

## 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 °C 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.

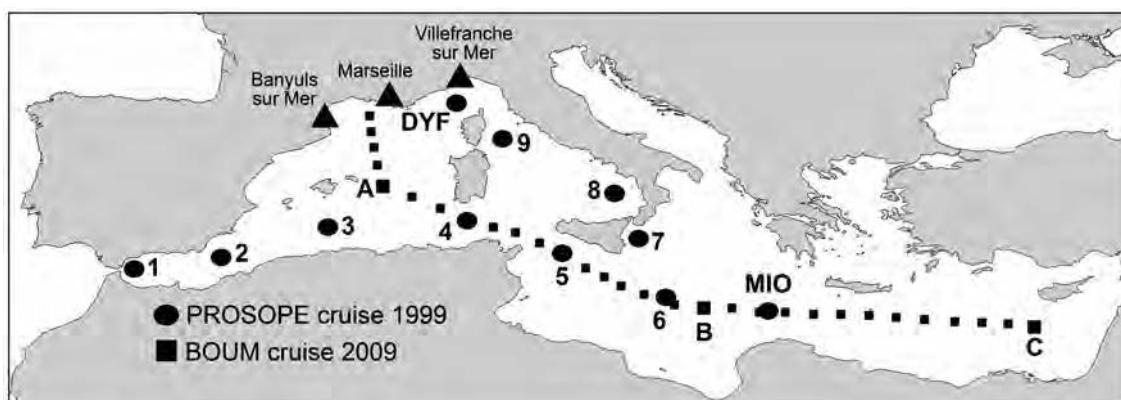


Figure 1. Map of sampling stations during the PROSOPE and BOUM cruises, and phytoplankton monitoring sites at Villefranche-sur-mer, Marseille and Banyuls-sur-mer.

## 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.

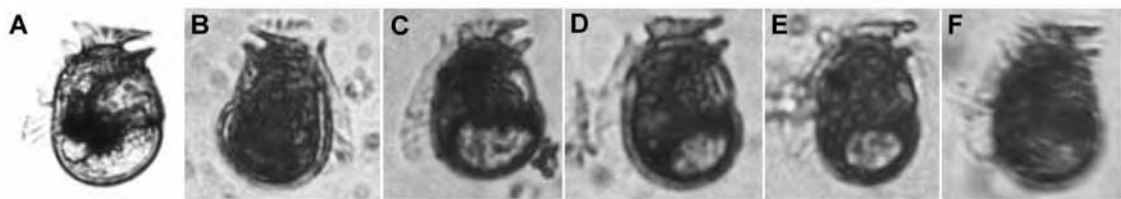


Figure 2. The tropical dinoflagellate *Citharistes regius*, found for the first time in the Gulf of Lions and in open waters of the Ionian and Levantine basins. **A.** Live specimen from Banyuls-sur-mer. **B-F.** Lugol-fixed specimens from the BOUM cruise.

### 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.

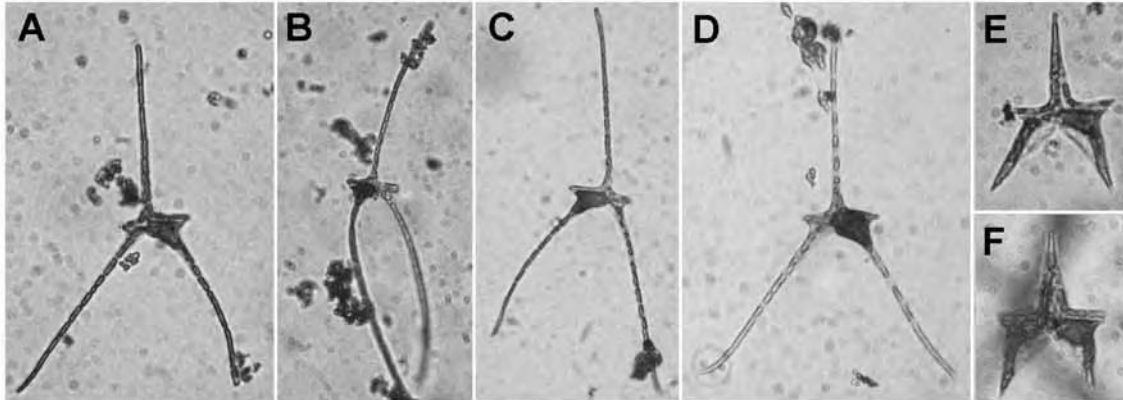


Figure 3. The dinoflagellate genus *Microceratium*, found for the first time in the Mediterranean. It is here considered to be a “tropical morphotype” of the local species, currently under the genus *Karenia*.

### 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).

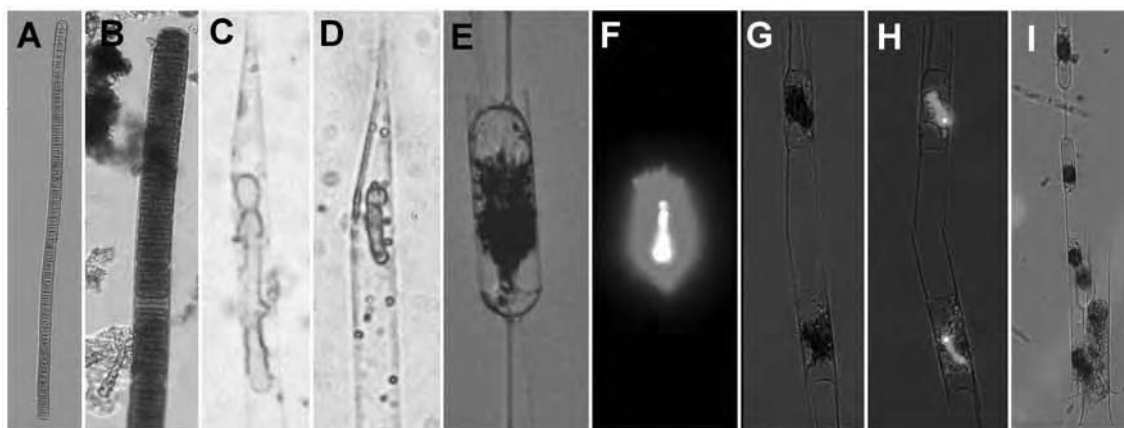


Figure 4. **A-B.** Tentatively, the cyanobacterium *Trichodesmium* from Banyuls-sur-mer. **C-D.** *Richelia intracellularis* endosymbiont in *Rhizosolenia clevei* from Endoume, Marseille and from the open Mediterranean during the BOUM cruise. **E-F.** *Richelia intracellularis* in *Hemiaulus hauckii*. **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

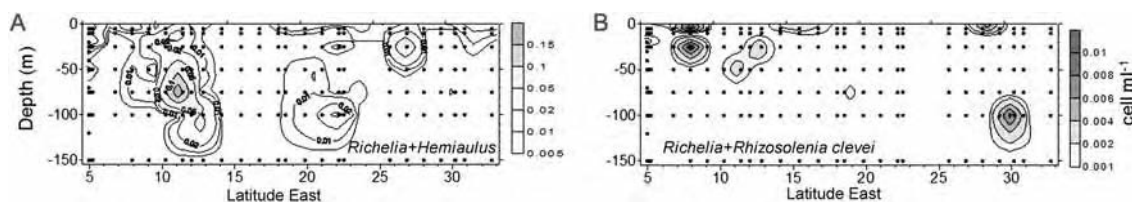


Figure 5. Distribution and abundance of *Richelia intracellularis* as endosymbiont in *Hemiaulus hauckii* (A) and *Rhizosolenia clevei* (B) during the BOUM cruise.

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.

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