sea

Ivona Marasović, Živana Ninčević-Gladan, Branka Grbec and Sanda Skejić

Institute of Oceanography and Fisheries, Split, Croatia

ABSTRACT

Since the early 1960s, regular monthly observations of phytoplankton abundance, the structure of the phytoplankton community, and primary production have been carried out at two stations in the middle Adriatic which are influenced to varying degrees by coastal and open Adriatic waters. These long-term data series have been elaborated in relation to the impacts of anthropogenic eutrophication and climatic changes. Our analysis shows an increase of phytoplankton biomass and abundance in the period from the mid-1980s to the mid-1990s. The shifts in primary production have been connected with hemispheric and regional scale climate variations, and support the hypothesis that atmospheric variability can trigger ecosystem changes.

INTRODUCTION

Marine ecosystem changes provoked by climatic variability are a focus of very high interest for marine scientists. Changes in climate over the last few decades have already affected the coastal and open sea ecosystems. Most of the shifts in the marine ecosystem are attributed to changes in sea temperature, salinity and circulation controlled by the regional atmospheric variations and large-scale teleconnections. The eastward shift of the northern hemispheric sea level pressure pattern around 1980 (Hilmer and Jung, 2000), a consequence of the increasing trend of the North Atlantic Oscillation index during the last decades of the 20th century (Peterson *et al.*, 2003), causes hydro-climatic changes in the Mediterranean, including the Adriatic. Numerous studies provide evidence that changes in primary production, plankton abundance and community structure are related to climate changes (Edwards *et al.*, 2002; Yunev *et al.*, 2007; Möllmann *et al.*, 2008; Katara *et al.*, 2008). The best-studied pattern of atmospheric variability in our region is the North Atlantic Oscillation (NAO) (Hurrel, 1995), which has been related to changes in phytoplankton biomass (Reid *et al.*, 1998; Barton *et al.*, 2003), to primary production, and to toxic algal blooms (Belgrano *et al.*, 1999). Phytoplankton, because of its short generation time and sensitivity to temperature variations, is a very good target for studies of the response of marine ecosystems to changes in the climate system.

Analyses of the long-term data series of physical, chemical and biological parameters from the Adriatic also show significant interrelated variability (Marasović *et al.*, 1995; Grubelić *et al.*, 2004; Grbec *et al.*, 2007). The observed increase of primary production in the Adriatic by the end of the 1970s was first attributed to increase of eutrophication (Pucher-Petković and Marasović, 1988). However, more recent investigations suggest that the increase of primary production may also be related to climate changes (Marasović *et al.*, 2005). Both, continuous and abrupt climate changes and shifts have been recognized as key factors which control variations in the pelagic ecosystem (Grbec *et al.*, 2008).

Since the early 1960s, regular monthly observations of basic hydrographic, chemical and biological parameters have been carried out at two middle Adriatic stations, which are under contrasting coastal and open sea influences (Fig. 1). The first station is located in Kaštela Bay (KBS), one of the largest and most eutrophic bays on the eastern Adriatic coast. Water depth ranges from 50 m at its deepest to between 10 and 25 m at its shallowest part. Water exchange with the adjacent basin is mainly wind driven (Gačić, 1980; Gačić *et al.*, 1987). The average water renewal time is about one month, while under strong wind conditions it can be as short as five days (Zore-Armanda, 1980). The narrow coastal strip of the bay is a highly developed area where urban and rural populations total about 350,000. As intensive industrialization and urbanization of this area (the population increased six fold from 1950 to 1990) have not been accompanied by an adequate development of necessary urban infrastructure, large quantities of urban and industrial waste waters are released untreated into the Bay. Increasing effluent discharges have brought considerable changes to the Bay ecosystem, resulting in changes in the abundance and diversity of phytoplankton (Pucher-Petković, 1976; Pucher-Petković and Marasović, 1980), increase of primary production (Marasović *et al.*, 1995), occurrences of red tide, “mucillagine“, temporary oxygen depletion, mortalities of fish and shellfish, and DSP toxicity of mussels (Marasović, 1989; Marasović *et al.*, 1991; Marasović *et al.*, 1998; Šolić *et al.*, 1997).

Figure 1. Area studied with sampling locations.

The second monitored station (STS) is located in the open part of the middle Adriatic over Palagruža sill (Fig. 1). This part of the middle Adriatic is strongly influenced by incoming Mediterranean water masses known as Levantine Intermediate Water (LIW) (Zore-Armanda, 1963; Artegiani *et al.*, 1993). Even though this station is not affected by land derived materials, increased primary production was observed in the 1980s (Pucher-Petković and Marasović, 1988).

The rate of primary production in the coastal area (station KBS) is approximately double than at the open sea (STS). In spite of the strong anthropogenic influences in the coastal area, the fluctuations at both stations show a significant degree of correspondence, with a correlation coefficient between monthly primary production values of 0.37 ($P<0.001$) (Grbec *et al.*, 2009).

Investigations of Buljan (1957) and Buljan and Zore-Armanda (1976) have shown that the eastern middle Adriatic region is occasionally exposed to strong Mediterranean water inflow. The inflow takes place in the intermediate layer (100 - 400 m depth) of the Otranto Strait, and protrudes northwards to the shelf break of the Palagruža sill (Middle Adriatic) where it bifurcates; one branch is deflected southwards while the remaining part continues towards the north (Manca and Giorgetti, 1999). The basic properties of the inflowing water mass are high salinity ($S > 38.80$ psu), and elevated temperature (Θ = 14.0 °C) (Tzipperman and Malanotte-Rizzoli, 1991; Manca and Giorgetti, 1999), and phosphate concentrations (Buljan and Zore-Armanda, 1979) with respect to the Adriatic waters. Based on its origin, the water mass is called Levantine intermediate Water (LIW). The latter is suggested by the studies of Roether *et al.* (1996) and Klein *et al.* (1999), who link the recent changes in the thermohaline circulation in the eastern Mediterranean to the occurrence of new deep water in that area (Cretan Sea Overflow Water; S>38.8 psu). Its inflow into the bottom layers of the Ionian and Levantine Basins causes upwelling of the nutrient-rich deep water to the upper water column, which could explain the phosphate increase recorded in the middle Adriatic. The outflow of Adriatic water to the Mediterranean occurs along the western coast, and is supported by the Etesian wind (Zore-Armanda, 1969). More detailed oceanographic properties and circulation characteristics for the Adriatic and its sub-basins are given by Franco and Michelato (1992); Orlić *et al.* (1992); Artegiani *et al.* (1993), Kovačević *et al.* (1999). The Adriatic shows strong interannual variability of temperature and salinity caused by the presence of Levantine Intermediate Water (LIW) in the intermediate layer of the eastern coast (Vilibić and Orlić, 2002).

RESULTS AND DISCUSSION

On the basis of our long-term data series, the primary production of Kaštela Bay was estimated about 430 mg C m^{-2} day^{-1} (Pucher-Petković and Zore-Armanda, 1973). Based on a general classification of the world seas into five categories of productivity (Koblentz-Mishke *et al.*, 1970), Kaštela Bay was categorised as productivity category four. At the same time, the open middle Adriatic water productivity was estimated at about 150 mg C m^{-2} day^{-1}, i.e. somewhere between categories two and three (Karlovac *et al.*, 1974). Primary production has continued to increase steadily, with minor fluctuations, exceeding 600 mg C m^{-2} day^{-1} by the beginning of the 1980s (Pucher-Petković and Marasović, 1988) and placing Kaštela Bay in production category five. An analysis of data for the period 1982-1996 showed a further increase, to over 700 mg C m^{-2} day^{-1} (Fig. 2).

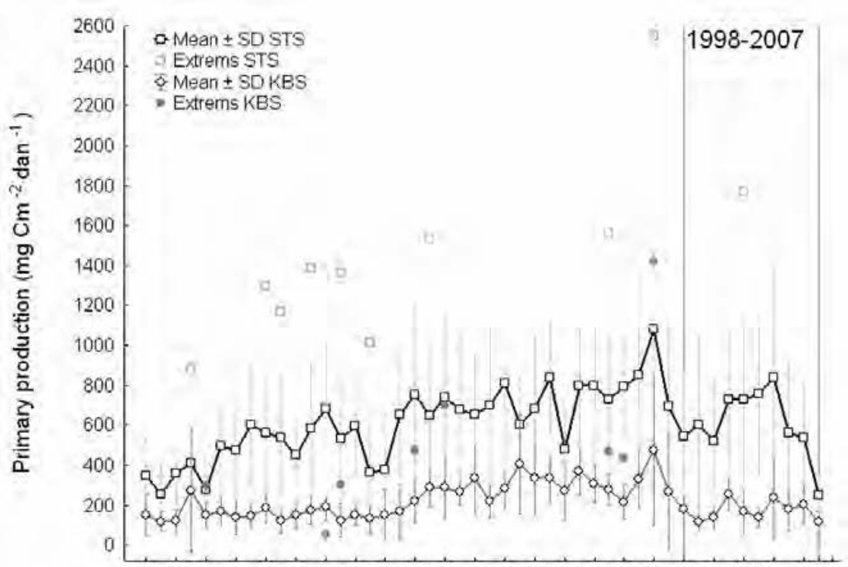

Figure 2. The mean annual values of primary production (mg C m^{-2} d^{-1}) in Kaštela Bay (KBS) and open sea (STS-Stončica) during period of 44 years.

The observed increase in primary production was not gradual, but characterised by frequent occurrences of exceptionally high values (>1,000 mg C m^{-2} day^{-1}) (Fig. 3). The increase in primary production coincided with changes in the seasonal cycle of phytoplankton in summer, rather than spring, so the summer maximum in phytoplankton abundance became dominant (Fig. 4). The first occurrence of a red tide, in summer 1980, was accompanied by a fish kill (Marasović and Vukadin, 1982). During the 1980s, red tides were recorded on several occasions and were always due to intensive blooms of *Gonyaulax (Lingulodinium) polyedra* Stein (Marasović, 1989; Marasović *et al.*, 1991). A comparative analysis of the structure of the phytoplankton community from summer 1975 to summer 1984 (Marasović and Pucher-Petković, 1991) shows a significant increase in the presence of dinoflagellates in relation to diatoms, whereas the winter structure of the Kaštela Bay phytoplankton community showed no changes (Fig. 5). Analysis of the diatom/dinoflagellate ratio to primary production in the warmer part of the year (May - October) over a period of twenty years, shows that the proportion of dinoflagellates in the phytoplankton community increased coincidentally with primary production (Fig. 6). It is apparent that the maxima in primary production coincided with the minima in diatom/dinoflagellate ratio, that is, a higher proportion of dinoflagellates. Up to the beginning of the 1990s, the environmental changes in Kaštela Bay were mainly attributed to substances added to the sea by man. However, this view started to be questioned at the beginning of the 1990s, when the results of analyses for the open Adriatic showed similar changes. The increase in primary production in the open Adriatic occurred with a lag of about 10 years behind that in Kaštela Bay (Fig. 2). As in Kaštela Bay, increased production in open waters was also caused by frequent occurrences of high productivity which was quite unusual for the open waters (Fig. 7).

Pucher-Petković and Zore-Armanda (1973) had already observed that temporary increases of the middle Adriatic primary production were highly affected by so-called "Adriatic ingressions" (Buljan, 1957), which are increased inflows of south Adriatic and/or Mediterranean water into the middle Adriatic. Water coming from the southern Adriatic contains higher quantities of nutrients. Since intensified inflow of the south Adriatic water depends on meteorological factors (Zore-Armanda, 1963; 1969), it is possible that global climatic changes have affected the frequency of "Adriatic ingressions". Global warming of the air and ocean (Béthoux *et al.*, 1990; Bindof and Church, 1992) could be another cause of increased primary production in the middle Adriatic, since higher temperatures positively affect photosynthetic processes.

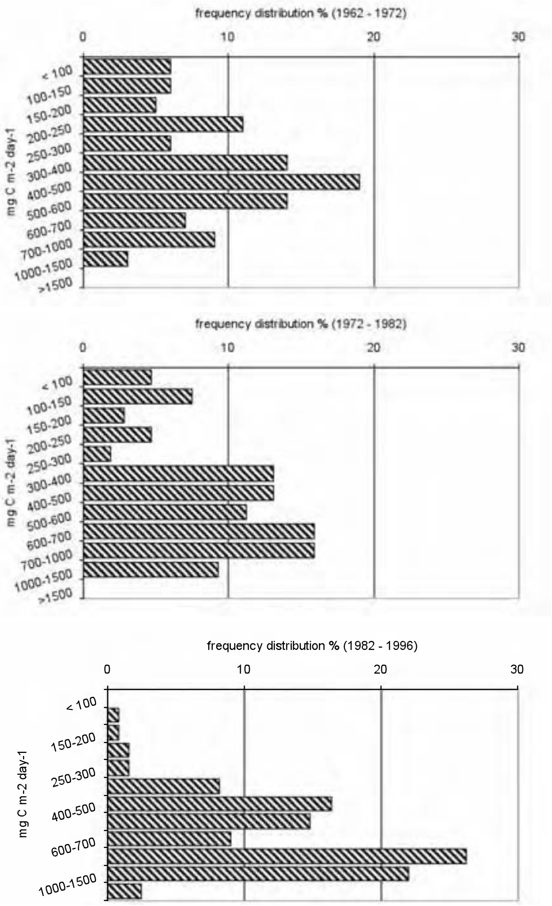

Figure 3. The frequency distribution of primary production values (mg C m^{-2} d^{-1}) recorded in Kaštela Bay during the three periods investigated (Marasović *et al.*, 2005).

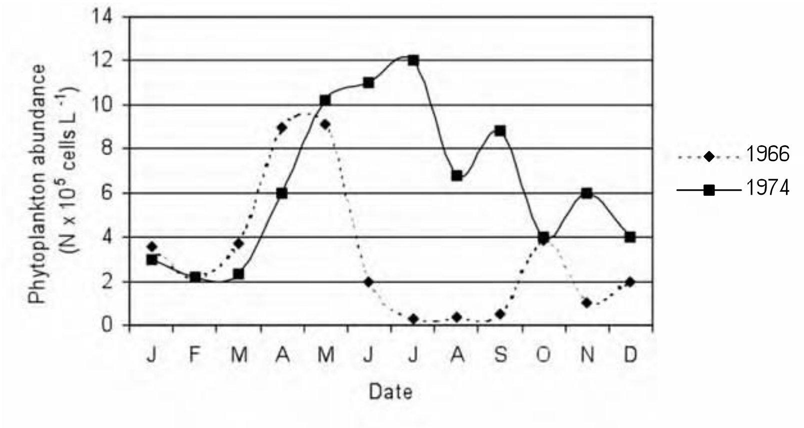

Figure 4. Comparison of phytoplankton seasonal cycles (N cells dm^{-3}) recorded in Kaštela Bay in 1966 and 1974 (Marasović *et al.*, 2005).

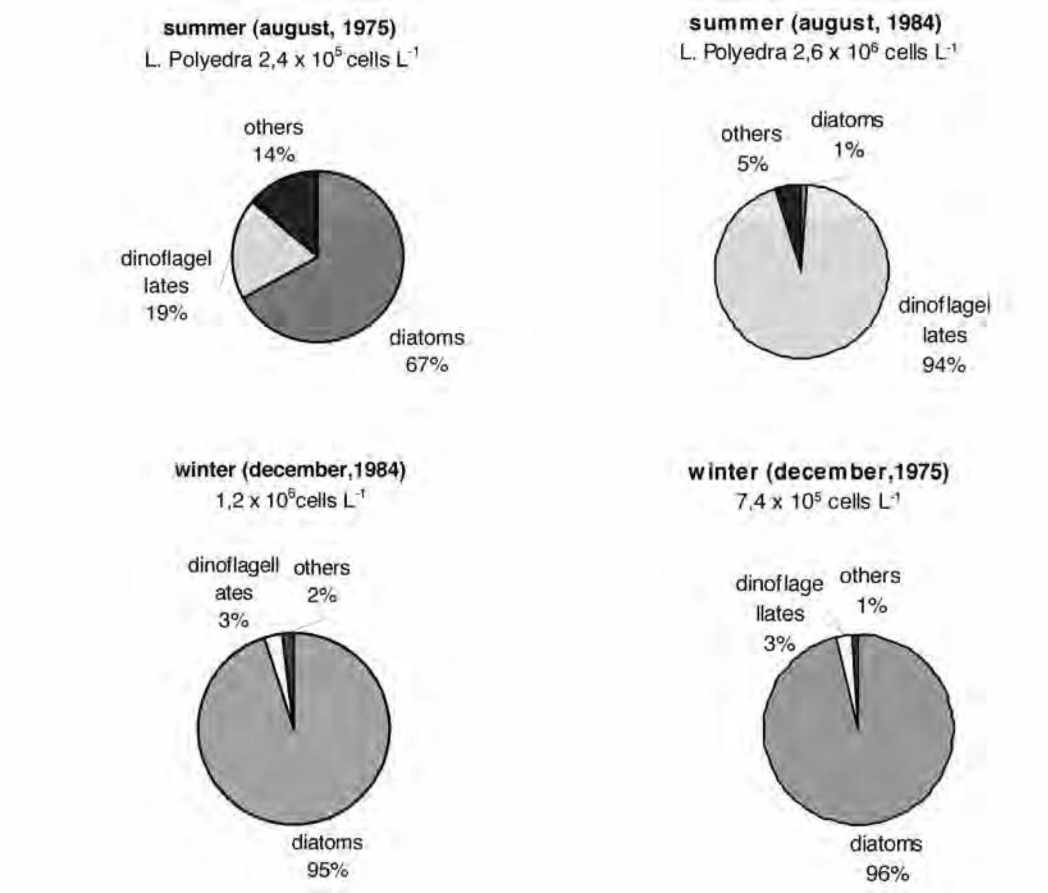

Figure 5. Phytoplankton community structure in Kaštela Bay during the summer and winter period in 1975 and 1984 (Marasović *et al.*, 2005).

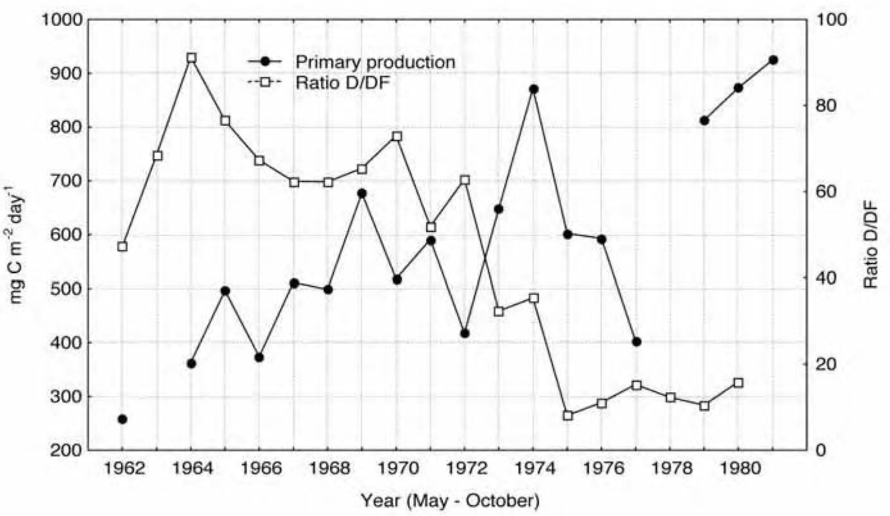

Figure 6. Ratio between diatom and dinoflagellate abundance (D/DF) and primary production (mg C m^{-3} d^{-1}) in Kaštela Bay (six month mean values May-October) (Marasović *et al.*, 2005).

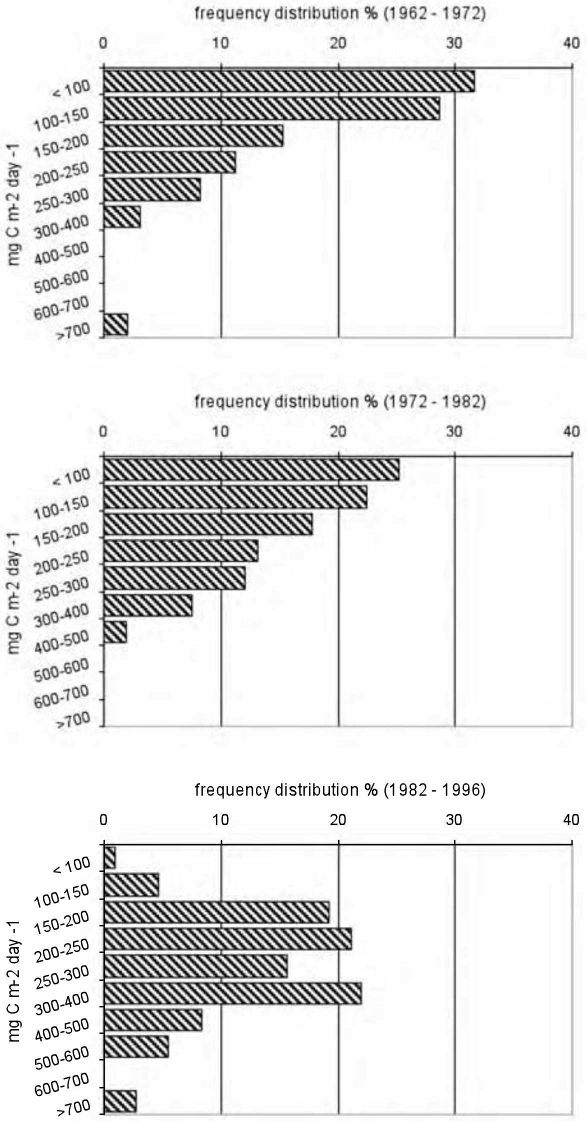

Figure 7. The frequency distribution of primary production values (mg C m^{-3} d^{-1}) recorded at the open sea station (STS) of the middle Adriatic during the period of 44 years (Marasović *et al.*, 2005).

Brankart and Pinardi (2001) proposed that, at the beginning of the 1980s, larger quantities of east Mediterranean water entered the south Adriatic, and caused a strong increase in salinity in the north Aegean Sea and south Adriatic. There is a high probability that this strong intrusion of east Mediterranean water is strongly connected with the abrupt shift of primary production in the middle Adriatic in the period from 1980 to 1996 (Marasović *et al.*, 1995; 2005; Grbec *et al.*, 2009).

Even though all the relevant data point to the fact that the changes in Kaštela Bay are closely related to an increase of anthropogenic nutrient loading, similar changes in the open sea suggest that the increase in primary production in the Bay might be partly due to global climatic changes. According to our results, changes in phytoplankton community composition, as well as the changes in seasonal cycles of phytoplankton in Kaštela Bay are mostly related to anthropogenic influences, while increased primary production in the Bay is a consequence of interaction between coastal eutrophication and general changes occurring in the Mediterranean and Adriatic Sea open waters (Marasović *et al.*, 2005). Fig. 8 shows a strong connection between the NAO index and primary production in the middle Adriatic. Primary production showed an inverse relation with the NAO winter index depending on the season and time series period (Grbec *et al.*, 2009; Ninčević Gladan *et al.*, 2010). Primary production in May was negatively correlated with the NAO winter index during the entire investigated period (Fig. 8; Table 1). Primary production in the winter period (December and January) showed a positive correlation with the NAO winter index (Fig. 8; Table 1).

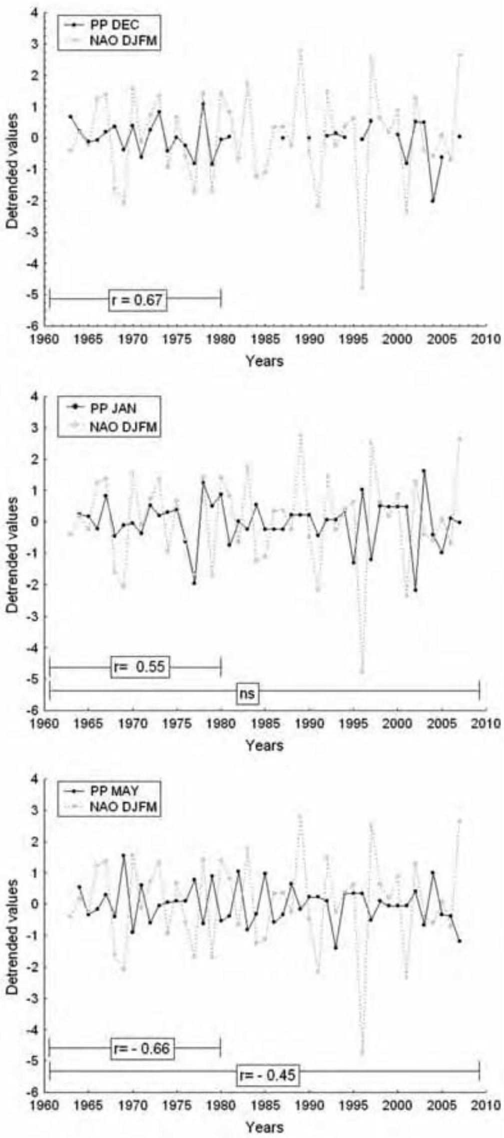

Figure 8. Time series of NAO winter index and primary production (PP) in December, January and May, with Spearman R correlation coefficients at a significance level p<0.05 (Ninčević Gladan *et al.*, 2010).

Table 1. Spearman R correlation coefficients between NAO winter index (DFJM) and primary production (PP) in December, January and May, and between NAO winter index (DFJM) and Diatom/Dinoflagellate ratio (D/DF) in May.

	NAO winter index
PP (December) 1962-1980	0.63 (p=0.005; N=18)
PP (December) 1962-2007	0.46 (p=0.002; N=45)
PP (January) 1962-1980	0.54 (p=0.026; N=17)
PP (May) 1962-1980	-0.68 (p=0.002; N=17)
PP (May) 1962-2007	-0.44 (p=0.003; N=44)
D/DF May 1962-2007	-0.25 (p=0.099; N=44)

Diatoms were the most abundant functional group in Kaštela Bay. In the period from 1956 to 1970, increasing abundances of diatoms were observed and remained relatively constant until the mid-1980s (Fig. 9). Time series of phytoplankton group abundances, particularly dinoflagellates, showed increased abundances in the period from the mid-1980s to the mid-1990s (Marasović *et al.*, 2005; Ninčević Gladan *et al.*, 2010) (Fig. 9). Diatom prevalence was established again after 1997. The same trend has been observed in the northern Adriatic (Kamburska and Fonda-Umani, 2009).

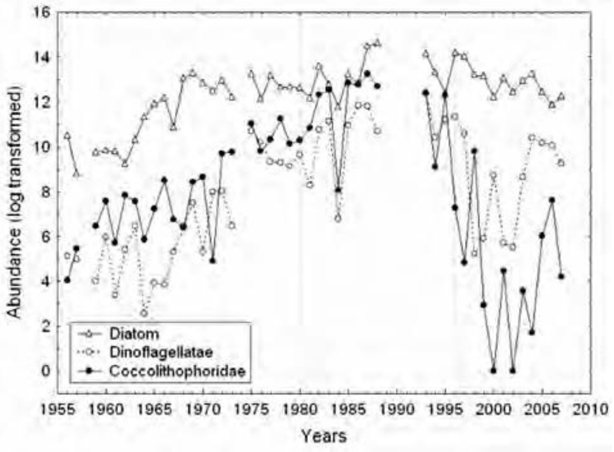

Figure 9. Time series of log(x+1) transformed abundances of the main phytoplankton functional group (Ninčević Gladan *et al.*, 2010).

"Spread of potentially toxic benthic dinoflagellates in the Mediterranean Sea: a response to climate change?"

Katerina Aligizaki

Department of Botany, Aristotle University of Thessaloniki, Greece

ABSTRACT

Records of toxic benthic dinoflagellates are increasing along the Mediterranean coasts, especially during the last decade. On the western coasts, *Ostreopsis ovata* blooms have been associated with human health problems due to palytoxin-like (p-PLT) toxin production, whereas, on the eastern Mediterranean (Greek) coasts, the presence of dense *Ostreopsis* spp. populations has resulted in shellfish contamination by similar (p-PLT) compounds. Furthermore, the recent detection of dinoflagellates once considered as tropical, such as the ciguatera causing genus *Gambierdiscus*, raises issues regarding changes in the biogeographical distribution of tropical benthic dinoflagellates. The possible relationship of climate and other relevant environmental changes to the increased records and intense blooms of temperate benthic dinoflagellates, and the detection in the Mediterranean of species previously considered tropical is discussed.

INTRODUCTION

Benthic dinoflagellates are characterized as a group of special interest since they comprise a high number of toxic and/or potentially toxic species (Yasumoto *et al.*, 1980b; Nakajima *et al.*, 1981). The biogeographical distribution of the majority of benthic dinoflagellate species was, until recently, limited mainly to tropical and subtropical areas, especially to coral reef ecosystems (Besada *et al.*, 1982; Faust, 1991; 1995; Faust *et al.*, 1996; Chinain *et al.*, 1999a; Chang *et al.*, 2000; Rhodes *et al.*, 2000; Pin *et al.*, 2001), while some others are known from temperate or even cold waters (Meunier, 1919; Hoppenrath and Leander, 2008).

Regarding their toxicity, it is worth pointing out that species of the genus *Ostreopsis* are capable of producing palytoxin analogs, with palytoxin being one of the most potent biologically synthesized marine toxins (Usami *et al.*, 1995; Ukena *et al.*, 2001; Taniyama *et al.*, 2003; Katikou, 2008); humans are affected with poisonous or even fatal consequences after consumption of fish contaminated with palytoxin and/or analogs, as in the case of clupeotoxism (Onuma *et al.*, 1999). Additionally, toxins produced by the benthic dinoflagellate genus *Gambierdiscus* (Yasumoto *et al.*, 1977; Bagnis *et al.*, 1980) are responsible for ciguatera, a disease that causes illness to more than 50,000 people each year after fish consumption, mainly in circumtropical areas (Glaziou and Legrand, 1994; Lewis, 2001).

BENTHIC DINOFLAGELLATES IN THE MEDITERRANEAN SEA BEFORE THE YEAR 2000

Prorocentrum lima was described from the Gulf of Sorrento in Italy (Ehrenberg, 1860) and *C. monotis* has been detected in the Mediterranean Sea since the early '60s (Halim, 1960a) following its initial description from North European waters (Meunier, 1919). On the contrary, the majority of the representatives of the toxigenic benthic dinoflagellate genera *Ostreopsis, Prorocentrum* and *Gambierdiscus* have been considered inhabitants mainly of tropical areas.

The first records of *Ostreopsis* species in the Mediterranean Sea go back to 1972 (Taylor in Zingone *et al.*, 2006) and 1979 (Taylor, 1979; Abboud-Abi Saab, 1989) on both the western and the eastern coasts. In the next two decades (1980-2000), records were relatively limited and concerned mainly the Italian coasts (for details see Table 1 *in* Zingone, this volume), while in some cases high cell densities were reported (Tognetto *et al.*, 1995). On the other hand, *Gambierdiscus* had never been detected, neither on the northern and more studied coasts nor in the southern and warmer areas of the Mediterranean; this absence was often used to discriminate benthic dinoflagellate assemblages of the Mediterranean Sea from similar ones in circumtropical areas (BENTOX-NET, 2007; Ismael and Halim, 2007).

BENTHIC DINOFLAGELLATES IN THE MEDITERRANEAN AFTER THE YEAR 2000

During the last decade, records of *Ostreopsis* spp. have increased rapidly on both western (Vila *et al.*, 2001c; Masó *et al.*, 2003; Sansoni *et al.*, 2003; Turki, 2005; Turki *et al.*, 2006; Zingone *et al.*, 2006; Monti *et al.*, 2007; Mangialajo *et al.*, 2008) and eastern Mediterranean coasts (Aligizaki *et al.*, 2004; Aligizaki and Nikolaidis, 2006; Ismael and Halim, 2006; Aligizaki *et al.*, 2009a). Furthermore, *Ostreopsis* spp. (*O. ovata* & *O.* cf. *siamensis*) (Penna *et al.*, 2005) are not only detected in more geographical areas inside the Mediterranean Sea, but they also form intense blooms, reaching abundances comparable or even higher to those of tropical areas (Vila *et al.*, 2001c; Aligizaki and Nikolaidis, 2006; Mangialajo *et al.*, 2008). Recently, *C. monotis* was found proliferating in Tunisian waters (Armi *et al.*, 2009); in this latter case, the cell densities of *C. monotis* were quantified in water column samples, while high epiphytic abundances of this species have been recorded in Greek and Spanish waters (Vila *et al.*, 2001c; Aligizaki and Nikolaidis, 2006).

Coolia monotis strains from the Mediterranean Sea have been shown not to produce toxins (Penna *et al.*, 2005; Aligizaki, 2008; Aligizaki *et al.*, 2009a), unlike strains from Australia that have been found to produce cooliatoxin (Holmes *et al.*, 1995). On the other hand, Mediterranean *Ostreopsis* strains are capable of producing palytoxin analogs (Ciminiello *et al.*, 2006; Riobó *et al.*, 2006; Aligizaki *et al.*, 2008b; Ciminiello *et al.*, 2008; Riobó *et al.*, 2008; Ledreux *et al.*, 2009), as has been shown for *Ostreopsis* spp. from other areas (Usami *et al.*, 1995; Rhodes *et al.*, 2000; Taniyama *et al.*, 2003; Lenoir *et al.*, 2004).

However, *Ostreopsis* toxicity is expressed differently in the Mediterranean compared to circumtropical areas, where palytoxin originating from *Ostreopsis* cells has been associated with human poisonings or even death after fish consumption (Onuma *et al.*, 1999). In Greek waters, bivalve mollusc (mussels, clams, hairy mussels) tissues have been found contaminated with putative palytoxin (Aligizaki *et al.*, 2008b), but without any human poisoning after shellfish consumption. This fact can be attributed to the restriction of fisheries and commercial activities implemented by the appropriate authorities based on the results of the Greek Monitoring Program on Marine Biotoxins (National Reference Laboratory on Marine Biotoxins, Laboratory Unit for Toxic Marine Microalgae-AUTH). In contrast, on western Mediterranean coasts, and especially along Italian coasts, *Ostreopsis ovata* blooms have been associated with health problems (respiration, skin and eye irritations, fever) in swimmers (Brescianini *et al.*, 2006; Ciminiello *et al.*, 2006) resulting in bans on swimming and closure of several kilometres of coastline. Both expressions of *Ostreopsis* toxicity in the Mediterranean have direct consequences not only for human health but also for socioeconomic activities, since they afflict fisheries, aquaculture and tourism.

Apart from the obvious increase of *Ostreopsis* spp. records in the Mediterranean Sea, another important issue is the detection of species formerly considered as tropical. Species of the ciguatera causing *Gambierdiscus*, as well *Prorocentrum borbonicum, P. levis* and *Sinophysis canaliculata*, have very recently been recorded in this area, and specifically in Greek coastal waters (Aligizaki and Nikolaidis, 2008; Aligizaki *et al.*, 2008a; Aligizaki *et al.*, 2009b).

Based on these findings, the first record of *Gambierdiscus* sp. in the Mediterranean goes back to 2003 in Crete (Figure 1), indicating a northward expansion of *Gambierdiscus* “entering” the Mediterranean basin (Aligizaki and Nikolaidis, 2008). Prior to this record, the northernmost one concerned the Canary Islands in the NE Atlantic Ocean (Fraga *et al.*, 2004; Aligizaki *et al.*, 2008a). More recently (February 2009), *Gambierdiscus* was recorded in Saronicos Gulf, Salamina Island (Figure 1), at a latitude of about 38 ºN extending the northern geographical boundaries of *Gambierdiscus* distribution even further within the Mediterranean basin (Aligizaki *et al.*, 2009a).

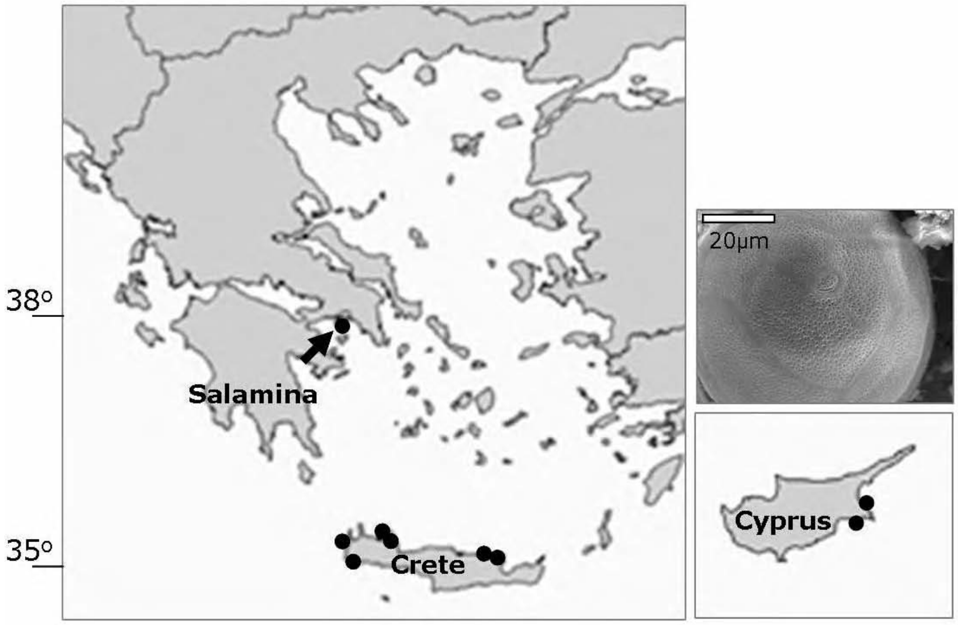

Figure 1. SEM micrograph and distribution of *Gambierdiscus* (KC81ALCCAUTH) in Greece and Cyprus. Arrow indicates the current northernmost record for the Mediterranean Sea.

Regarding the taxonomic identity of *Gambierdiscus* from the Mediterranean, it is possible that more than one species is present; in this context, several strains have been isolated, and morphological and molecular analyses are in progress, in view of recent new species descriptions and reinvestigation of the type species *G. toxicus* (Tester *et al.*, 2006; Litaker *et al.*, 2009). It is worth noting that preliminary toxicity analyses on two *Gambierdiscus* strains isolated from Greek waters indicated low toxicity compared to other strains from Malaysia and the Canary Islands based on mouse bioassay and cytotoxicity tests (Aligizaki *et al.*, 2009a; Caillaud *et al.*, 2009).

IS CLIMATE CHANGE RESPONSIBLE FOR BENTHIC DINOFLAGELLATES EXPANSION IN THE MEDITERRANEAN SEA?

The increased number of potentially toxic benthic dinoflagellates in the Mediterranean could reflect the apparent global increase of harmful algal blooms (Hallegraeff, 1993). Scientific interest in the impacts of climate variability on harmful algal blooms, and on marine ecosystems in general, is increasing (Navarra *et al.*, 2007; Moore *et al.*, 2008; Paerl and Huisman, 2008; Rabalais *et al.*, 2009; Shears and Ross, 2009; Valdes *et al.*, 2009). Regarding benthic dinoflagellates and their impact on human health, most surveys have focused on the link between climate change and ciguatera caused by *Gambierdiscus*-toxins (Hales *et al.*, 1999; Hales *et al.*, 2000; Llewellyn, 2010). Most authors suggest the possible spread of ciguatera to formerly non-endemic areas due to geographical expansion of its causative agent (*Gambierdiscus* spp.).

The fact that *Gambierdiscus* detection in the Canary Islands (Fraga *et al.*, 2004) was followed by ciguatera incidents in the same area (Pérez-Arellano *et al.*, 2005), indicates the potential hazard for future occurrence of ciguatera in the Mediterranean, since *Gambierdiscus* has been a Mediterranean inhabitant for at least six years (Aligizaki and Nikolaidis, 2008). But against this possibility is the fact that the first Mediterranean *Gambierdiscus* strains examined have shown low toxicity (Aligizaki *et al.*, 2009a; Caillaud *et al.*, 2009).

It is worth noting that suspected cases of ciguatera have been reported in the Mediterranean (Israel coast) in 1971-1972 (Herzberg, 1973) and 1988 (Spanier *et al.*, 1989), but they have not been associated with *Gambierdiscus* species. However, the latter report was questioned by Chevaldonné (1990), who suggested the possibility of misinterpretation of this case with "*Caulerpa* poisoning". Recently, two cases of another type of ichthyosarcotoxism, ichthyoallyeinotoxism, were described and distinguished from ciguatera poisoning based on the typical clinical signs and symptoms (De Haro and Pommier, 2006). The causative agents of ichthyoallyeinotoxism are still unknown, although representatives of the family Caulerpaceae have been suspected (Chevaldonné, 1990). Furthermore, the presence of ciguatoxin-like substances in edible fish of the eastern Mediterranean has been suggested (Bentur and Spanier, 2007). However, the aforementioned authors point out the necessity for additional analyses in order to determine whether these substances are ciguatoxins or related polyethers.

The detection of tropical representatives of *Gambierdiscus, Prorocentrum* and *Sinophysis* in the Mediterranean may represent biological signs of climate change in this area; this accords with discussion of "tropicalization" of the Mediterranean (Bianchi, 2007). In this context, the possibility of geographical expansion of tropical microalgae, such as *Gambierdiscus*, due to climate change was somehow "foreseen" (Fraga, 2007).

However, climate change is not a new issue; the climate has always been changing on scales of million of years and shorter times. But what is new during the last decades is the anthropologically induced component of global warming. Regardless of the mode of climate change, temperature range alterations may have an impact on microalgae, since each species/taxon has a preferred temperature range in which it can survive or even bloom. In this context, it can be suggested that the gradual increase in sea surface temperature in the Mediterranean (Salat and Pascual, 2002) facilitated the acclimation and settlement of such tropical species in this area, as in the case of other marine organisms (e.g. mollusca, fish, macroalgae) (Occhipinti-Ambrogi, 2007).

Furthermore, taking into account that *Ostreopsis* species had already been recorded in the Mediterranean Sea since the 1970s (Taylor, 1979), but did not form enhanced blooms until the last decade, it can be proposed that even a small-scale shift in the surface temperature may have increased prevalence of the optimal growth conditions for these species.

Additionally, temperature alterations might trigger a series of changes in parameters, such as stratification, upwelling, freshwater run-off and cloud cover, which affect microalgal communities (Dale *et al.*, 2006). Diatoms can be disadvantaged due to silicate limitation that results from limited rainfall and freshwater run-off, while flagellates (e.g. dinoflagellates, raphidophytes) are favoured in stratified conditions against diatoms due to their vertical migration ability (Anderson and Stolzenbach, 1985; Levandowsky and Kaneta, 1987). In the same context, it has been shown experimentally, with all the restrictions that laboratory conditions can have, that climate change in the North Sea will increase the risk of harmful dinoflagellate and raphidophyte blooms (Peperzak, 2003).

However, the role of the climate change focuses mainly on the prevalence or otherwise of conditions that permit the organisms to survive and establish viable populations; environmental changes are not responsible for organisms' transport itself. Thus, when it comes to tropical taxa in the Mediterranean, it is tempting to try to understand how they were at first transferred into this area.

Microalgae are known to be transported in several ways (e.g. ships' ballast waters, aquaculture products, etc) mainly in the form of resting cysts (Hallegraeff *et al.*, 1988; Hallegraeff and Gollasch, 2006). Information on cyst formation in benthic dinoflagellates is limited (Pearce *et al.*, 2001; Aligizaki and Nikolaidis, 2006), but this does not exclude the fact that they may form cysts. Nevertheless, benthic dinoflagellates are able to "travel" attached to floating material, e.g.

macroalgae or even plastic debris (Bomber *et al.*, 1988; Masó *et al.*, 2003). Thus, in the case of *Gambierdiscus*, it may have invaded the Mediterranean along with "alien" macroalgae, which are increasing in Mediterranean waters (Galil, 2000; Tsiamis *et al.*, 2008).

However, *Gambierdiscus* most probably invaded the Mediterranean at a specific, though unknown, time; this hypothesis is based on the fact that research on benthic dinoflagellates was underway some years before its finding, not only in the northern Mediterranean countries (e.g. Spain, Italy, France), but also in the warmer southern areas (e.g. Egypt, Tunis, Lebanon), and *Gambierdiscus* cells are usually noticeable due to their large size and characteristic shape. Furthermore, the limitations that usually arise when examining "microalgal invasions" due to taxonomic ambiguities do not apply in the case of *Gambierdiscus* since no taxon assigned to this genus had previously been observed in the Mediterranean.

CONCLUDING CONSIDERATIONS

It is apparent that records of benthic dinoflagellates in the Mediterranean have increased in recent years, in number of species, abundance, and geographical distribution. This situation is most probably related to climate change, but many issues have to be examined to come to accurate conclusions. It cannot be ruled out that the increasing records of benthic dinoflagellates in recent years reflect intense research in this field, especially in temperate areas (Shears and Ross, 2009).

Regardless of how and why, potentially toxic and/or toxic benthic dinoflagellates nowadays constitute a significant component of benthic communities in the Mediterranean. This fact requires further studies and collaboration among Mediterranean countries in order to understand the environmental changes that are occurring and to protect human health and human activities from the potential hazards of benthic dinoflagellate toxicity.

Climate-induced changes and Harmful Algal Blooms in the Mediterranean: perspectives on future scenarios

Catherine Legrand [1,2] and Raffaella Casotti [3]

[1] *Alfred Wegener Institute for Polar Research, Bremerhaven, Germany*
[2] *University of Kalmar, Kalmar, Sweden*
[3] *Stazione Zoologica Anton Dohrn, Napoli, Italy*

ABSTRACT

Climate-induced changes in water temperature, stratification and other physical properties are likely to strongly impact the physiology and behaviour of harmful algae bloom (HAB) species, in terms of occurrence, physiology and toxin production. Although it is impossible to predict and eventually prevent outbreaks of HAB species, based on climate studies, one can expect that features such as increased stratification periods, modified circulation patterns and ocean acidification may affect algal physiology, causing an enhancement of toxin production and amplification through the food web. Apart from toxin production and transfer to final consumers, chemically-mediated interactions among and between species are likely to be modified by the physical environment. In this context, an integrated approach which considers the relationships between different players in the plankton is needed. All this complexity calls for a need of monitoring programmes complemented by suitable experimental approaches to test possible scenarios. In the Mediterranean, coastal areas are already experiencing increased occurrence of HABs, and often "novel" nuisance species are recorded. The improvement of plankton taxonomy skills and/or better monitoring programmes, and the interplay with anthropogenic nutrient inputs also add complexity to the problem. Improving HAB in the Mediterranean calls for integration of basic knowledge of coastal and regional differences with marine policy programmes, and to heighten public awareness of these issues and the potential economic and societal impact.

Recently, the potential impact of climate-induced changes on phytoplankton, and especially on HABs, has raised attention in the scientific community (Riebesell *et al.*, 2000; Edwards and Richardson, 2004; Hays *et al.*, 2005; Dale *et al.*, 2006; Riebesell *et al.*, 2008; Moore *et al.*, 2008). Future research directions to take in this field are often driven by human health concerns due to the potency of some algal toxins that are transferred through the food web. It is widely agreed, climate induced changes will impact phytoplankton physiology, community composition and interactions with the other components of the marine systems. However, we do not really understand the consequences of these modifications since the future dynamics of the physical and chemical environment is not fully predictable, nor are the physiological adaptations of HAB species to these changes known. Here we outline selected potential interactions between climate driven changes and the chemical ecology of HABs, and their potential consequences in the Mediterranean.

Harmful Algal Blooms are a recurrent phenomenon caused by a small fraction of phytoplankton causing a range of negative physiological, environmental and economical effects. Some HAB species, mainly dinoflagellates and diatoms, produce potent toxins, such as paralytic or neurotoxic shellfish poisons, that can be accumulated and transferred through the food web, inducing death or illness in fish and higher animals such as birds and mammals (including humans) (Hallegraeff, 1993). The dynamics of their toxicity is not always clear, as some toxins are toxic only to final consumers (e.g. fish, humans) but apparently are neutrally transferred through intermediate consumers. Additionally, other phytoplankton species produce compounds that are not toxic to humans but negatively impact competitors and grazer reproduction and therefore impact recruitment and population dynamics of fish species of commercial interest (Legrand *et al.*, 2003; Tillmann *et al.*, 2007; Selander *et al.*, 2008). Diatoms in general appear to produce a whole array of different compounds with such effects, directly derived from fatty acid metabolism, in which they are rich (e.g. the diatom *Skeletonema)* (Miralto *et al.*, 1999; Fontana *et al.*, 2007). The same metabolites are shown to have a role as infochemicals, and are involved in signaling adverse conditions within diatom populations (Vardi *et al.*, 2006), act as allelochemicals against competitor algae (Ribalet *et al.*, 2007), or regulate bacterial community composition and metabolism (Ribalet *et al.*, 2008). In general, there is a wealth of secondary metabolites produced by phytoplankton which are involved in the so-called "chemical warfare" that rules interaction among organisms at any level of the pelagic food web (reviewed by Ianora *et al.*, 2006). This field of research is a key to understanding how aquatic ecosystems function, and can also lead to discovery of biotechnologically exploitable molecules.

Other non-toxic HAB species can attain extraordinarily high cell numbers, negatively impacting coastal environments and economies (e.g. *Phaeocystis* in Northern Europe) (see overview by Zingone, this volume). In the Adriatic Sea, a recurrent accumulation of mucilage is apparently due to an imbalance in phosphorus metabolism, which affects bacterial degradation of organic matter from phytoplankton (Pugnetti *et al.*, 2005; Fonda Umani *et al.*, 2007). Mucilage events have also been recently reported in the Black Sea (Aktan *et al.*, 2008). The spreading of marine mucilage has been linked to surface seawater warming (Danovaro *et al.*, 2009).

In the last three or four decades, coastal HABs appear to have increased on a global scale. Several reasons have been suggested: a better knowledge of toxic species, better monitoring and alerting systems, the transport of algal cysts in ballast waters, the development of aquaculture, the stimulating effect of urban and industrial activities and/or atypical climate conditions (Smayda, 1997; Glibert *et al.*, 2005). New approaches and additional data are needed in order to distinguish the effects of climate from other biological and environmental factors driving occurrence of HABs (Wyatt, this volume). The response of HABs to climate changes will highly depend on the ecological and physiological diversity of the species involved and on the time scales.

In the Mediterranean, coastal areas subject to high nutrient discharge are hotspots for algal blooms including HABs, and some of them are at high risk for the possible impact on very delicate ecosystems such as the NW Catalan Sea, the Po river delta, the Nile river delta, and sectors of the Aegean Sea. Recently, the high abundance of dinoflagellates, especially the toxic *Karenia selliformis* in the Gulf of Gabes, Tunisia, has been attributed to increased nitrogen input (Turki *et al.*, 2006; Drira *et al.*, 2008). Additionally, the lack of deep mixing along the coast, and the ultra-oligotrophy of the Gulf of Gabes contributes to its extreme sensitivity to anthropogenic nutrient load. Summer outbreaks of the dinoflagellate *Alexandrium taylorii* are recurrent events in both the central and eastern Mediterranean Sea (Giacobbe *et al.*, 2007). Detrimental to both the tourism industry (water discoloration) and the shallow marine systems (hypoxia), the expansion of this HAB species is linked to high nutrient load.

Climate change triggers an increase in frequency of extreme and dramatic events such as floods, sudden and strong precipitation, and even intense freezing events in wintertime. This climatic variability has an immediate effect on coastal areas where it modulates stratification, general circulation, nutrient availability and also resuspension of sediments, on which some HAB species depend for their spreading. Climatic variability has also an indirect effect on periodic or long-term oceanographic features such as stratification periods or circulation patterns. One of the effects of temperature increase is an enhanced stratification, which will at first select species adapted to low turbulence, high light and to rapid utilization of available nutrients. Increased stratification of the water column, for instance, may favour phytoplankton species such as dinoflagellates, among which many HAB species are present, segregating them in the surface water, favouring blooms and enhancing accumulation. In a second phase, when nutrients are consumed, species able to migrate vertically and access nutrients in deeper layers will be favoured. However, this scenario still needs to be tested. On the other hand, off the coast of Spain, blooms of the HAB dinoflagellate *Gymnodinium catenatum* are driven by the supply of inorganic nutrients through upwelling and wind (Fraga and Bakun, 1990). In case of weakening of the upwelling, blooms of the toxic dinoflagellate could decrease in abundance. However, limiting concentrations of N or P are known to enhance toxin production of some HAB species, thus increasing their negative effects even at low cell concentrations (Graneli and Johansson, 2003; Uronen *et al.*, 2005; Poulton *et al.*, 2005; Adolf *et al.*, 2009). Temperature also has an effect on growth and division of both phytoplankton and their grazers, and this may result in an altered synchrony, inducing encounters between toxic species and predators that were temporally segregated in the past. For example, the seasonal window for growth of the paralytic shellfish toxin (PST) producer *Alexandrium catenella* could increase from about 65 to over 260 days per year following a 4-6°C temperature increase (Moore *et al.*, 2008).

Warmer waters may also increase the geographical range of dangerous benthic species, such as the tropical ciguatera producer *Ostreopsis*, which has now been observed in the Adriatic (see Aligizaki, this volume). Other examples are the pelagophyceaen *Chrysophaeum taylorii* associated with mucilaginous layers found in Sardinia (Luglie *et al.*, 2008), or the toxic dinoflagellate *Gambierdiscus* sp., the range of which is apparently spreading eastwards in the Mediterranean as far as Crete (Aligizaki *et al.*, 2008a). In many cases, doubts arise as to whether such species have never been reported due to lack of occurrence or to lack of observations. For these reasons, long-term time series of plankton observations are necessary to identify new arrivals and signal increased presence of HAB species.

The climate-induced variability in terms of temperature rise and pH lowering will most likely add to the human-induced effects such as continental runoff, fishing, introduced species, exponentially enhancing the impact of HABs. The extent of our knowledge on the ecological effects of ocean acidification is limited (see CIESM, 2008b), and drawing conclusions on the impact of decreasing pH on HABs would be premature. Some studies have tested the positive effect of high pH on growth or toxin production (Hinga, 1994; Hansen *et al.*, 2007). However, different species have different pH optima (Hansen *et al.*, 2007), which calls for changes in phytoplankton composition with decreasing pH (Engel *et al.*, 2008). However, the resilience to slow changes must be taken into consideration, since microalgae may adapt to altered conditions if the time scale of the change is increased. These aspects, and their interactions with temperature rise, are an important field of research that needs to be fostered.

Most research has concentrated on nutrient-phytoplankton interactions under different scenarios of N or P increase, as related to human activities, but there is little information on the synergistic effects of other ecosystem forcing in combination with excess nutrients as induced, for instance, by increased stratification. Future research should also focus on the interactions between HAB species and other components of the ecosystem such as viruses, bacteria, microzooplankton and zooplankton. In fact, it is evident that grazing is involved in control of blooms, but the interplay of mechanical and chemical defense must be further investigated in HABs (e.g. Ribalet *et al.*, 2009). In addition, heterotrophic bacteria often appear to be strongly associated with HAB species and insensitive to their toxins. The understanding of these links and the molecular mechanisms underlying them may lead to increased HAB prediction abilities and eventually bioremediation strategies.

In a scenario where the water column is both warmer and rich in nutrients, algal blooms can potentially last longer and decrease in diversity. However, warmer conditions, and weakening of nutrient upwelling in coastal conditions may produce contradictory responses at the clonal level. Therefore, it becomes crucial to understand the genetic variability of populations and what factors drive selection of a few genotypes within the same potentially toxic species. The link with toxin production is an important field of research, as well as the complex environmental factor interactions underlying the selection. However, ecophysiological experiments seldom include physiological plasticity and genetic strain/population diversity. For the diatom *S. marinoi* in the

Adriatic, it appears that highly genotypically different strains are responsible for blooms in different years, and the link to the observed effect with copepods needs to be established (Casotti and Gohde, pers. comm.). In the North Sea, evidence of the genotypic uniqueness of many clones of the PST producer *Alexandrium tamarense* in mixed plankton assemblages was recently demonstrated (Alpermann *et al.*, 2009). This is a milestone to follow the success of certain phenotypic traits, e.g. toxicity, in ecophysiological experiments or during bloom development.

In conclusion, predicting the impact of climate changes on HABs in the Mediterranean is very challenging because many factors and their combination are involved. Is our society ready to face up to major changes in the extension of HAB events, in the composition of HAB species and toxins, and in the resulting decrease of seafood safety? This calls for increased awareness in both HAB and seafood monitoring programmes, but also to increased attention to the interactions among species and between HAB species and abiotic factors.

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Phytoplankton response to anthropogenic nutrient discharge in a southwestern Mediterranean lagoon: implication for toxic algal blooms

Asma Sakka Hlaili [1], Mohamed-Ali Chikhaoui [1], Ines Sahraoui [1],
Stéphane Bates [2] and Hassine Hadj Mabrouk [1]

[1] *Laboratoire de Cytologie Végétale & Phytoplanctonologie, Département des Sciences de la Vie, Faculté des Sciences de Bizerte, Tunisia*
[2] *Fisheries and Oceans Canada, Gulf Fisheries Centre, Moncton, Canada*

Abstract

Effects of nutrient enrichments (+NSi/P; +P/NSi) on phytoplankton of Bizerte Lagoon were examined during July 2004, during a potentially toxic diatom (*Pseudo-nitzschia*) bloom. The seasonal dynamics of these algae was also investigated monthly from March 2004 to March 2005. Enrichment experiments showed that treatments with both +NSi/P and +P/NSi supported higher net growth rates, with corresponding increases in Chl *a* and algal abundance. N and Si seemed to be the major nutrients affecting phytoplankton since their addition (i.e. +NSi/P treatment) induced more pronounced enhancement in algal growth. Dinoflagellates exhibited the highest growth with +P/NSi fertilisation. In contrast, diatom growth and abundance were mostly stimulated by +NSi/P enrichment. The proliferation of *Pseudo-nitzschia* species was markedly stimulated when nutrients were added. These algae bloomed in July 2004, under summer conditions (high water temperature and salinity) and increased Si and P concentrations. The causative species of the July peak was identified by scanning electron microscopy as *Pseudo-nitzschia calliantha*. Our results suggest that increased anthropogenic nutrient loads in the lagoon may influence algal dynamics as well as productivity in different ways, and can also initiate proliferation of toxic diatoms.

1. Introduction

In recent decades, human activities have increased nutrient loads in coastal systems. Anthropogenic nutrient enrichment has been identified as an environmental problem in several Mediterranean coastal areas (Pinckney *et al.*, 2000; Smith, 2003). Eutrophication frequently results in harmful algal blooms (HABs), which have become more frequent in recent years. These events can seriously impact public safety, fisheries, wildlife and aquaculture, resulting in considerable economic losses (Liu *et al.*, 2001). This ecological problem has led to extensive research on the effects of nutrients on primary producers. While nutrient availability can control algal growth, biomass and species composition (Sakka *et al.*, 1999; Caron *et al.*, 2000; Lagus *et al.*, 2004), there is no general consensus as to which element (N or P or Si) impacts the phytoplankton, and which nutrient can initiate HAB events. Thus, evaluation of phytoplankton responses to different nutrient discharges is an important stage in the development of efficient strategies of ecosystem management and protection.

Bizerte Lagoon, which supports intensive fishery activities and several aquaculture farms, is experiencing increasing anthropogenic nutrient loading from expanding urban, agricultural and industrial development along its shores. Furthermore, potentially toxic algae have been identified several times in the lagoon (Turki and El Abid, 2001), and recent work shows that the nutrient supply stimulates the biomass and growth of the potentially toxic diatom *Pseudo-nitzschia* (Sakka Hlaili *et al.*, 2006). On longer time scales, changes in nutrient status of the lagoon can increase the incidence of harmful microalgae, raising an obstacle to further development of shellfish farming in the area. So, our focus was first on the effects of nutrient fertilisation on phytoplankton, especially on potentially toxic diatoms, and secondly to investigate the seasonality and bloom dynamics of these diatoms in the lagoon.

2. MATERIAL AND METHODS

2.1. Study site

Bizerte Lagoon (37°8' - 37°14' N, 9°48' - 9°56' E) is located on the northern coast of Tunisia (Fig. 1). It has a surface area of 150 km^2 and a mean depth of 8 m. Exchanges with the Mediterranean occur through a 7-km-long channel with a depth of 12 m. Bizerte Harbour, which has considerable regional importance, lies on this channel. The marine inflow is strong in summer (Harzallah, 2002). The lagoon also receives freshwater from several surrounding rivers and from Lac Ichkeul. The lagoon supports high chlorophyll *a* (Chl *a*) concentrations (1.54-3.84 µg L^{-1}) (Sakka Hlaili *et al.*, 2006). This high Chl *a* biomass may reflect high algal growth rates due to the relatively high nutrient availability ($NO_2^- + NO_3^-$: 0.96-4.35 µM, NH_4^+: 1.2-2.4 µM, PO_4^{3-}: 0.40-0.64 µM) (Harzallah, 2002). Concentrations of total (organic and inorganic) nitrogen and phosphorus have been reported to be 32-52 µM and 3.8-5.4 µM, respectively (Harzallah, 2002).

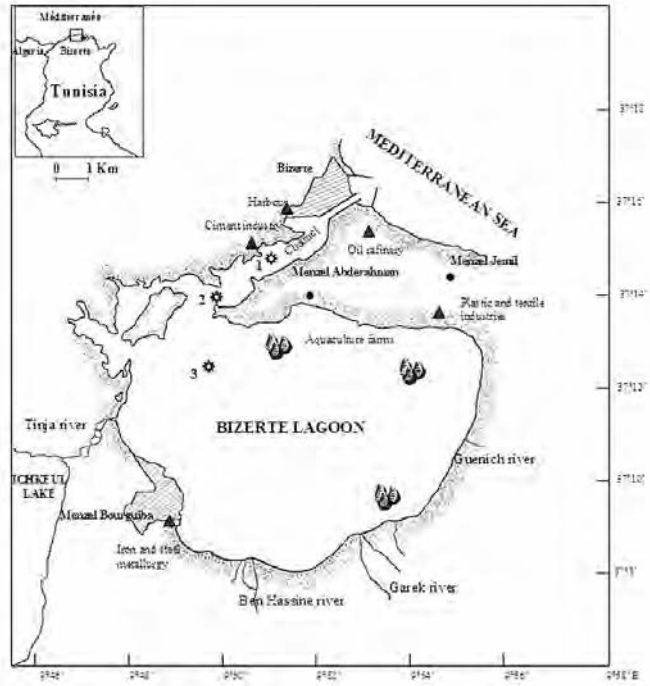


Figure 1. Location of the study stations in the Bizerte Lagoon, Tunisia.

2.2. Nutrient enrichment experiments

To test the effects of nutrients on phytoplankton, enrichment experiments were performed during summer 2004 (July, period of *Pseudo-nitzschia* bloom) at three stations (Fig. 1): Station 1 is situated in the Channel of Bizerte, which carries intensive marine traffic and receives various industrial effluents; station 2 is a site for collecting wild mussels (*Mytilus galloprovincialis*); station 3 is located in the open lagoon. During each experiment, pre-sterilised 2-L Nalgene polycarbonate bottles were filled with filtered (200 µm mesh) water from the depth of the Chl *a* maximum (2 m) and enriched according to different treatments: +P/NSi (addition of 20 µM NO_3, 20 µM SiO_4 and 2.5 µM PO_4; N:Si:P<16:16:1), +NSi/P (addition of 40 µM NO_3, 40 µM SiO_4 and 1.5 µM PO_4; N:Si:P>16:16:1,) and C (control, no nutrient addition). The bottles were incubated *in situ* at 2 m for six days. The replicates of each treatment were sampled three times (at 2, 4 and 6 days of incubation) for analyses of Chl *a* (spectrophotometric method, Parsons *et al.*, 1984) and phytoplankton identification and enumeration (inverted microscopic technique). Initial samples were also taken to determine nutrient concentrations (NO_2^- + NO_3^- and PO_4^{3-}), Chl *a* and phytoplankton abundance and taxonomic composition in the ambient water. The net growth rates of phytoplankton in the different treatments were estimated using the exponential model of Landry and Hassett (1982).

2.3. *Pseudo-nitzschia* dynamics

The temporal distributions of phytoplankton and the genus *Pseudo-nitzschia* were investigated at the three stations (Fig. 1), where enrichment experiments were also conducted. Sampling was carried out monthly from March 2004 to March 2005. During sampling, water temperature, salinity, nutrient and Chl *a* concentrations (spectrophotometric method, Parsons *et al.*, 1984) and algal abundance (inverted microscopy technique) were determined.

2.4. Culture and identification of *Pseudo-nitzschia*

Clonal nonaxenic isolates of *Pseudo-nitzschia* were established from the July 2004 bloom. Cultures were grown in f/2 medium (Guillard and Ryther, 1962), at temperature of 25°C and at light intensity of 100 µmol photons m^{-2} s^{-1} (12h:12h light:dark cycle). For identification to the species level, aliquots of *Pseudo-nitzschia* cultures were prepared and examined by scanning electron microscopy (SEM), using the method of Kaczmarska *et al.* (2005).

2.5. Statistical analyses

For the enrichment experiments, the significance of the nutrient treatments on algal variables was tested using a repeated-measures two-way ANOVA. The ANOVA was followed by the *a posteriori* contrast test of Dunnett, to identify which treatments were significantly different from the control. The conditions of normality of distribution (Kolmogorov-Smirnov test) and homogeneity of variance (Bartlett-Box test) were respected. A multivariate data analysis (non-parametric multi-dimensional scaling ordination, MDS) was performed on the species/group abundance (on last day of incubation) to determine if there was any change in the final algal composition among treatments. An Euclidean distance similarity measure was then used to determine the differences in the final algal community composition among treatments.

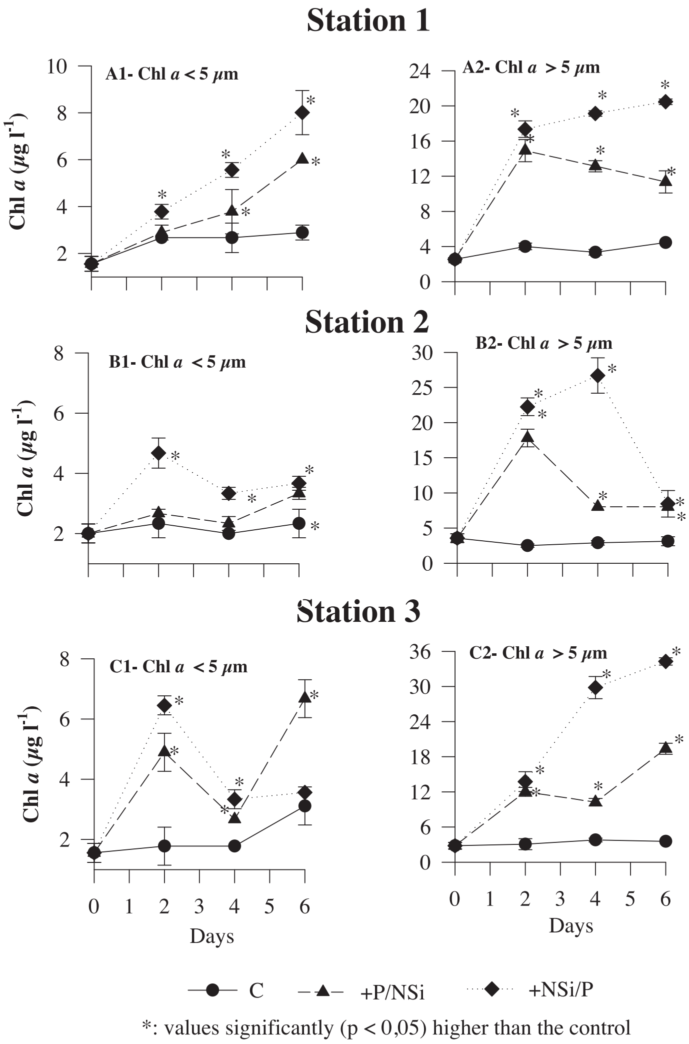
To investigate *Pseudo-nitzschia* dynamics, two-way ANOVA was used to test the temporal and spatial variation of abiotic and algal variables. Spearman's correlation analysis was performed to assess whether *Pseudo-nitzschia* distribution was linearly related to abiotic conditions.

3. RESULTS AND DISCUSSION

3.1. Phytoplankton responses to nutrient enrichments

3.1.1. Response of phytoplankton biomass (Chl a) and growth

At the three stations, Chl *a* concentrations (for ≤ 5 µm and > 5 µm algal size fractions) measured after +NSi/P and +P/NSi fertilisations were significantly ($p<0.01$) higher than in the control, but the +NSi/P treatment induced more pronounced enhancements in Chl *a* than those observed when Si was deficient (i.e. +P/NSi treatment) (Fig. 2).



*: values significantly (p < 0,05) higher than the control

Figure 2. Temporal variations of Chl *a* concentrations for < 5µm and > 5µm size fractions in the control (C, no nutrient addition) and nutrient enrichments (+NSi/P and +P/NSi) during the July 2004 experiment. (Means ±SD, n = 3)

In the control, net growth rate of phytoplankton varied from 0.020 to 0.088 d^{-1} at different stations. After +NSi/P and +P/NSi enrichments, phytoplankton growth was enhanced 2-6 fold at the three stations (Table 1). The most pronounced growth stimulation was observed in the +NSi/P treatment.

Table 1. Net growth rates of algae (d^{-1}) in different treatments during the July 2004 experiments at the three study stations.

	C	**+NSi/P**	**+P/NSi**
Station 1	0.088 ± 0.002	**0.298 ± 0.012**	**0.214 ± 0.002**
Station 2	0.020 ± 0.001	**0.122 ± 0.020**	**0.072 ± 0.001**
Station 3	0.072 ± 0.009	**0.348 ± 0.010**	**0.260 ± 0.016**

Values in bold type are significantly higher than the control.

3.1.2. Response of algal taxonomic groups

There were also significant ($p<0.05$) effects of nutrient additions on various algal groups. Diatoms responded strongly to the +NSi/P treatment, and their abundance was 2.5 fold higher than in the control (Fig. 3). The growth of several diatom taxa was significantly stimulated by both treatments, but the most pronounced growth rates were observed after +NSi/P fertilisation (Table 2). Among these diatom taxa, the potentially toxic species of *Pseudo-nitzschia* were observed, with higher growth rates in +NSi/P (5 fold) and +P/NSi (3 fold) treatments than in the control. Dinoflagellate proliferation was also favoured by both treatments, but the most pronounced increase in their abundance was detected after +P/NSi addition (Fig. 3). Furthermore, the growth of dominant dinoflagellate taxa (*Prorocentrum*, *Gyrodinium*, and *Gymnodinium*) was strongly stimulated by the +P/NSi enrichment (Table 2). Unlike diatoms and dinoflagellates, the abundance and net growth rates of autotrophic flagellates after nutrient enrichments were not significantly different from the control (Table 2; Fig. 3).

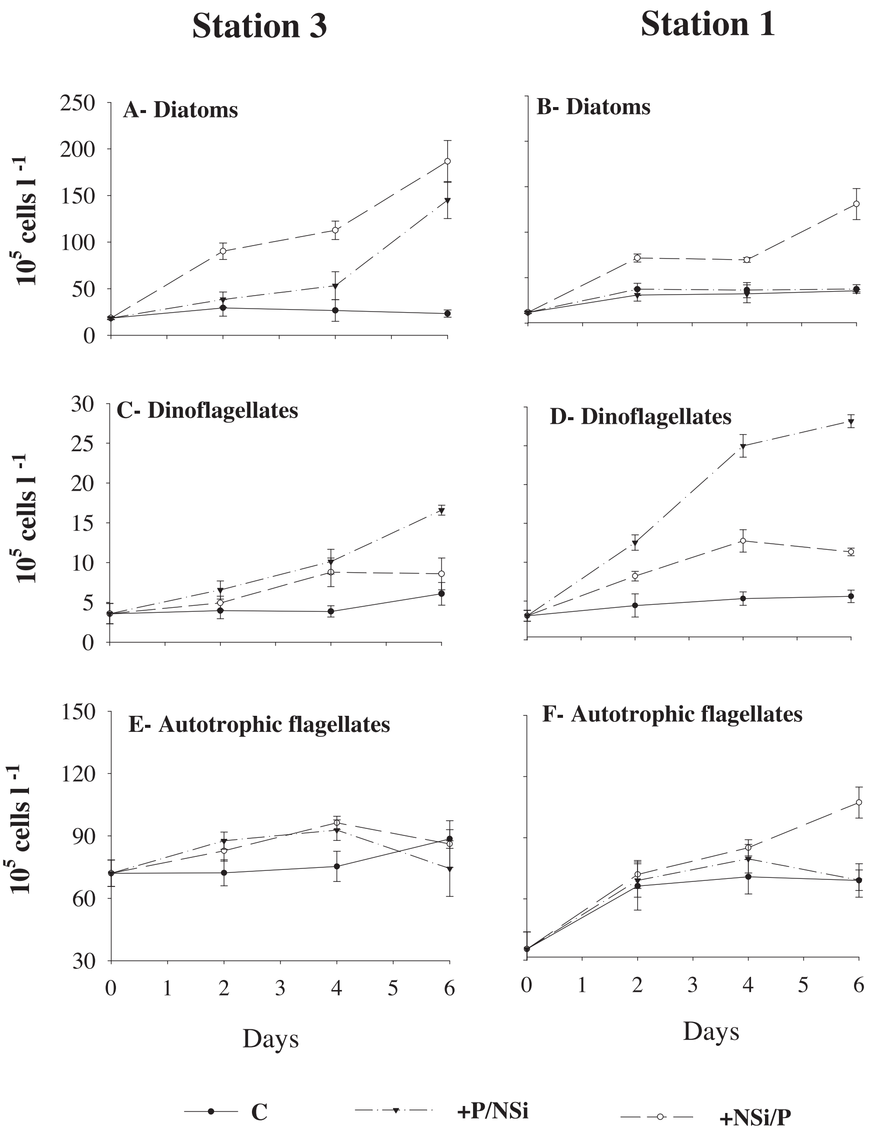


Figure 3. Temporal variations of abundances of diatoms, dinoflagellates and autotrophic flagellates in the control (C, no nutrient addition) and nutrient enrichments (+NSi/P and +P/NSi) during the July 2004 experiment. (Means + SD, n = 3)

Table 2. Net growth rates (d^{-1}) of different taxonomic groups in the control and nutrient enrichments during the July 2004 experiment at station 3.

Diatoms			
Chaetceros	0.101 ± 0.001	**0.281 ± 0.010**	**0.180 ± 0.002**
Skeletonema costatum	0.001 ± 0.001	**0.220 ± 0.015**	**0.430 ± 0.011**
Entomoneis	0.080 ± 0.005	**0.390 ± 0.015**	0.040 ± 0.005
Gyrosigma	0.020 ± 0.002	**0.200 ± 0.010**	**0.110 ± 0.005**
Attheya	-0.020 ± 0.002	**0.201 ± 0.010**	**0.080 ± 0.006**
Leptocylindrus	0.130 ± 0.010	**0.172 ± 0.015**	**0.240 ± 0.015**
Guinardia	0.110 ± 0.008	**0.221 ± 0.010**	**0.240 ± 0.014**
Dactyliosolen	0.030 ± 0.005	**0.130 ± 0.012**	0.030 ± 0.005
Cylindrotheca closterium	0.020 ± 0,005	**0.400 ± 0.010**	**0.260 ± 0.014**
Nitzschia	0.090 ± 0,009	**0.340 ± 0.012**	0.120 ± 0.008
Pseudo- pnitzshia	0.060 ± 0.003	**0.300 ± 0.010**	**0.210 ± 0.008**
Dinoflagellates			
Gymnodinium	0.080 ± 0.001	**0.160 ± 0.006**	**0.260 ± 0.003**
Gyrodinium	0.200 ± 0.021	**0.520 ± 0.150**	**0.730 ± 0.010**
Autothrophic flagellates	0.100 ± 0.007	0.170 ± 0.005	0.110 ± 0.010

Values in bold type are significantly higher than the control.

The diverse responses of the various algal taxa to the nutrient supply were followed by a change in the final phytoplankton assemblage. Initially, phytoplankton was dominated by autotrophic flagellates which represented 68-70% of algal abundance; diatoms and dinoflagellates contributed, respectively, 23-26 and 5-6% of total abundance (Fig. 4). After both treatments, the relative contribution of autotrophic flagellates decreased while that of diatoms increased, particularly under +NSi/P enrichments (60-62% of algal abundance), and that of dinoflagellates reached a high value after +P/NSi addition (20% of algal abundance) (Fig. 4). The changes in the final algal composition following the nutrient additions were confirmed by the MDS results (Fig. 5). The replicates of all nutrient treatments clustered far from the control. Moreover, high dissimilarities were observed between each nutrient enrichments and the control and between both nutrient treatments (Table 3).

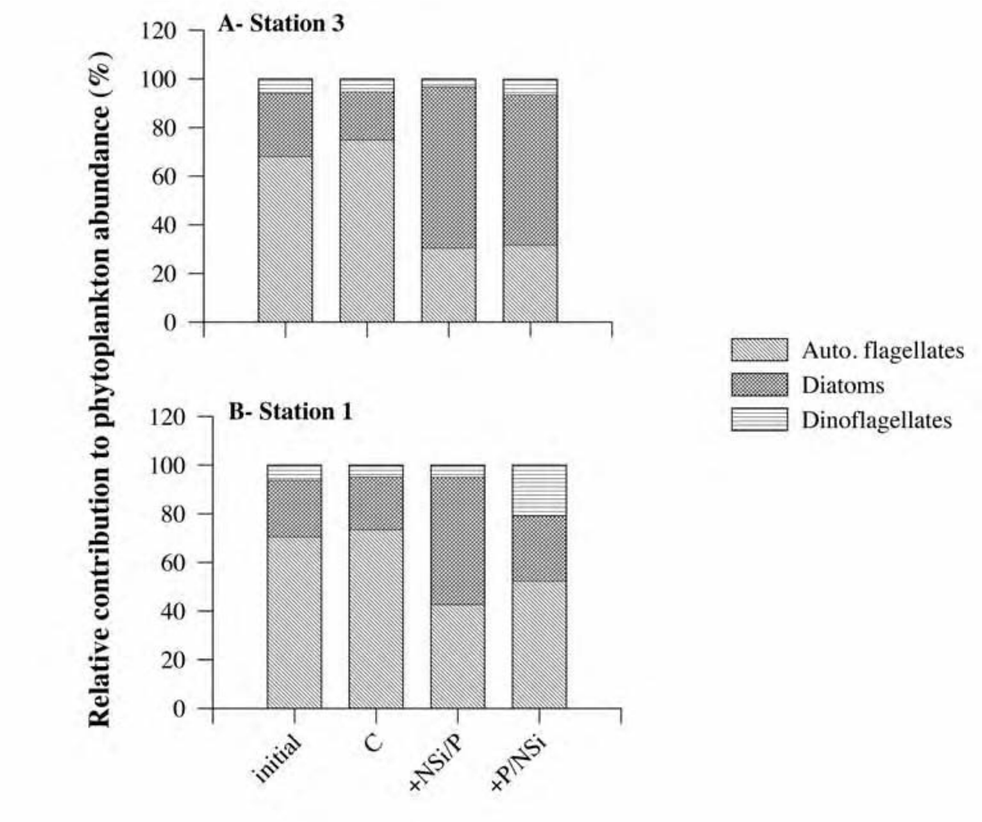


Figure 4. Algal taxonomic composition at initial state and at the end of incubation in the control (C, no nutrient addition) and enrichments (+NSi/P and +P/NSi) during July 2004 at stations 1 and 3.

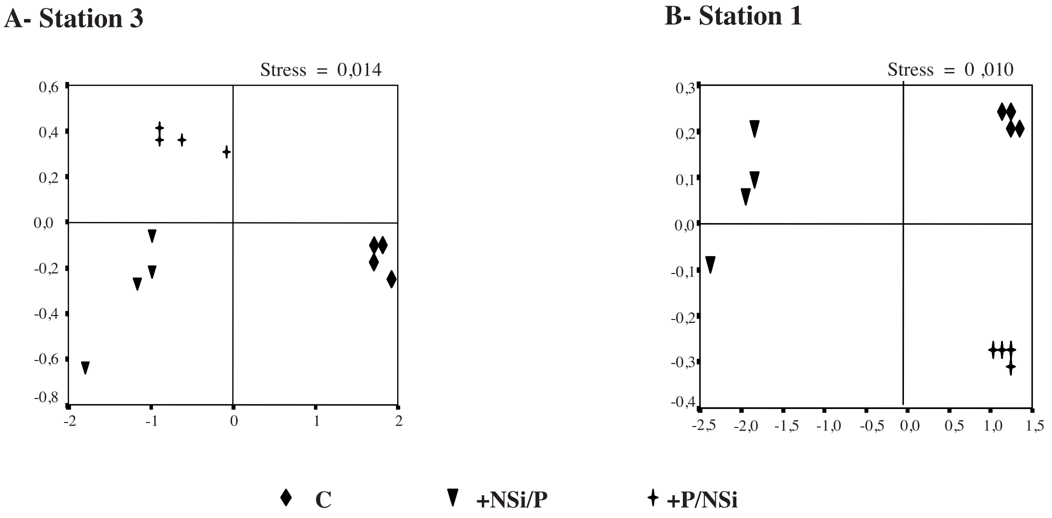


Figure 5. Non-metric MDS ordination of algal species abundance data from the control (C, no nutrient addition) and +NSi/P and +P/NSi treatments during July 2004 experiment.

Table 3. Average Dissimilarity in the final community composition of phytoplankton among treatments during the July 2004.

	C	**+NSi/P**	**+P/NSi**
Station 3			
C			
+NSi/P	129.7 ± 5.0		
+P/NSi	103.8 ± 2.5	40.8 ± 3.7	
Station 1			
C			
+NSi/P	99.0 ± 9.0		
+P/NSi	67.6 ± 3.5	68.7 ± 5.7	

These results clearly show that phytoplankton of the lagoon was limited by N, Si and P, since +NSi/P and +P/NSi treatments supported higher net growth rates with corresponding increases in Chl *a* and algal abundance. In summer, N and Si seemed to be more limiting than P since their addition (i. e. +NSi/P treatment) induced more pronounced enhancement in algal growth. The lagoon was characterized by a summer proliferation of diatoms. Moreover, the N nutrients were shown to be the major factor affecting diatom dynamics in the lagoon during winter and summer (Sakka Hlaili *et al.*, 2006). This can explain the strong response of algae to the simultaneous addition of Si and N (i. e. +NSi/P treatment). The most important increase in abundance and growth of diatoms was detected after +NSi/P treatment. This enrichment was also followed by a significant growth increase of a potentially toxic diatom. Compared with diatoms, dinoflagellates have a stronger response to P addition during summer. The abundance and growth of dinoflagellates was strongly enhanced in the +P/NSi enrichment (Table 2; Fig. 3), leading to an increase of their relative contribution to algal abundance (Fig. 4). This may be attributed to the high P requirement of dinoflagellates, which have a high DNA content (Berman and Dubinsky, 1985). The dinoflagellate dynamics in the lagoon seems to be strongly tied to P availability. Thus, nutrient fertilisations affect the algal structure in different manners, as several algal taxa have specific responses to each nutrient. This implies that the increase in anthropogenic nutrient loading to the lagoon may influence algal dynamics as well as productivity in different manners, and also initiate proliferation of toxic diatoms.

3.2. *Pseudo-nitzschia* dynamics

3.2.1. Monthly variation of phytoplankton

Chl *a* concentration showed significant ($p<0.05$) seasonal variation (Fig. 6A). Highest levels were recorded in March 2005 at stations 1 and 3 (7-8 µg L^{-1}). Total phytoplankton abundance remained low (1.5 - 7.1 x 10^5 cells L^{-1}) and relatively constant during late autumn and winter (Fig. 6B). It peaked during April 2004 (19.4 x 10^5 cells L^{-1}), July 2004 (19.9x 10^5 cells L^{-1}) and March 2005 (12.2 x 10^5 cells L^{-1}), with pronounced concentrations at Station 3. Over the year, changes in both total phytoplankton and diatom abundance paralleled each other in a significant fashion ($p<0.01$) (Fig. 6B, C). However, diatoms did not account for the April peak of phytoplankton abundance, which was composed of flagellate species (*Plagioselmis* spp.). Diatom abundance was generally moderate (~3 x 10^5 cells L^{-1}) and increased considerably during summer 2004 and in March 2005 (Fig. 6C). The highest values were attained in July 2004 (17 x 10^5 cells L^{-1}, at Station 3). The July peak of diatoms was mainly composed by *Pseudo-nitzschia* and *Chaetoceros* species.

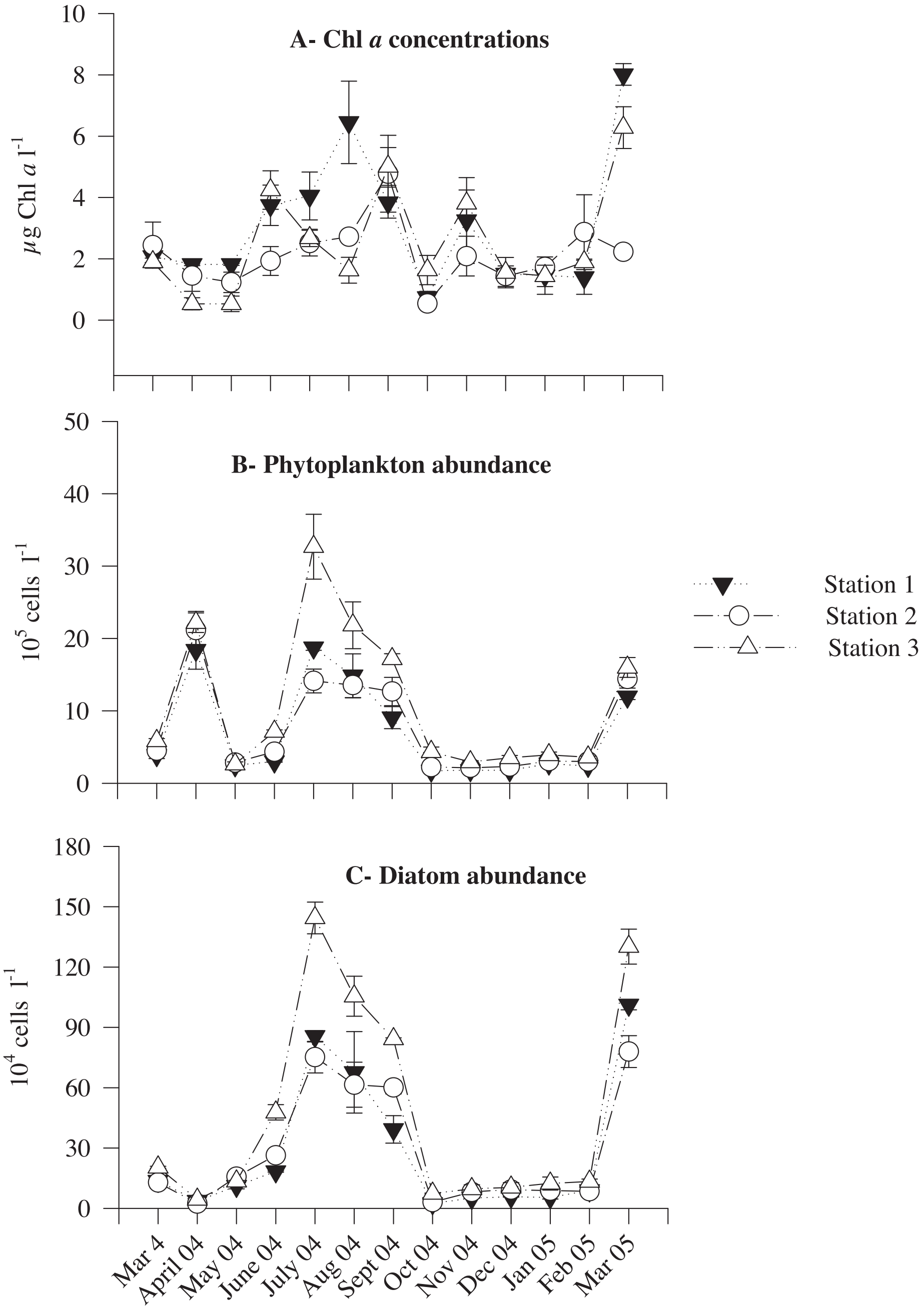


Figure 6. Monthly variation in Chl *a* concentration (A), total phytoplankton abundance (B) and diatom abundance (C) at the three study station. (Mean ± SD).

Changes in algal communities among seasons are an obvious consequence of seasonal changes in physical, chemical and biological factors (Sommer, 1996; Macedo *et al.*, 2001). The summer proliferation of diatoms is probably a common feature of Tunisian coastal waters (Daly Yahia-Kéki, 1998) and of several Mediterranean areas like the Mar Menor lagoon (Gilabert 2001) and Orbetello lagoon (Nuccio *et al.*, 2003). Increased water temperature and salinity may enhance the specific growth rates of these algae. Indeed, laboratory and field studies have shown that diatom growth increases with high values of both factors (Montagnes and Franklin, 2001; Macedo *et al.*, 2001). Nutrient availability can also affect diatom dynamics. Indeed, diatom density was positively correlated with silicate levels at stations 2 and 3 ($r= 0.69$; $p<0.05$). This emphasises that Si availability was the main factor in the regulation of diatom seasonal dynamics.

3.2.2. Monthly variation of Pseudo-nitzschia

The presence of *Pseudo-nitzschia* species was highly variable over time (Fig. 7A). These algae were abundant from May to September 2004 (2-23 x 10^4 cells L^{-1}) and they bloomed in July 2004 at stations 2 and 3 (Fig. 7A). However, *Pseudo-nitzschia* showed low concentrations from November 2004 to January 2005, and was absent in March 2004, April 2004, February 2005 and March 2005 (Fig. 7A). The relative contribution of *Pseudo-nitzschia* to total diatom numbers was maximal in July (30-34%) at station 2 (Fig. 7B). The July peak of *Pseudo-nitzschia* spp. was exclusively composed of members of the *P. delicatissima* complex. Abundance of these cells was positively correlated with temperature ($r=0.60$, $p<0.001$), salinity ($r=0.63$, $p<0.001$) and PO_4^{3+}, $Si(OH)_4$ concentrations ($r=0.60$, $p< 0.001$). These results reveal that blooms of potentially toxic diatoms were favoured by summer conditions (increased water temperature and salinity) in the lagoon, and suggest that P, Si nutrients are the main elements initiating this algal proliferation.

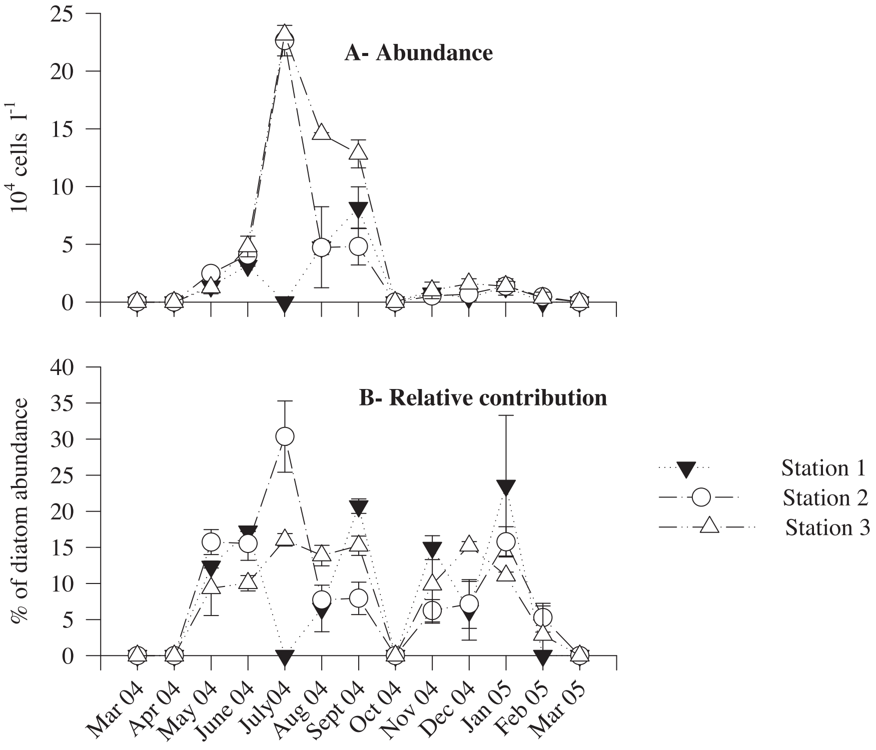


Figure 7. Monthly variation in abundance of *Pseudo-nitzschia* species (A) and their relative contribution to total diatoms number (B) at the study stations. (Mean ± SD).

3.2.3. Pseudo-nitzschia identification

Four monospecific cultures of *Pseudo-nitzschia* were obtained from the July bloom. The strains Psn-5 and Psn-13 were obtained from station 3, and Psn-6 and Psn-11 from station 2. SEM observation of these isolates revealed two striae (37-40/ 10 µm) per fibulae (17-20/10 µm), one row of large poroids (4-5 per 1 µm) in each stria, and the presence of a central interspace with a central nodule (Fig. 8). The hymen of the valve poroids exhibits a flower pattern. These morphometrics were consistent with the recently described *P. calliantha* (Lundholm *et al.*, 2003).

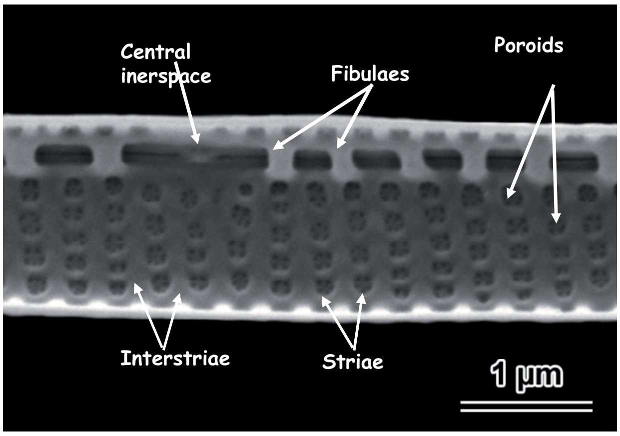


Figure 8. Scanning electron micrograph of *Pseudo-nitzschia calliantha* (strain Psn-5), showing detail of central part of the cell.

Studies and changes of phytoplankton populations in Lebanese coastal waters - a brief overview

Marie Abboud-Abi Saab

National Council for Scientific Research, National Center for Marine Sciences, Batroun, Lebanon

ABSTRACT

Regular studies of phytoplankton in the Lebanese coastal area have been carried out since 1979 at the National Center for Marine Sciences, to describe seasonal, vertical and spatial variations in primary production in relation to environmental conditions. Attention is focused on toxic algae. This paper gives an overview of the factors affecting the development of phytoplankton and the main characteristics of these populations. Interannual changes in surface density are principally due to the hydrological conditions and to an oil spill accident in 2006. Increased abundance of some harmful algae is noted.

INTRODUCTION

Studies of phytoplankton in Lebanese coastal waters started with Gruvel (1931) using net samples. A later study by Taslakian and Hardy (1976) investigated the effect of pollution on phytoplankton populations in relation to the sewage of Beirut. A parallel zooplankton study (Lakkis and Novel-Lakkis, 1980) covered a larger area, but the use of nets of 53µm mesh size and the monthly frequency did not adequately sample the most important biomass.

Regular study of phytoplankton has been carried out since 1979, at the National Center for Marine Sciences, to describe seasonal, vertical and spatial variations in primary production in the Lebanese coastal area, at offshore and inshore stations. Information is given on the distribution, abundance, biomass and biodiversity of phytoplankton populations in this eastern Mediterranean region. An STD probe, water sampler and net samples were used to collect data. Data was arranged as time series (day-to-day, weekly, monthly, and yearly), surface data for some stations and at different levels between surface and 100 m for offshore stations). There are 50 stations (20 littoral, 3 in fishing harbours and 27 coastal and offshore stations). The distribution of abundance, biomass and productivity of phytoplankton in Lebanese coastal waters has been explored in many studies. Here particular attention is given to toxic algae.

The Lebanese coast (220 km) (Fig. 1) has several distinguishing features: 1) the absence of large rivers, so that nutrient inputs are generally very modest; it is mostly in spring that rising temperatures causings now melt add fresh water to the sea: in summer, this nutrient source is not available, since most rivers are dry; 2) the thermocline is of long duration (more than 6 months), and deep (40-50 m); 3) the continental shelf in Lebanon is in general narrow, and widest (12 km) in the north; 4) the Lebanese coastline is generally unprotected with few bays and creeks; there are no closed bays and water is continuously mixed, thus promoting dispersion of nutrients and pollutants and preventing eutrophication. Thus widespread offshore eutrophication cannot be expected. These combined conditions make the ecosystem at large scale relatively homogeneous. 5) massive urbanization of coastal areas and accompanying sewage, solid and industrial wastes, with or without primary treatment; 6) intense human activities: tourist complexes, dumping, and inshore and offshore sand dredging.

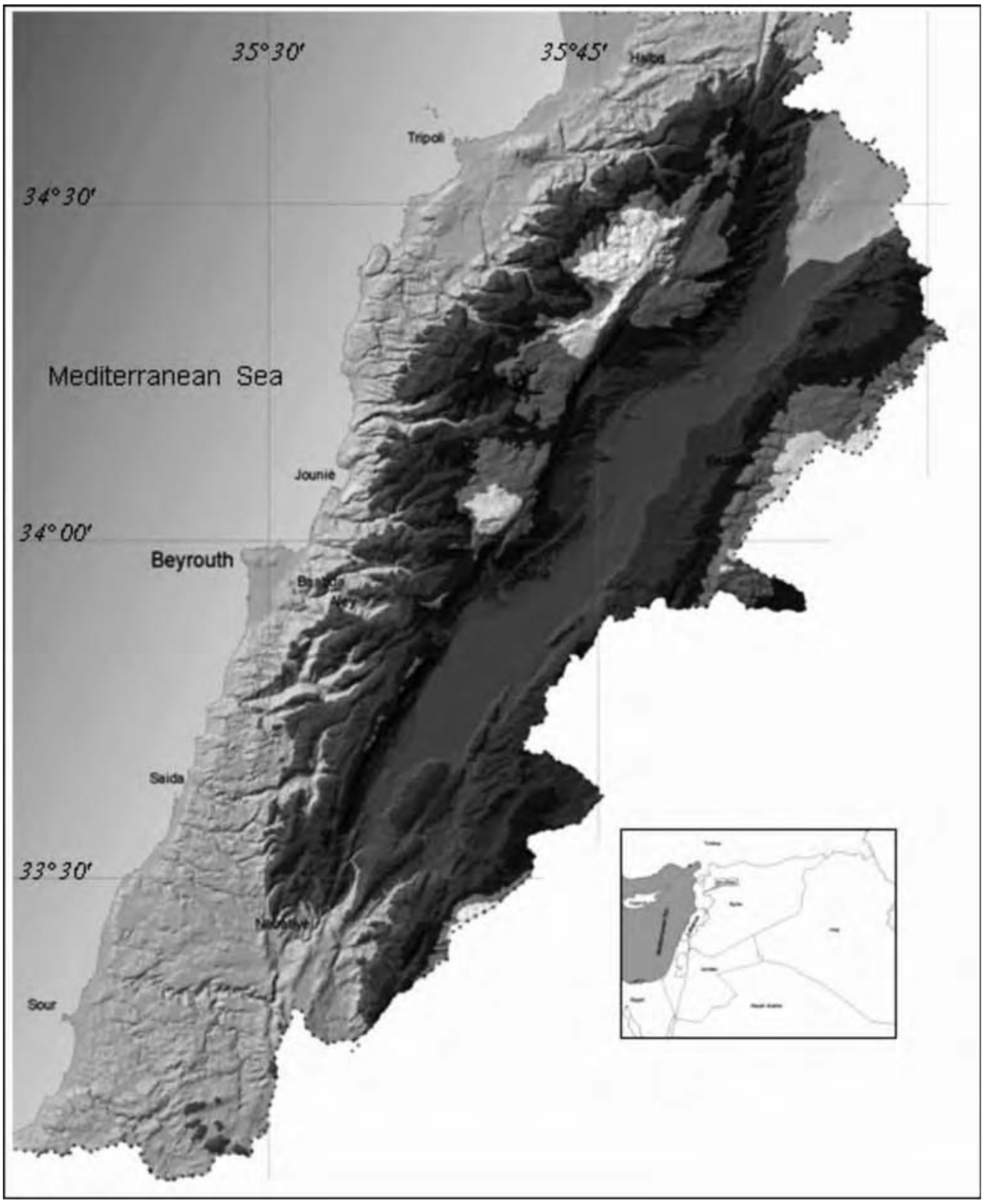


Figure 1. Lebanese coastal area.

The most important factors which affect development of planktonic populations in the eastern Mediterranean are: 1) seasonality of the Mediterranean climate (dry season and wet season); 2) presence of seasonal thermocline and permanent thermocline; mixing of water between the surface layer and deep water is very restricted; 3) influence of Red Sea waters through via the Suez Canal; 4) domestication of Nile waters.

MAIN PHYTOPLANKTON CHARACTERISTICS

Earlier studies by Abboud-Abi Saab, (e.g. 1985a;b; 1986; 1987; 1988a;b; 1989), and by Abboud-Abi Saab and Nabbhan (1994) have revealed large fluctuations in the seasonal and weekly abundance of phytoplankton. The effect of meteorological variations is more important in coastal and shallow stations.

Studies of potentially toxic microalgae, and relationships between environmental conditions and the ecology and occurrence of harmful algal blooms (HABs) in fishing harbours are summarized by Abboud-Abi Saab and El-Bakht (1998) and Abboud-Abi Saab *et al*. (2006).

The results of the studies cited above can be summarized as follows: 1) Microphytoplankton populations are mainly comprised of diatoms and dinoflagellates; other groups are present mainly in summer in low densities, e.g., silicoflagellates (Abboud-Abi Saab, 1992), cyanophytes, and coccolithophorids (Abboud-Abi Saab, 1985a); 2) the spring bloom is characterized by centric (*Skeletonema costatum*, *Leptocylindrus danicus*, *L. minimus* and *Chaetoceros curvisetus*) and pinnate diatoms (*Pseudonitzschia pseudodelicatissima, P. pungens*), and some neritic dinoflagellates (*Scrippsiella trochoidea, Prorocentrum triestinum*); a large variety of naked dinoflagellates dominate in summer throughout the water column (Abboud-Abi Saab, 1986); 3) Nanoplankton (2-20 µm) is relatively abundant (Abboud-Abi Saab, 1988b); 4) Phytoplankton is in general typically Mediterranean, rich in species, mainly dinoflagellates, and with some similarities to that of tropical regions in (Abboud-Abi Saab, 1989; 5) the presence of rare species and newly described species *Ceratoperidinium mediterraneum* and *Asterodinium libanum* (Abboud-Abi Saab, 1989; Gomez *et al*., 2003); 6) Presence of significant proportion of well-established Indo-Pacific species derived from the Lessepsian migration (from Red Sea to Mediterranean) (Abboud-Abi Saab, 1985b); 7) Diversity index showed an annual minimum in May and a maximum in September (Abboud-Abi Saab, 1988a); 8) At a biogeographical level, some species develop particularly in neritic zones and others in both neritic and oceanic zones; 9) Compared with other Mediterranean areas, cell densities in oceanic waters are low.

MAIN CHANGES IN LEBANESE PHYTOPLANKTON

Qualitative changes include 1) A continuous appearance of rare species such as *Dicroerisma psilonereiella* (Taylor and Cattell, 1969), *Brachydinium capitatum* and *Protoceratium reticulatum*. 2) An exceptional mixed bloom of *Skeletonema costatum* reaching 1.6 × 10^7 cells L^{-1} and *Heterosigma akashiwo* reaching approximately 9 x 10^6 cells L^{-1} was detected in May 2007 (Abboud-Abi Saab *et al*., 2008). Maximum cell concentrations were located in surface water opposite; chl-a values reached 32 mg m^{-3}. Prior to this bloom, *H. akashiwo* was present in some urbanized areas at low densities.
3) Increased frequency of *Ostreopsis siamensis;* this species was present at the beginning of our sampling in 1979, and began to increase in density at most of the rocky littoral stations between May and November, reaching 10560 cells L^{-1} in July 2001 and 5740 cells L^{-1} in September in the same year at another station. In 2008 and 2009, this species was found in February, March and April in small numbers.

After a catastrophic oil spill in July 2006, a decrease in chl-*a* concentrations was recorded at most stations in August and September, while in October, concentrations were generally higher, indicating recovery (Khalaf *et al*., 2006); in the first samples collected after the oil spill, most microphytoplankton cells were distorted and empty, particularly near the spill site; there was no general decrease in total numbers of phytoplankton, but a decrease in specific diversity; it seems that some species did not resist this situation and disappeared from the environment. After two years, environmental parameters (nitrates, nitrites, orthophosphates and chl-*a*) did not show significant differences ($p > 0.5$). In conclusion, and compared with studies in the benthic zone, the apparent impact of oil spill in the pelagic zone was limited to the first months after the accident. Analyses of trace elements in fish, mussels, sediments and fuel oil north of the spill site are still ongoing because they still contaminated.

Recent changes in phytoplankton during the period 1999-2009 can be summarized as follows:
At the offshore station, changes in phytoplankton density in the surface layer were related to rainfall (e.g. 1999, 2003, 2004, etc.). At 40 m and 60 m, variations were slight. The same diatom species appear with variable timing every year during spring, and form variable proportions of total microphytoplankton; In summer and autumn, the dominant species vary from year to year, and naked dinoflagellates often dominant, especially in autumn. At 40 m and 60 m (above and below the thermocline), diatom species are usually dominant in spring and occasionally in autumn and winter (e.g. 2004).
At these depths too, naked dinoflagellates are always dominant during summer and autumn.
At coastal stations, interannual variations in abundance and in dominant species are related to meteorological conditions.

Toxic phytoplankton response to warming in two Mediterranean bays of the Ebro Delta

Margarita Fernández-Tejedor [1], Maximino Delgado [1,2], Esther Garcés [2], Jordi Camp [2] and Jorge Diogène [1]

[1] IRTA, Sant Carles de la Ràpita, Spain
[2] Institut de Ciències del Mar, Barcelona, Spain

ABSTRACT

The analysis of phytoplankton and environmental parameters of the Alfacs and Fangar bays time series (northwestern Mediterranean) from 1990 to 2009 reveals certain trends. There is an increase in the average water column temperature by 0.11, 0.01, 0.80 and 0.23 ºC for spring, summer, fall and winter respectively in Alfacs Bay and by 1.76, 0.71, 1.33, 0.89 ºC for spring, summer, fall and winter in Fangar Bay. The trends in phytoplankton populations show a shift in the timing of occurrence of *Karlodinium* spp. blooms and an increase of *Pseudo-nitzschia* spp. abundance. There is no correlation between the average seasonal temperatures and toxic phytoplankton abundance.

INTRODUCTION

The linear warming trend of global surface temperature over the 50 years from 1956 to 2005 is 0.13 [0.10 to 0.16] °C per decade (IPCC, 2007). In the Western Mediterranean, during the same period of time the linear trend is 0.002 - 0.009 ºC per year, 0.025 ºC per year for the period 1973-2005 at a station in Estartit on the Catalan coast (Vargas-Yáñez *et al.*, 2008). In other areas, Peperzak (2003) suggests an increase in toxic phytoplankton blooms due to climate change as a conclusion of different laboratory studies. Analysis of data from the Continuous Plankton Recorder in the northeast Atlantic shows that climate oscillations and warming play an important role in governing fluctuations of some harmful algal species, from exceptional blooms to long-term decadal trends (Edwards *et al.*, 2006).

Increase in temperature is a factor to consider affecting the seasonal composition of phytoplankton and the position of biogeographic boundaries; each species has a temperature window for survival and optimal growth, although we know that many phytoplankton species from coastal regions tolerate changes in temperature. Other factors such as stratification, upwelling, freshwater run-off from land, and cloud cover, need to be considered as indirect effects of climatic variations, and these factors may be as important as temperature increase alone to explain responses of phytoplankton to climate change (Dale *et al.*, 2006). Other factors to consider are pH - surface ocean pH has decreased by ~ 0.12 units to 8.2 (Hays *et al.*, 2005) - and the increase in atmospheric CO_2 from a pre-industrial level of 280 µatm to the present 370 µatm.

Time series of environmental parameters and phytoplankton abundance are needed to assess trends in phytoplankton populations. Unfortunately, most toxic phytoplankton time series in the

Mediterranean have started only recently (since the 1990s), associated with European legislation on shellfish growing areas; and longer series, more than 30 years are necessary to reveal convincing relationships between climate and harmful algal blooms (Dale *et al.*, 2006).

Alfacs Bay, located in the south of the Ebro delta, has a surface of 50 km^2 and a maximum depth of 6 m. Fangar Bay, located in the north of the Ebro delta, has a surface of 12 km^2 and a maximum depth of 4 m (Camp and Delgado, 1987). Both are important aquaculture areas and receive freshwater through irrigation channels used for rice cultivation. The first studies of phytoplankton in the Ebro delta area, outside the bays, were by Herrera and Margalef (1963) and Margalef and Herrera (1964), and later by Estrada (1972) and López and Arté (1973) in Fangar Bay; and by Delgado (1987) in both Alfacs Bay and Fangar Bay. In August 1987 a programme was established to monitor the quality of the shellfish growing areas in Catalonia; since then phytoplankton and oceanographic parameters have been monitored weekly.

Toxic events in these bays are related to the presence of *Alexandrium minutum*, *A. catenella*, *Dinophysis sacculus*, *D. caudata*, *Protoceratium reticulatum*, *Karlodinium veneficum*, *K. armiger*, and *Pseudo-nitzschia* spp. (Delgado, 2003; Delgado *et al.*, 1996; 2000; 2004; Diogène *et al.*, 2008; Fernández-Tejedor *et al.*, 2004; 2008; 2009; Garcés *et al.*, 2006). Species of *Pseudo-nitzschia* found in both bays are *P. calliantha*, *P. delicatissima*, *P. fraudulenta* and *P. pungens* (Quijano-Scheggia *et al.*, 2008). There is seasonality in the bloom period for the different phytoplankton species (Fernández-Tejedor *et al.*, 2008). Moreover shellfish mortalities occur in both bays associated with warm events in summer (Lleti *et al.*, 1995; Ramón *et al.*, 2007). Water discolorations due to *Noctiluca scintillans* were observed in the area in 1971 (López and Arté, 1971) but toxic phytoplankton events were not reported before 1989.

The first PSP (Paralytic Shellfish Poisoning) event in the Ebro Delta area occurred in May 1989 (Delgado *et al.*, 1990), associated with a bloom of *Alexandrium minutum*. *Alexandrium* was known to be linked to PSP since 1927 (Sommer and Meyer, 1937); the species *A. minutum* was described from samples taken in Alexandria Harbour in 1956 (Halim, 1960b). The first PSP event on the Mediterranean coast of Spain occurred in 1987 (Bravo, 1993). PSP was not reported in the Mediterranean before 1970 (Hallegraeff, 2003). DSP (Diarrhetic Shellfish Poisoning) is known since 1978 (Yasumoto *et al.*, 1978) and was linked to *Dinophysis* in 1980 (Yasumoto *et al.*, 1980a). The first DSP event on the Spanish Catalan coast occurred in 1998 in Alfacs Bay; DSP was reported on the French Catalan coast in 1987 (Belin and Raffin, 1998). *Dinophysis caudata* was abundant in the western Mediterranean during the Thor cruises in 1909-1910 (Navarro and Bellón Uriarte, 1945), but only since 1980 has it been known that certain cases of diarrhoea in consumers of molluscs are not due to bacteria or viruses, but to DSP toxins, as in the Adriatic (Ciminiello *et al.*, 2003).

This study analyzes the phytoplankton and environmental parameters of the time series from Alfacs and Fangar bays (north western Mediterranean) from 1990 to 2009.

METHODS

In Alfacs Bay and Fangar Bay water temperature, salinity and oxygen have been measured every week since May 1990 at surface and bottom at one station at the centre of each bay. Since January 1992 these parameters are also measured at every meter in the water column, and in June 1992 more sampling stations were added to the weekly monitoring. Since May 1990 phytoplankton species composition has been determined every week at the same stations. The depths for phytoplankton samples are surface, bottom and integrated water column for the central station, but only at the surface for the rest. In 2006, surface samples were replaced by integrated water column samples to adapt the sampling scheme to the European legislation (EC, 2004). Phytoplankton samples are preserved using formalin, and the Utermöhl procedure is used for identification and quantification using magnifications 100x, 200x and 400x (Hasle, 1978). The main references used for the identification are Tomas (1997), Throndsen *et al.* (2007) and the IOC-UNESCO Taxonomic Reference List of Harmful Microalgae (Moestrup *et al.*, 2009). Since October 2000, chlorophyll was measured in duplicate samples, unpreserved and maintained in darkness, by in vivo fluorescence using a TURNER fluorometer (Lorenzen, 1966; Jeffrey and Welschmeyer, 1997).

The average temperature of the water column at the central stations of each bay (Alfacs n = 8-24, Fangar n = 6-18, per week, 8/6 depths per sampling day and 1-3 sampling days per week) was calculated for each week and used to obtain the seasonal average for each year between 1990 and 2009 (6762 and 4661 temperature measurements in total for Alfacs and Fangar bays respectively). Linear regression was used to evaluate the increase or decrease in seasonal average temperature. Winter average refers to the year when the season begins.

Water temperature anomalies were calculated for each week and depth, the water column average of the anomalies was calculated for each week, and used to obtain seasonal anomalies for each year. The mean of all the standard deviations for each week and depth per season was used to determine which years were different from the average due to the lack of significant differences between different years for each season. The Kruskal-Wallis One Way Analysis of Variance on Ranks result was H = 18 with 18 degrees of freedom (P = 0.456) for spring, fall and winter; H = 19 with 19 degrees of freedom (P = 0.457) for summer.

The temperature and salinity measurements were used to calculate sigma-t for each week and depth. The difference between sigma-t at 6m and at 0.5 m depth was used as a stratification index. The average stratification index was calculated for each season.

The average phytoplankton species abundances of the surface and bottom weekly samples at the central station of each bay were used to calculate the seasonal average per year.

The Spearman Rank Order Correlation was employed to test the correlation between the seasonal temperature and species abundances or the seasonal stratification index and species abundances.

RESULTS

Temperature

In Alfacs Bay, average seasonal temperatures for spring, summer, fall and winter in the period covered were respectively 18.9 ± 0.5, 26.1 ± 0.6, 17.4 ± 1.1, 11.3 ± 1.1 ºC (mean ± SD, n = 19-20 years). In Alfacs Bay the linear trend from spring 1990 to spring 2009 shows a non-significant increase of 0.006, 0.0003, 0.042, 0.012 ºC per year ($R^2 = 0.00, 0.00, 0.05, 0.00$) for spring, summer, fall and winter respectively (Figure 1). In Fangar Bay, average seasonal temperatures were 18.3 ± 0.9, 25.3 ± 0.7, 16.6 ± 1.4, 10.9 ± 1.1 ºC (mean ± SD, n = 19-20 years). The linear trend shows a non-significant increase of 0.093, 0.037, 0.070, 0.047 ºC per year ($R^2= 0.33, 0.10, 0.08, 0.06$) for spring, summer, fall and winter respectively (Figure 2). In these 19 years, from 1990 to 2009, the seasonal water column temperature increased 0.11, 0.01, 0.80 and 0.23 ºC for spring, summer, fall and winter respectively in Alfacs Bay. In Fangar Bay the increase was 1.76, 0.71, 1.33, 0.89 ºC for spring, summer, fall and winter. The means of the standard deviations of the seasonal water temperatures were 1.8, 1.4, 1.3, 1.9 in Alfacs Bay and 1.8, 1.8, 2.4, 2.1 in Fangar Bay for spring, summer, fall and winter respectively; these means were used to determine the years with positive and negative seasonal temperature anomalies. From summer 1990 to summer 2009, in Alfacs Bay, positive water temperature anomalies occurred in fall 1995 and 2006 and negative temperature anomalies occurred in fall 1993 and 1999 and winter 2004-2005. Positive temperature anomalies also occurred in Fangar Bay during winter 1996-1997 and fall 2006. The average water column temperature was higher than 28ºC during 10 weeks for the decade 1990-1999 and 17 weeks for the decade 2000-2009.

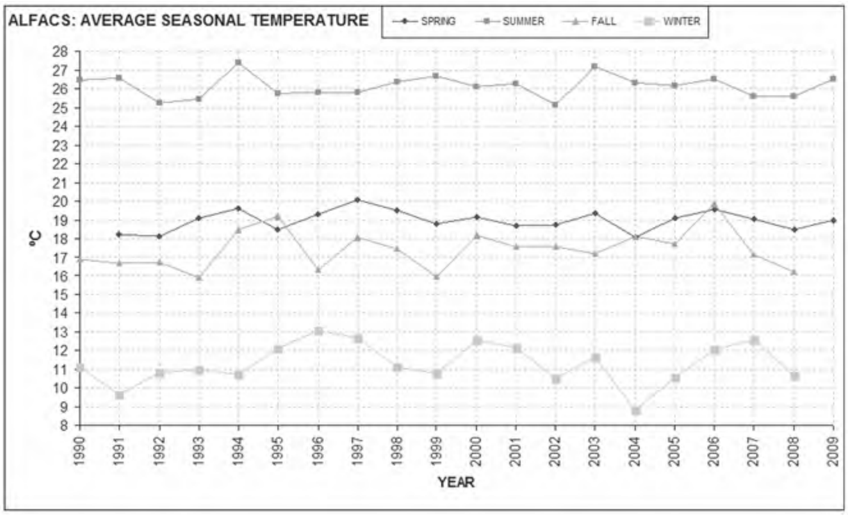


Figure 1. Average seasonal temperature for the period spring 1990-summer 2009 in Alfacs Bay (n = 12-14 weeks).

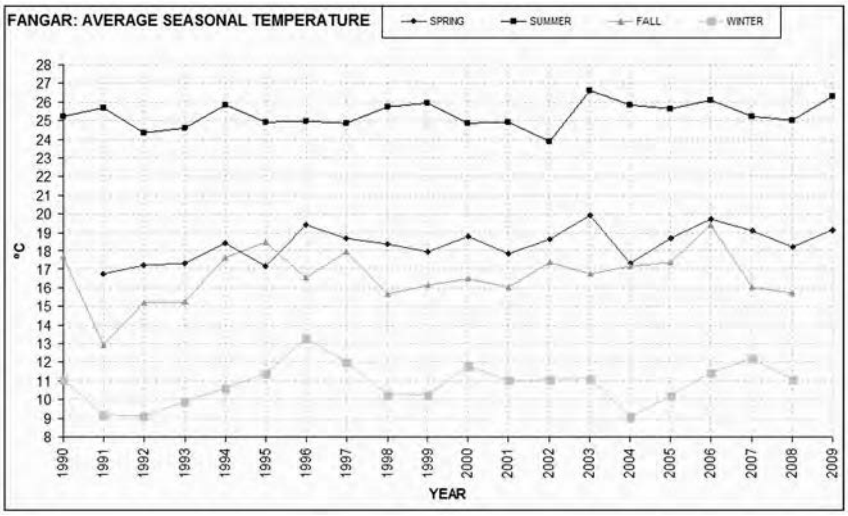


Figure 2. Average seasonal temperature for the period spring 1990-summer 2009 in Fangar Bay (n = 12-14 weeks).

Salinity

For the period 1990-2009, salinity in Alfacs Bay has increased 0.019, 0.030, 0.010 units per year, for spring, summer and fall and decreased 0.014 units for winter, this negative value of the linear trend in winter is due to very low salinities in winter 2008-2009, while the linear trend for winter is positive, 0.039 units for the period 1990-2007.

Stratification

In Alfacs Bay mean seasonal stratification indices were 1.83 ± 0.44, 2.06 ± 0.36, 1.83 ± 0.43, 1.16 ± 0.58 for spring, summer, fall and winter. The maximum was measured in spring 2003, stratification was also strong in winter 1996, summer and fall 1992 (3.2, 3.1, 2.9, 3.0 respectively).

Toxic phytoplankton

In Alfacs Bay there is a shift in the time of occurrence of *Karlodinium* spp. blooms, from winter in 1994-1999 to spring in 2000 and spring-summer in 2003-2009 (Figure 3). Positive correlation exists between *Karlodinium* spp. abundance and the stratification index in spring (Spearman rank 0,682 P= 0,003). There is a trend to an increase in the abundance of *Pseudo-nitzschia* spp. (Figure 4), more important in summer and fall. *D. sacculus* and *A. minutum* are more abundant in spring and winter. There is a lack of correlation for the averaged temperatures of each season per year and the phytoplankton cell abundance. In Fangar Bay there is a decrease in *D. sacculus* abundance during all seasons of the year, while *D. caudata* increases the abundance during the summer season. In Fangar Bay *Pseudo-nitzschia* spp. blooms do not show the increase in abundance observed in Alfacs Bay.

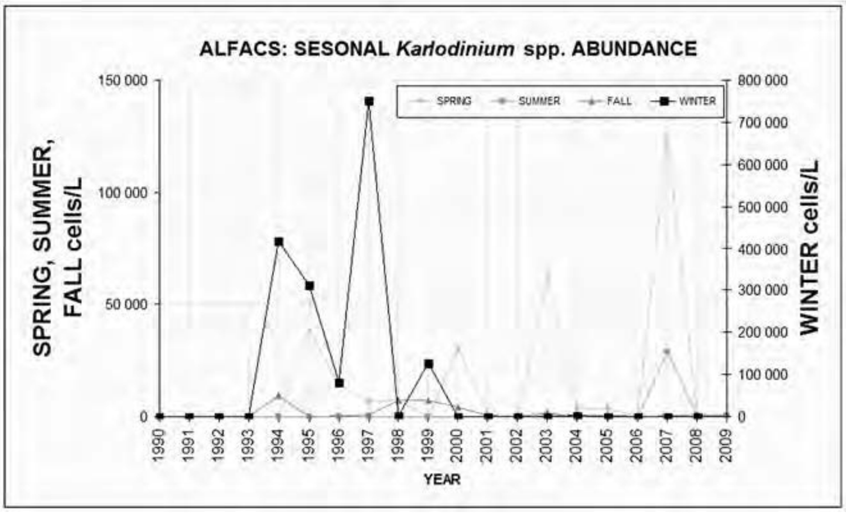


Figure 3. Seasonal *Karlodinium* spp. cell abundance in Alfacs Bay for the period 1990-2009 (n= 12-14 weeks).

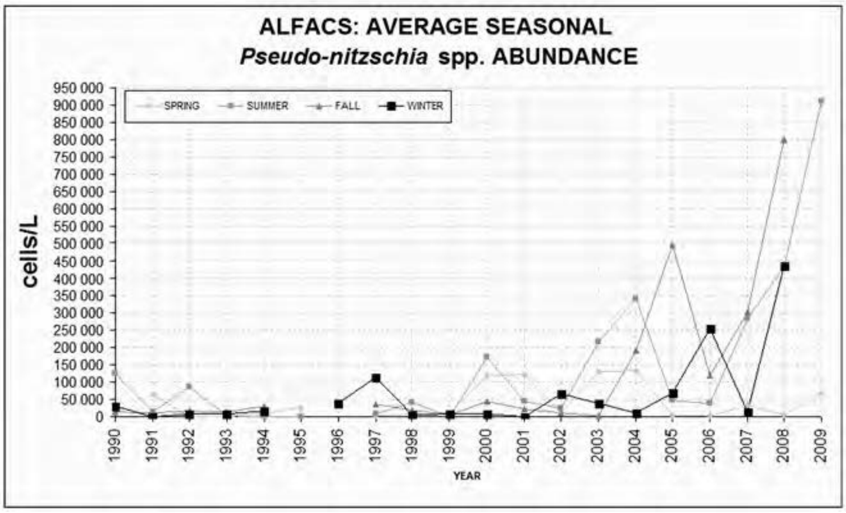


Figure 4. Seasonal *Pseudo-nitzschia* spp. abundance in Alfacs Bay for the period 1990-2009 (n = 12-14 weeks).

DISCUSSION

Long-term changes related to global warming are usually statistically modeled by means of linear regression. Vargas-Yáñez *et al.* (2009) showed that the results reported in different studies on the trends for salinity and temperature in the western Mediterranean depend on the period of time considered and on the data analysis methods used. With linear regression, we detect a significant increase of temperature for the 1990-2009 time series in Alfacs and Fangar bays.

Warming is more intense in Fangar Bay than in Alfacs Bay for all seasons of the year, especially in summer and spring. In Thau Lagoon (Mediterranean coast of France), water temperature increased 0.091, 0.061, 0.052 ºC per year for spring, summer and fall for the time series from 1972 to 2006 (Collos *et al.*, 2009). The increases in temperature in Thau Lagoon for spring and fall are similar to those in Fangar Bay but the summer increase in water temperature in Fangar Bay is only half that in Thau.

Average seasonal water column temperatures for the period 1990-2009 were 18.3, 25.3, 16.6, 10.9 ºC for spring, summer, fall and winter in Fangar Bay. For comparative purposes, López and Arté (1973), who measured the water column temperature at the same station during the years 1968-1971 calculated seasonal water column temperatures as 17.9, 24.5, 14.2, 10.7 ºC for spring, summer, fall and winter. Comparison between the averages for the periods 1968-71 and 1990-2009 shows respective increases of 0.5, 0.8, 2.4, 0.1 ºC (Figure 5).

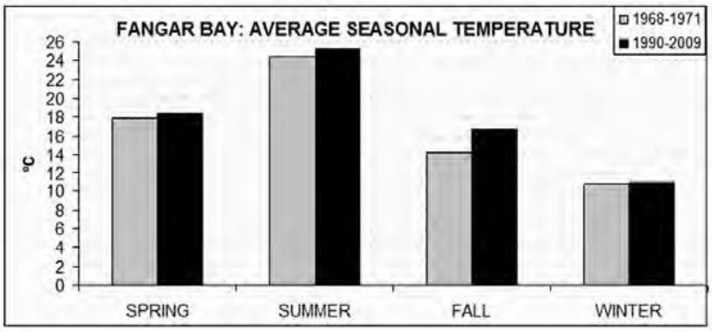


Figure 5. Average seasonal temperature in Fangar Bay. The average values for the period 1968-1971 are shown by grey bars, black bars show the average for the period 1990-2009. The frequency of measurements was monthly for 1968 and bimonthly for 1969-71, in total 27 days of measurements in 4 years.

Positive thermal anomalies, even of short duration, may represent an important physiological change for some organisms such as *Mytilus galloprovincialis*. During warm events, a reduction in grazing together with an increase in nutrients could lead to the high phytoplankton abundance observed in 2003 in Fangar Bay (Ramón *et al.*, 2007). The effect of water temperature on mussel (*M. galloprovincialis*) mortality was studied in laboratory experiments by Anestis *et al.* (2007), who showed that animals started to die as water temperature reached 26ºC, and that when temperature exceeds 25ºC filtration falls significantly. In Alfacs and Fangar bays, summer shellfish mortalities occur due to high water temperatures (Lleti *et al.*, 1995; Ramón *et al.*, 2007). The average water column temperature in summer for the period 1990-2009 for Alfacs and Fangar bays were 26.1 ± 0.6 and 25.3 ± 0.7 (mean ± SD), exceeding the threshold for filtration rate in both bays and for mortality in Alfacs Bay.

There is no correlation between the averaged temperatures of each season per year and toxic phytoplankton cell abundances. The analysis of the toxic phytoplankton species present in Alfacs and Fangar show a shift in the bloom period for the ichthyotoxic *Karlodinium* (Figure 3), and an increase in *Pseudo-nitzschia* (Figure 4) densities in the last seven years. Spring *Karlodinium* blooms are correlated with stratification. The main factor controlling stratification in Alfacs Bay is the status of the irrigation channels discharging into the bay (Solé *et al.*, 2009). The management of the channels is controlled by the associations of rice producers and shellfish producers, which generates two main period, an ‘open channel’ - (more stratified water column) and a ‘closed channel’ - (less stratified water column).