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A brief and personal history of plankton research in Roscoff

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Abstract: In the last forty years, marine plankton studies have considerably developed at the Roscoff Biological Station, leading to the development of one of the largest research group in the world working on this topic. This has been possible through a suite of technological advances that allowed us to have a better view of marine protist communities, from flow cytometry to high throughput sequencing. In this paper, I retrace the growth of the Roscoff Plankton Group and present some of the key scientific advances made during these four decades.

Résumé : *Une histoire brève & personnelle de la recherche sur le plancton à Roscoff*. Au cours des quarante dernières années, les travaux sur le plancton marin se sont considérablement développés à la Station Biologique de Roscoff, aboutissant à la formation d'un groupe de recherche qui est sans doute le plus important au monde sur ce sujet. Cela a été rendu possible grâce à une suite d'avancées technologiques, de la cytométrie en flux au séquençage haut débit. Dans ce papier, je retrace les étapes de la croissance du "groupe Plancton" et présente quelques-unes des avancées scientifiques clées au cours de ces quatre décades.

Keywords: Plankton ● Marine station ● Flow cytometry ● Molecular methods ● History of science

Introduction

When, during the winter 1961, Guy Jacques and four other students from the newly created Biological Oceanography graduate program ("3ème cycle" in French) arrived at the Station Biologique de Roscoff (SBR) to start two joint theses on plankton, they were greeted by the Director Georges Teissier by these words: "Vous venez étudier le plancton de la Manche. Mais il n'y en a pas et il est bien connu" (You are coming to study the plankton of the English Channel, but there is none - G. Jacques remarked that he probably meant little - and it is well known", Jacques, 2022). In a somewhat brutal way, G. Teissier was following the assertion of John Gerould who remarked in the journal Science in 1899, when touring the Biological Station of Brittany, that "As regards to the fauna, the fact is to be emphasized that for plankton studies Roscoff is badly situated " (Gerould, 1899). In some way, the situation on the other side of the English Channel was much better for plankton studies, with the Plymouth Marine Laboratory of the Marine Biological Association pursuing very active research on phytoplankton production and taxonomy (e.g., Harvey et al., 1935; Manton, 1959).

A bit more than sixty years after the arrival of G. Jacques and his friends, the Roscoff laboratory has become one of the most important research centres for plankton studies, extending from marine protists to viruses. Over all these years it has produced over 800 papers, including in the most prestigious journals such as Science and Nature

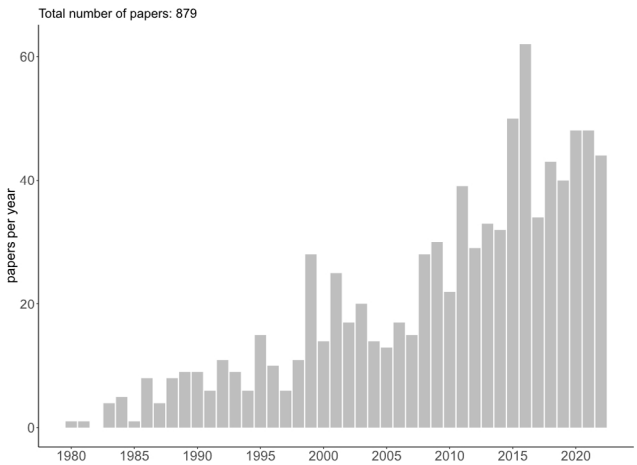

Figure 1. Plankton papers co-authored by members of the plankton group over the years. The full list is available at: https://www.zotero.org/groups/312573/roscoff_plankton_group/library

(Fig.1). I am going to retrace some of the steps of this adventure. This is a very personal point of view and all participants may be not named, but still they will know that they were part of this "épopée" in one way or the other.

The pioneers

Although they were not really studying plankton, two names must be mentioned, since they were the first researchers involved in protistology in the Roscoff laboratory. Enrique Balech (Dolan, 2022) was an Argentinian phycologist who spent two months in Roscoff in the summer of 1952 to study the dinoflagellates from the sand of the intertidal zone. He described several new species and in particular *Roscoffia capitata*, named in the honour of Roscoff (Balech, 1956). Gilbert Deroux was a "Maître-Assistant" (Junior Professor) in Roscoff between 1955 and 1985. His research focused in particular on ciliates (e.g., Deroux, 1974 & 1978), and several species have been named in his honour (e.g., *Dysteria derouxi*, Gong & Song, 2004).

However, research on plankton really started with the arrival of five students sent like a commando squad by Pierre Drach, who was in charge of the newly created graduate program in Biological Oceanography at the University of Paris. Guy Jacques and Jean-René Grall were to study phytoplankton, while Claude Razouls, France Bodo and Alain Thiriot were to focus on zooplankton (Fig. 2). They achieved a "thèse de 3ème cycle collective" (corresponding more or less to a Master thesis by current standards) according to a practice that was quite current back then, i.e. to write "collective" thesis manuscripts. Two papers were published summarizing the observations they carried out from February 1962 to September 1963 (Grall & Jacques, 1964; Bodo et al., 1965), presenting some key features of plankton off Roscoff which were confirmed in later studies (Martin-Jézéquel, 1983; Sournia & Birrien, 1995) such as the occurrence of two phytoplankton blooms, one in the spring and one, more unusual, in the fall, these blooms co-occurring

Figure 2. Top. Four of the initial 5 graduate students that studied plankton in Roscoff in 1962-1963: J.R. Grall, C. Razouls, G. Jacques (first three from left) and F. Bodo (last on the right) during a cruise on the Thalassa in 1960 (reprinted from Jacques, 2022). **Bottom.** Seasonal succession of diatoms in Roscoff (reprinted from Grall & Jacques, 1964).

with a zooplankton surge or the importance of diatoms such as *Rhizosolenia delicatula* Cleve, 1900 (now *Guinardia delicatula*) and of copepods such as *Centropages*, *Temora* and *Acartia*.

Of the five plankton musketeers, only one, Jean-René Grall, stayed in Roscoff and continued to work on phytoplankton of the English Channel, successfully defending a PhD thesis (thèse d'Etat, Grall, 1972). In the late 70's, two students started a 3rd cycle thesis, Véronique Martin-Jézéquel and Mohideen Wafar, who respectively studied phytoplankton and nutrients in

the waters around Roscoff. Véronique was the first in Roscoff, during her thesis, to create a collection of phytoplankton strains for research purpose (see below), isolating diatom species from coastal waters. At the same time, Catherine Riaux was pursuing a PhD thesis on the taxonomy of benthic diatoms at the SBR. Unfortunately, J.R. Grall passed away in 1980 and this jeopardized the construction of a strong plankton group in Roscoff.

The plankton group from the 80's to present

One can really anchor the birth of the Plankton Group at the SBR with the arrival in the 80's of Serge Poulet (Fig. 3), a zooplanktonologist who had worked previously in Canada, in particular developing methods to measure size and abundance of plankton with the Coulter Counter, an instrument based on electrical impedance. In Roscoff, he developed research on the chemosensory grazing of zooplankton in particular around amino acid composition and signalling (Poulet & Ouellet, 1982) and then moved to look at the negative interactions between diatoms and copepod reproduction, leading to a paper in Nature (Miralto et al., 1999). Serge also initiated closer relationships with the Plymouth Marine Laboratory across the Channel. He retired in 2008.

In 1983, Alain Sournia, a phytoplankton specialist and in particular of dinoflagellate taxonomy, who had previously worked at the French National Museum of History (MNHN), obtained a position at CNRS (Centre National de la Recherche Scientifique) and moved to Roscoff. He started the time-series of plankton observation off Roscoff (Sournia & Birrien, 1995) and pushed for studies of the frontal regions in the English Channel and the Mediterranean Sea (Sournia, 1993). Alain also published during this time the monumental multi-volume "Atlas of Marine Phytoplankton" (Sournia, 1986). Unfortunately, he stayed only a few years in Roscoff, moving back to Paris in 1987, but remained still affiliated with the SBR lab for a few more years. Alain passed away in 2018 (Partensky & Vaulot, 2018).

Véronique Martin-Jézéquel obtained a CNRS position in the plankton group in 1984. She continued studying ecophysiology of phytoplankton populations of English Channel and Atlantic waters. Working with Serge Poulet on phyto-zooplankton relationships, she developed a novel approach to analyse amino acids in cultures and natural population of phytoplankton. Her work progressively focused on the physiology of diatoms using laboratory cultures. She moved to Brest in 1997 and then to Nantes in 2003, where she concentrated on the metabolism of silica and nitrogen in diatoms. She retired in 2020.

Figure 3. Serge Poulet during the Donghai cruise in Dec 1985 - Jan 1986 in China that aimed to analyse biogeochemical processes at the interface between the Chang Jiang (Blue river) and the China Sea.

I first arrived at the SBR in the summer of 1984, but really moved to the laboratory in early 1985. I had been recruited at CNRS a couple of years before in the follow-up of Mitterrand's election, initially to study fish populations in the lagoons around Montpellier. At that time, I was doing my PhD at the Massachusetts Institute of Technology (MIT) in the laboratory of Penny Chisholm, working on the control of phytoplankton cell growth and division by external factors such as light and nutrient. I managed to convince CNRS to let me first finish my thesis and then to move to a laboratory where I could continue working on phytoplankton. As my wife had just got a position at IFREMER Brest, Roscoff seemed to be a good option.

I must say that my first months at the SBR were a bit dismal after the effervescence of MIT. At that time, Roscoff researchers lived in a kind of monastic atmosphere in that sense that they had usually an office immediately next to their laboratory, or even their office and laboratory in the same room (called a "stalle", due to their similarity to horse stalls) from which they rarely emerged. There was virtually no equipment to work on phytoplankton and those available, e.g. microscopes, were locked out in "stalles" where they were very difficult to access. The first real break came at the end of 1985 when, through the SBR director Pierre Lasserre, I had the chance to be invited to participate in the Donghai project which aim was to study the interactions between the Chang Jiang river and the China Sea (Fig. 3). This allowed me to start building a network in France as well as obtain some

Figure 4. Top. The Plankton group at the end of the 80s. Michel Viollier, Catherine Riaux-Gobin, Jean Louis Birrien, Bert Klein, Alain Sournia, Véronique Martin-Jézéquel, Serge Poulet, Daniel Vaulot, Suzanne Roy, Ana Maria Hapette. **Middle.** The Plankton group in 2002. Front row: Claire Carré, Florence Le Gall, Khadidja Romari, Dominique Marie, Isabelle Biegala, Delphine Doussal, Frédéric Partensky, Nathalie Simon, Fabrice Not. Back row: Isabelle Mary, Alexis Dufresne, Christophe Six, Bastien Simonnet, Daniel Vaulot. **Bottom.** The Plankton group in 2009. Front row: Yoshiyuki Ishitani, Antonio Pagarete, Océane Dahan, Priscillia Gourvil, Xiaoli Shi, Florence Le Gall, Dominique Marine. Second row: Yurika Ujiié, Sarah Romac, Sylvie Masquellier, Chrsitophe Boutte, Laure Guillou, Fabrice Not, Fabienne Jallabert. Back row: Shuhei Ota, Aurélie Chambouvet, Manon Viprey, Estelle Bigeard, Miguel Frada, Anne-Catherine Lehours, Christophe Six, Cécile Lepère.

funds to purchase basic equipments such as a good microscope.

Another key element for the development of the plankton group was the arrival of Frédéric Partensky in 1986 who started a PhD thesis under the joint direction of Alain Sournia and me. His thesis focused on two dinoflagellates that often bloom in the English Channel, *Gyrodinium* cf. *aureolum* kott, 1983 and *Gymnodinium nagasakiense* H.Takayama & M.Adachi, 1985, (Partensky et al., 1988), now gathered under the common species name *Karenia mikimitoi*. His presence brought the plankton group to a critical size. Claude Courties, a research engineer at CNRS who had worked previously with the SBR director Pierre Lasserre, helped us to operate our recently acquired flow cytometer (see below).

The year 1990 turned out to be critical as Alain had left for Paris and both Frédéric and I were away, Frédéric doing a post-doc at the Bedford Institute of Oceanography in Dartmouth (Canada) and myself spending a year at the University of Hawaii where I set up a flow cytometry facility and participated in the early years of the HOT project (Hawaii Ocean Time series). The next year, however, 1991 saw the real consolidation of the plankton group with my return to the SBR, the recruitment of Frédéric by CNRS, followed by the arrival of new PhD students Nathalie Simon in 1991, Laure Guillou in 1995, Stéphan Jacquet and Laurence Garczarek in 1996. These arrivals led soon to an expansion of the group with the recruitment on permanent positions of Laure, Nathalie and Laurence (Fig. 4), Stéphan getting a position at INRA (now INRAE) in Thonon. During this period the group focused on picophytoplankton in particular developing the use of flow cytometry to measure these tiny cells (see below). Dominique Marie, a CNRS research engineer who had worked previously on plants in Gif-sur-Yvette near Paris, joined the group in 1992 and played a key role in the development of flow cytometry. At the same time, we also began to develop a collection of strains that later became the Roscoff Culture Collection.

One key ingredient for the development of the plankton group at the end of the 90s was the funding provided the European Union with medium-size projects such as PROMOLEC (*Prochlorococcus* Molecular Ecology, 1998-2001) and PICODIV (Diversity of Picoplankton, 2000-2003) that not only allowed us to finance PhDs and post-doctoral positions but more importantly built an enduring network of European colleagues with whom we collaborated and published, such as David J. Scanlan from Warwick University, Wolfgang Hess from the University of Freiburg, Ramon Massana from the ICM-CSIC in

Barcelona, and Bente Edvardsen and Wenche Eikrem from the University of Oslo. This support allowed us to get a head start in applying molecular methods in oceanography, in particular cloning and sequencing of natural populations, as well our first steps into the genomics universe (see below). During this period, we also restarted sample the time series at the Roscoff Astan buoy that had been initiated by Alain Sournia (Sournia & Birrien, 1995) and later became incorporated into the SOMLIT((Service d'Observation en Milieu Littoral) monitoring network.

The new century saw a novel expansion of the group with the recruitments of Christophe Six, Fabrice Not, both of which had done their thesis in the group, Colomban de Vargas, Anne-Claire Baudoux and the transfer from Brest of Christian Jeanthon, a microbiologist working previously on deep-sea bacterial communities and who had done his thesis at the SBR with Daniel Prieur. This allowed to diversify but also to expand the research portfolio beyond phytoplankton, from viruses and bacteria to heterotrophic protists. New themes such as symbiosis and parasitism were developed.

The development of high throughput sequencing at the end of the decade 2000 allowed a new revolution with the widespread use of metabarcoding, metagenomics and metatranscriptomics to examine microbial marine communities. This led to very large scale sampling projects such as Biomarks and especially Tara Oceans in which many members of the plankton group participated. An important area of development focused on the ecological and evolutionary genomics of picocyanobacteria under the leadership of Laurence and Frédéric, coupled with photo-physiological measurements, in which Christophe was instrumental, and genetic approaches developed thanks to the long-standing collaboration of the team with David M. Kehoe (University of Bloomington).

I retired in 2019 (but still active in research) and the plankton group is now called the ECOMAP team (for ECOlogy of MArine Plankton). The team is led by Christian Jeanthon and Laure Guillou and is part of the UMR7144 between CNRS and Sorbonne Université directed by one of its former PhD student Fabrice Not. It hosts more than sixty members including permanent researchers and research engineers as well as post-docs, PhDs and Master students. As seen in Figure 1 it continues to have a very abundant production of scientific publications. Members of the group have organized quite a few meetings in Roscoff, which is an ideal place to host small to medium-sized meetings, including Jacques Monod and Gordon Research Conferences. Over the years its members have received numerous individual awards including the CNRS Silver medal, the CNRS Crystal award, the medal of the Oceanography Institute, the medal of the Sciences of the Sea (IFREMER) or the Tregouboff medal awarded by the French Academy of Science.

Key topics in Plankton research

Picoplankton, a new paradigm in the 80s

Until the late-70's, the central oceans were seen as vast expanses nearly devoid of planktonic life, even though chlorophyll could be measured. Theories were abundant to explain the presence of this "detrital" chlorophyll. Around the turn of the decade, researchers had realized that these central regions (called oligotrophic because nutrients are very low) hosted very large populations of small photosynthetic cells in particular the cyanobacterium *Synechococcus* (Waterbury et al., 1979) that could be observed through the fluorescence of their photosynthetic pigments.

While at MIT, I had the chance to be exposed to a new technique called flow cytometry, initially developed in biomedicine, that allowed the measurement of the abundance and fluorescence of cells using laser illumination. My PhD supervisor, Penny Chisholm, had the intuition that this could be a very good way to measure phytoplankton abundance in natural environments since it contains pigments such as chlorophyll that fluoresce naturally under blue light. Penny obtained from the US Office of Naval Research funds to purchase a commercial flow cytometer, and I had the chance to be on the first cruise that took the instrument to sea. John Waterbury who had discovered the importance of *Synechococcus* was also part of this cruise and for the first time we were able to measure very easily the abundance of this cyanobacterium in natural waters as well determine its variation in size and pigments with depth (Olson et al., 1985).

When I left MIT to move to the SBR, I had the chance to attend a NATO conference in late 1985 in Italy organized by Trevor Platt and W.K.W. Li dedicated to picoplankton. I thought it was really a fascinating topic and that it would be worth pursuing, as nobody was working on it in France. However, none of the tools necessary for studying picoplankton were available in Roscoff. A first break came when funds for the Donghai project (see above) allowed me to purchase in 1986 an epifluorescence microscope that was critical to detect pico-phytoplankton based on their fluorescence, although this was far from a high throughput method. The second break came a bit later when, with the support of Pierre Lasserre, we were able to obtain funds from CNRS to purchase a large

flow cytometer, a Coulter EPICS 451, equipped with a 5 W laser (currently lasers used in flow cytometry have a 10 mW power, Fig. 5). This made us one of the first teams in Europe to possess such an equipment for oceanographic research. We decided very soon to take this instrument to sea and in the fall of 1987, we participated in the CHLOMAX cruise in the Sargasso Sea where we could detect *Prochlorococcus* (Neveux et al., 1989), the most abundant species in the world ocean, which had been discovered the year before by Penny Chisholm (Chisholm et al., 1988).

In the wake of this first successful cruise, we began to participate in other cruises to track picoplankton in the Mediterranean Sea (EROS and MINOS cruises in 1989 and 1996, respectively), in the Atlantic (EUMELI in 1991), the Pacific (OLIPAC and BIOSOPE in 1994 and 2004, respectively) and even the Arctic Ocean (MALINA, 2009). These cruises were made possible through strong collaborations with other French laboratories, in particular in Villefranche-sur-Mer with André Morel, Hervé Claustre and Marcel Babin. This led to a flurry of work on the distribution of picoplankton in a range of ecosystems establishing the importance of cyanobacteria, and in particular *Prochlorococcus*, in oceanic systems (reviewed in Partensky et al., 1999). We were also able to establish growth characteristics of these organisms based on measurement of their cell cycle, a continuation of my thesis work at MIT, establishing that it was dividing about once a day but that DNA replication was inhibited near the surface probably because of UV radiation (Vaulot et al., 1995), a hypothesis which was confirmed later by work on *Prochlorococcus* cultures.

In parallel with these measurements at sea, we developed novel methods to fix samples for later analysis (which allowed analysing samples without bringing the flow cytometer at sea) and to detect bacteria and viruses. This is mostly the work of Dominique Marie who saw all the benefit that could arise from new DNA markers, such as SYBR Green. The methods he developed have now become standards for the field (Marie et al., 1997 & 1999).

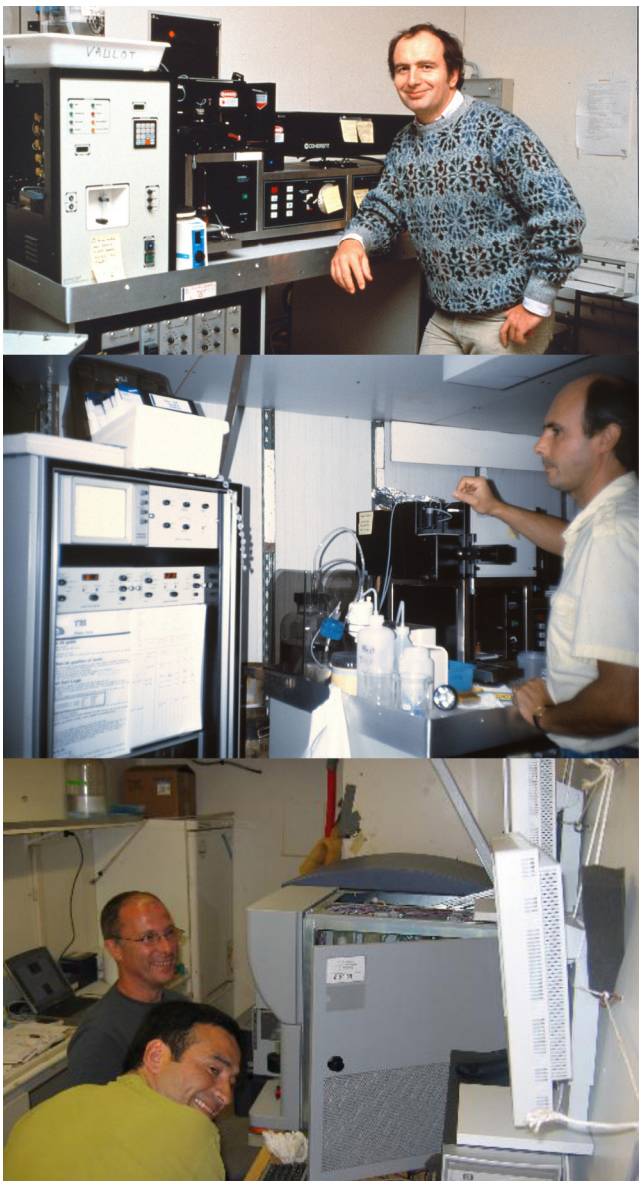

Figure 5. Top. Daniel Vaulot with the first flow cytometer in Roscoff, the Coulter EPICS 451 (Coulter). Middle. Claude Courties, CNRS Research Engineer, with the EPICS during the CHLOMAX cruise in 1987 in the Sargasso Sea. Bottom. Dominique Marie, CNRS Research Engineer, and Osvaldo Ulloa our colleague from Chile, with the FACS Aria (Becton Dickinson) during the BIOSOPE cruise in 2004 in the South East Pacific.

Culturing and describing phytoplankton

While at MIT, I had been fascinated by Bob Guillard who was at the origin of a large culture collection of phytoplankton strains initially located at Woods Hole and then later transferred to the Bigelow Laboratory (now NCMA, for National Center for Marine Algae). It was obvious that one could not really understand phytoplankton taxonomy and physiology if we could not cultivate it. It was as important that these cultures would be available for anyone interested in working with them. Our first attempts at culturing phytoplankton took place during the thesis of Frédéric Partensky who was very successful at growing dinoflagellates.

With our exploration of different oceanic regions, we began to try to isolate novel strains, especially from pico-phytoplankton. Our initial efforts were modest, but during the CHLOMAX we were able to isolate several strains of *Synechococcus*. Our most noticeable success was the isolation of several *Prochlorococcus*

strains by Frédéric both from the Mediterranean Sea during the EROS cruise and from the North Atlantic during his post-doc in Canada. We were helped in this by Penny Chisholm, who communicated the medium designed by Brian Palenik that had allowed them to grow *Prochlorococcus*. These strains proved invaluable as they gave us a head start in the field. In particular, in 1993, we organized under the leadership of Frédéric an international workshop during which a range of physiological measurements were done for the first time on this cyanobacterium. A second workshop was then held in 1999 to study the diel cycle of the first *Prochlorococcus* strain to be made axenic, a feat achieved some time earlier by Rosi Rippka (Pasteur Institute), as part of our partnership in the PROMOLEC program.

Besides pico-cyanobacteria, we also decided early on, to focus on eukaryotic pico-phytoplankton for which very few species had been isolated and described. Our first picoeukaryotic strains were isolated during the CHLOMAX cruise in 1987 and turned out to belong to the genus *Pelagomonas*, a eukaryotic alga that would only be formally described in 1993 (Andersen et al., 1993). The thesis of Nathalie Simon allowed us to better characterize the few cultures which were available in the early 90s (Simon et al., 1994). However, our isolation effort took a major turn with the OLIPAC and MINOS cruises that provided the material for the thesis of Laure Guillou. In particular Laure achieved the feast of describing a new class of algae, the Bolidophyceae, located at the base of the diatoms, characterized by fast swimming cells ("petits bolides" in French, Guillou et al., 1999). A few years later, another mysterious group of algae, the Parmales that are characterized by very small cells recovered by silica plates and had escaped cultivation, were isolated in culture by Japanese researchers and proved to also belong to Bolidophyceae. Following the footsteps of Laure, the Roscoff team described many new species, such as *Florenciella parvula* (Eikrem et al., 2004) or *Partenskyella glossopodia* (Ota et al., 2009). Almost two decades later, we described two novel classes of green algae, the Chloropicophyceae and the Picocystophyceae that are important in open oceanic waters (Lopes dos Santos et al., 2017).

As the strains accumulated, we decided to make them available more widely, leading in 1998 to the creation of the Roscoff Culture Collection (Vaulot et al., 2004). This collection grew slowly, first curated by Florence Le Gall then by Priscillia Gourvil and now managed by Ian Probert and Priscillia. It is currently one of the biggest collections in the world for marine microalgae, maintaining and distributing more than 5,700 strains. Along the year, it has developed a unique expertise in the domain of strain isolation (e.g. Le Gall et al., 2008) and cryo-preservation. It also participates in courses such as the Advanced Phytoplankton Course last held in Roscoff in 2019.

The molecular revolution

Another direction where the Roscoff plankton group played a pioneering role was the introduction of molecular techniques in Oceanography. This was initially resisted by oceanography pundits that did not see any interest to apply such sophisticated approaches. Our first exposure (our very first DNA gels) occurred in 1992 when our colleague from the University of Warwick, Dave Scanlan, came to the SBR to teach a 10-day course in molecular biology. A bit later, we were successful in obtaining an EU fellowship to study genetic diversity and gene expression in our recently isolated *Prochlorococcus*. First Wolfgang Hess came from Berlin to fill the position, but as he obtained a position in Germany he was soon replaced by José Manuel García-Fernández, who continued in the same line of research.

The next logical step was to investigate whole genomes to understand the link between genotype and phenotype. This was achieved under the leadership of Frédéric first for *Prochlorococcus* with the support of the Genoscope (Evry, France), which just had finished its involvement in the race for sequencing the human genome (Dufresne et al., 2003). This organism is particularly interesting because it possesses different ecotypes adapted to different light levels in the ocean, some thriving near the surface and some at the bottom of the euphotic zone (Partensky & Garczarek, 2010). However, the fact that it is virtually impossible to transform led Frédéric and Laurence to re-orient these studies towards *Synechococcus* which, besides being genetically amenable, displays an amazing genetic and pigment diversity, a clue to explain its capacity to grow in all marine ecosystems reached by solar light from the equator to the sub-polar regions.

On the pico-eukaryotes front, a major break occurred when we decided to perform for eukaryotes what had been done for bacteria ten years earlier, i.e. amplify, clone and sequence gene markers such as the 18S rRNA. Our goal was to compare the diversity that we knew about, i.e. from microscopy and culture, to the one in the water. We applied this for the first time on a few samples collected during the OLIPAC cruise in the equatorial Pacific Ocean, and the outcome by far exceeded our results with entire branches of eukaryotic phylogenetic tree appearing which had absolutely no representative in culture, in particular in the Alveolates and Stramenopiles lineages (Moon-

van der Staay et al., 2001). In the same Nature issue where these findings were reported, another group reached the same conclusion, but using samples from the Antarctic region (López-García et al., 2001). This new direction was further developed during the EU PICODIV project with our colleagues from Spain, UK, Germany and Norway during which we investigated picoplankton using a range of molecular approaches including the use of fluorescent *in situ* hybridization which led to establish the importance of the very small green alga *Micromonas* in the waters off Roscoff (Not et al., 2004), which were revealed to be much more interesting for phytoplankton than Georges Teissier had predicted.

The age of the metas

The advent of massively parallel sequencing techniques around 2005, first pioneered by 454 Life Sciences, soon bought by Roche, and then developed by Illumina, completely changed the game for sequencing microbial populations in the environment both for identifying their composition as well as their gene content. This led to the development of "meta" disciplines, in particular metabarcoding, metagenomics and metatranscriptomics. The Roscoff group very soon recognized the importance of these new approaches to better characterize marine microbial populations and, under the leadership of Colomban de Vargas, devised large scale projects that sampled oceanic waters, first BioMarks around European coasts and then the more ambitious *Tara* Oceans that circumnavigated the tropics on a sailing-vessel equipped with sophisticated sampling devices. The success of this global expedition is linked to several factors. First, it federated a large community of scientists from around the world. Second, its alliance with the French national sequencing centre, Genoscope, allowed it to obtain a massive amount of data both for metabarcoding and metagenomics to a degree that had not been reached before. Third, it adopted very soon an open-data policy, which made the raw data available as soon as the first papers were published. This policy and the wide sampling coverage encouraged many researchers to use these data to address questions at a global scale. Probably the most emblematic paper is that of de Vargas et al. (2015) that offered a very comprehensive view of protist communities in the surface layer of the ocean. The metagenomics data of *Tara* Oceans were also a key asset for the analysis of pico-cyanobacterial populations, offering an unprecedented view in their ocean wide diversity (e.g., Farrant et al., 2016).

The massive amount of data that resulted from these new approaches also led to the development of on-line databases that are necessary to analyse these data. The Plankton group played a key role in the development of these databases and in particular the Protist Reference Database (PR^2) that contains reference 18S rRNA sequence database that are taxonomically annotated by specialists of different protists groups (Guillou et al., 2013, cited more than 1000 times according to Google Scholar). With the development of metabarcoding for eukaryotes, this resource became a *de facto* standard for annotation. This database, still maintained by the group, is now complemented by a metabarcode database, metapr2 (Vaulot et al., 2022). The Plankton group has also developed a genome database, Cyanorak, which allows the manual curation and easy comparison of marine pico-cyanobacterial genomes (Garczarek et al., 2021) that can notably be used as references for global metagenomic studies.

A view of the future

I have always had much difficulty to predict what will be the major development in our field in the next 10 or 20 years, and I have always found "Prospectives" meetings quite vain and not very useful. Furthermore, I believe that science is strongly driven by technology and being a "good" scientist (well, "good" is very relative in any case) is to be able to see the potential of a novel technology to solve some of the questions we ask. I have always been fascinated by the diversity and distribution of plankton. The idea of "everything is everywhere" did not seem to be very satisfying, and my intuition is that there must be rules, factors that control the presence and abundance of any given species: it will not make sense to find *Prochlorococcus* for example off Roscoff because of the prevailing environmental conditions (and we never found). During all these years at the SBR, three techniques have really yielded new answers to these questions: flow cytometry to precisely identify and measure the abundance of well-defined groups, PCR that gave us the mean to sequence natural populations and finally high throughput sequencing coupled with novel software programs, and in particular open source software, permitting a very detailed and quantitative look at natural protist communities.

What will be the next frontier? Very hard to predict. There are still many protist groups for which we do not have any idea about the shape or size of the cells, e.g. oceanic Chrysophyceae, a group which seems abundant in marine waters but for which most described species are from freshwater, or clades of green algae still designated by a number such as prasinophytes clade IX. Among heterotrophic groups, the lack of

knowledge of marine groups is still wider, with entire lineages such as marine alveolates (MALV) or marine stramenopiles (MAST) with very few representatives isolated or described. Clearly, novel microscopy approaches coupled with single cell sequencing may help move forward.

Another area which has gained considerable momentum is that of "interactions". The time when each population was seen in isolation is gone, and scientists have now realized that different groups are constantly interacting. For example, while the first papers on mixotrophy, i.e. the capacity for some phytoplankton species to switch between photosynthesis and prey ingestion, originate from the 80s, this behaviour seems to be more the rule than the exception in some groups such as dinoflagellates or haptophytes. There is also the recognition that parasitic relationships are very ubiquitous and very important to explain the dynamics of many phytoplankton species. Taking into account viruses is essential to understand the dynamics of the supposedly well known diatoms off Roscoff. To study these phenomena, single cell approaches will also be very indispensable, in particular single cell transcriptomics, cryo-microscopy or nanoSIMS. However, these techniques still lack the massive output from flow cytometry and HTS and one can hope that novel more parallel approaches will be appearing in the near future.

Final remarks

What made the success of the Plankton Group? Probably being in France part of the CNRS was a key factor. The aim of CNRS is "Faire progresser la connaissance et être utile à la société (Improve knowledge and be useful to society)". At no point in my career, anyone has asked me to demonstrate that my research would result in a product or would solve an immediate question. What was really critical is that we produced papers, but even for this, the pressure was not huge. This gave us a lot of freedom to pursue long-term goals. Another important factor was the very congenial atmosphere of the SBR despite the fact that it is quite secluded and far off the main research centre of France. Although some crises occurred, there was always some pleasure to walk in the corridors of the SBR, say hello to colleagues, have a chat around a coffee or just to look at the incredible landscape shaped by the tides and ever-changing weather. Despite this seclusion, we were able to weave a lot of links with laboratories in France, in Europe, in the US, in South America and in Japan as Roscoff is a place where people enjoy coming. The SBR also hosted a number of students and post-docs from all over the world making it a truly cosmopolitan laboratory. It is necessary to emphasize also the role of Station Directors, Pierre Lasserre, André Toulmond and Bernard Kloareg who each in their individual styles helped to maintain this atmosphere. One final point is that the Plankton Group turned out to be quite endogenous. Students of initial members were recruited on permanent positions and themselves had students who are now part of the group. Although such endogamy could result in a very sterile atmosphere, it turned out to be quite the opposite. Maybe because each member of the group first went away after their thesis and when they came back developed quite independently, often switching to novel topics such as symbioses and parasitisms in marine protists.

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