

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).

The construction of new sewage treatment plants along Mediterranean coasts has increased greatly since the late 1980s, which has resulted in 'oligotrophication' of numerous coastal sites and the 'recovery' of benthos and fish populations. This oligotrophication signal has been documented by ongoing monitoring programmes at many coastal sites. On the other hand, aquaculture activity has also increased in many coastal zones, and is associated with increased nutrient inputs. Thus, in many areas we will not be able to distinguish the effects of local man-made shifts from those caused by global change for a long time.

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 as:

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 *Ethmodiscus* 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 *Neostreptothecca* 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 dinophysaceans 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 “opportunistic” 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 >