Training and Integration: Sabbatical Reports

MarPLAN on the move

Short-term sabbaticals are a MarBEF initiative to support integration of research effort in Europe. Here we hear from the RMP MarPLAN on two MarBEF sabbaticals and the research carried out by two PhD students: Alina Tunin-Ley and Elodie Foulon. Their sabbaticals involved short-term visits to and from the Laboratoire d'Océanographie de Villefranche in France.

Alina Tunin-Ley.

Phytoplankton: climate-change indicators

By Alina Tunin-Ley

I began my PhD thesis in October 2004 in the Laboratoire d'Océanographie de Villefranche (LOV, Observatoire Océanologique de Villefranche, Université de Paris VI, France), supervised by Frédéric Ibañez and Rodolphe Lemée. My PhD project focused on the impact of global change on the biodiversity of the genus *Ceratium* Schrank (planktonic dinoflagellates) in the NW Mediterranean, and its interest as a potential climate-change indicator.

Among planktonic organisms, a northward extension of species distribution areas has been demonstrated for copepods in the North Atlantic (Beaugrand *et al.,* 2002) from weighty CPR data; yet, little is known about phytoplanktonic species, although this compartment is the basis of the marine trophic web. This is mainly due to two factors: firstly, species identification remains very arduous and uncertain for most of the genera, with an increasing difficulty from microphytoplankton (from 20 to 200µm) to picophytoplankton (<2µm); secondly, long-time series recording phytoplankton species are still scarce. The genus *Ceratium* might pose a possible solution to the problem. It possesses numerous attributes (Fig. 1) that facilitate sampling and identification, with cell size ranging from 50 to 1,000µm (microphytoplankton), resistant cellulosic theca, cosmopolitan range, low variability in annual abundances and very well-documented and illustrated literature. Besides, it exhibits a very high specific and infraspecific richness, especially in the Mediterranean Sea, where it has been largely studied from the end of the 19th century. Thus, we benefited from the early establishment of several marine stations on the French and Italian coasts that allowed the profuse publication of works describing the annual composition of *Ceratium* species, among other phytoplanktonic species. From this old bibliography, chosen with several constraints depending on the nature of the data and the sampling, it was possible to extract ancient data, which would then be combined with recent data in order to constitute a long-term dataset. The recent data corresponded to net samples, realised in the framework of a long-term monitoring of the microphytoplankton in the bay of Villefranche at a station called 'Point B.' However, the bibliographic works which were retained described *Ceratium* species' occurrence in the Ligurian Sea but also in the Tyrrhenian basin. So it was really interesting for us to obtain recent data on *Ceratium* species' occurrence in this area. To that purpose, collaboration with the Stazione Zoologica di Napoli was proposed. Indeed, there was also a long-term monitoring of the phytoplankton at a fixed point (named Mare Chiara) in the bay of Naples that could provide the needed recent Tyrrhenian data.

I went to Naples in October 2004 for a short sabbatical (15 days), funded by the MarBEF project MarPLAN. There, I was welcomed by Adriana Zingone and her colleagues. She organised my stay in the Station of Naples and my work in the laboratory. I analysed Tyrrhenian net samples, using an inverted microscope. This short sabbatical offered me my first experience of presenting work from my PhD project outside of my own laboratory. I also benefited from acquiring new complementary skills and knowledge in this area. The final dataset that I acquired was composed of six "studies," each describing the monthly *Ceratium* species' richness throughout one or several annual cycles: four bibliographic studies (considered as old data) and two field studies (corresponding to recent data), resulting from the counting of samples from Villefranche bay and Naples bay. The analysis of this multidimensional set of binary data (presence/absence) required an ordination method, namely Multiple Correspondence Analysis (MCA). The results suggested that the species composition was specific to each site, with a division between the Tyrrhenian and the Ligurian sites. However, the composition of *Ceratium* species in Villefranche appeared to be more closely related to the Tyrrhenian sites' assemblages. Moreover, when the corresponding surface water temperatures were added to the analysis, we observed a relationship between recent data and positive temperature anomalies, whereas ancient data were globally linked to negative temperature anomalies. Thus, the work highlighted a warming of surface water in the NW Mediterranean that has affected the annual composition of *Ceratium* species in this area, although this change was more subtle than expected. Indeed, no typical warm-water or tropical species clearly appeared and developed during the time-period studied. Nevertheless, several of these species could act as potential indicators of climate change. It is also likely that recurrent events in the annual biodiversity of this genus, as occurrence of the minimal richness or seasonal dominance of some species, could constitute good indicators and should also be taken into consideration (Tunin-Ley *et al.,* 2007).

Using *Ceratium* species as biological indicators of global warming in the Mediterranean needs a very good definition of each taxon. Based on morphology, the genus has been subdivided into more than 120 infra-genus taxa including species, subspecies, forms or varieties. However, given the morphological variability observed within species, the definitions of those taxa are unresolved. Some authors have multiplied forms and varieties while others did not recognise any subspecific levels. While temperature is likely to be one of the main ecological factors controlling *Ceratium* species' distribution, this parameter may be associated with morphological variations; a study by Sournia (1967) suggested that some subspecific variability (i.e. varieties) of the genus *Ceratium* might reflect distinct thermal types. Sournia used a very specific and unofficial nomenclature to distinguish those varieties. For example, *Ceratium candelabrum*

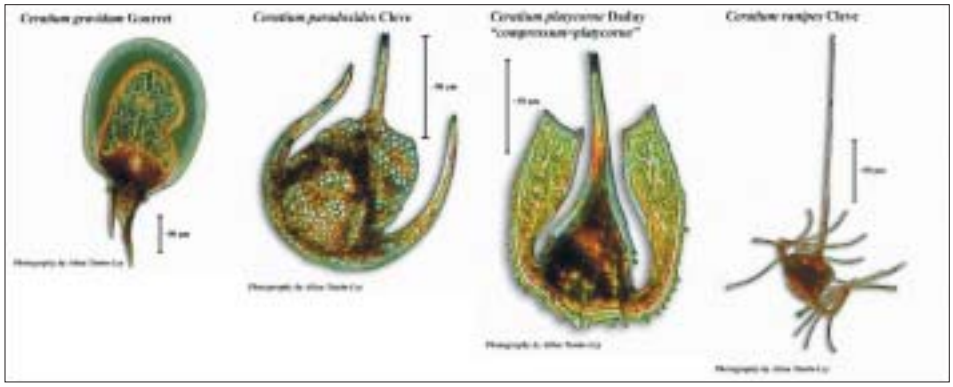

Figure 1. Sample of *Ceratium* species from the Villefranche bay (cells were stained in Lugol's acid solution).

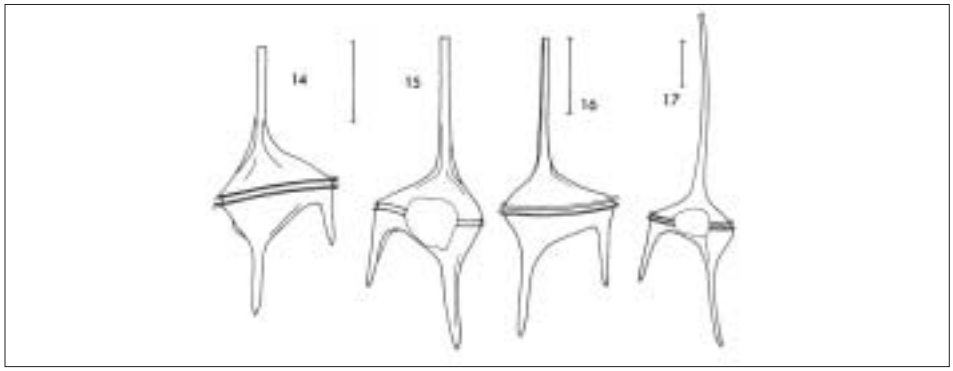

Figure 2. Varieties and intermediate taxa of *Ceratium candelabrum* from the unofficial para-systematic designation of Sournia (1967). Illustrations from Sournia (1967).

(Fig. 2) may have a psycrophylous variety named *C. candelabrum var. candelabrum* and a thermophylous variety named *C. candelabrum var. depressum*. When a morph between these two varieties was observed, it was named *C. candelabrum "candelabrum–depressum."* When the morph was nearer to the second variety, it was called *C. candelabrum "candelabrum >depressum."*

Whether those morphological infraspecific variations really reflect thermal preferences or correspond to pseudo-cryptic species is unknown. To answer this question, our Villefranche team contacted another MarPLAN member, Dr Nathalie Simon, from Observatoire de Roscoff, France. Rodolphe Lemée, one of my PhD advisors and associate professor at University of Paris VI (Observatoire de Villefranche), spent three sabbaticals (autumn 2005 & 2006 and spring 2007) at Roscoff to study molecular techniques and to work on *Ceratium* phylogeny. Studies were initially made on LSU rDNA and then on the Intergenic Transcribed Spacer (ITS), which was considered as more accurate for reconstructing phylogenetic trees, in order to evaluate the intraspecific genetic diversity in *Ceratium* genus. A Master's student, Anne-Lise Cras, worked on this subject during the first semester of 2007 and has obtained more than a hundred sequences of *Ceratium's* ITS, using the single-cell method. This method allowed her to clearly relate molecular diversity to morphological diversity. The preliminary results suggest (1) the presence of both cryptic species and ecomorphs in *Ceratium* taxa and (2) the need for a complete review of the subgenus definition. These hypotheses will be confirmed using other molecular markers. Anne-Lise Cras has just received financial support from the University of Paris VI to carry out a PhD on the "Relationship between morphological diversity, molecular diversity and biogeography of marine microplankton."

References

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Infra-specific genetic variability within *Micromonas pusilla,* a green picoplanktonic alga

By Rodolphe Lemée

As part of the MarPLAN project, our team also participated in the study of infra-specific genetic variability within *Micromonas pusilla,* a green picoplanktonic alga *(Prasinophyceae)*, through a second sabbatical, where Elodie Foulon (PhD student) travelled to Villefranche. *Micromonas pusilla* is cosmopolitan and widespread in marine systems and has been proved to dominate the picoplanktonic assemblages in coastal systems such as the English Channel. The *Micromonas* taxon is genetically diverse and includes several genetically distant clades.

Elodie Foulon is working on the infra-specific ecological, morphological and physiological diversity of *Micromonas pusilla* in the frame of her PhD thesis, directed by Nathalie Simon and Frédéric Partensky at the Station Biologique de Roscoff (SBR). Wiebe Kooistra (Statione Zoologica di Napoli) is an advisor on this project and visited the SBR in December 2006 and July 2007 to share his expertise on cryptic species in phytoplankton.

In order to identify potential separate niches for these genetic clades, Elodie Foulon monitored the densities of three genetic clades in different water masses, including the waters of the Villefranche bay. She was able to sample in the Villefranche bay in the frame of the MarPLAN collaboration with Rodolphe Lemée (one week in spring 2006). Our Villefranche team also sent further samples to be analysed in Roscoff.

It appeared that *M. pusilla's* densities decreased during the stratification event and that all the genetic clades were present in the Villefranche bay, but one clade was dominant and another clade was only detected at depth. The results obtained, together with other results obtained in other biogeographical regions, will be published soon (Foulon *et al.*, in prep.).

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