Annexe 4

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Opération DIAP’ATHIO

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OPERATION DIAP'ATHIO

Coordinateur du projet : Cécile DUPOUY

L' opération DIAP'ATHIO est destinée à réunir des chercheurs de DIAPAZON intéressés par le suivi d'une efflorescence de cyanobactéries. Un ensemble de mesures et d'expériences dans le milieu naturel et au laboratoire sera réalisé à la période la plus favorable de l'année (15 novembre-4 décembre) dans les eaux au large de la Côte Est de Nouvelle Calédonie, plus propices à l'apparition d'efflorescences que la zone de Nouméa. Un laboratoire léger adapté aux expérimentations sera installé à THIO pendant la durée de l'étude (15 novembre-4 décembre 2002). Ce projet s'appuie sur la disponibilité d'un navire océanographique permettant d'échantillonner l'efflorescence et de récupérer des échantillons pour expérimentations.

La série d'observations et d'échantillonnages sera constituée des mesures faites pendant les campagnes DIAPALIS (biomasses, productions, taux de fixation de N2 et régénération, etc..), mais permettra également de développer de nouvelles expérimentations qui pourront ensuite être reprises lors des campagnes. Elle permettra également de réaliser des objectifs de DIAPAZON non encore abordés tels que - les causes et mécanismes de la fin d'une efflorescence - la caractérisation des exopolymères transparents (TEP) et de la matière organique colorée dissoute (CDOM). Trois projets sont détaillés en annexes.

Mots clés

Trichodesmium –– suivi et fin de l'efflorescence ––flux de carbone et d'azote – Fer - TEP et CDOM-couleur de la mer

Liste des personnes et des Laboratoires associés au projet

UR CYANO, UR 99 IRD, Nouméa

Aubert Le Bouteiller, Jacques Neveux, Martine Rodier, Marcio Tenorio, Italo Masotti, Robert Le Borgne

UR LODYC, UR 86, IRD, Nouméa

Cécile Dupouy, Xavier Mari

Laboratoire d'Océanographie de Villefranche sur Mer

Emma Rochelle-Newall

Université de Virginie

Margaret Mulholland

Université de Rutgers

Ilana Berman-Frank

Aide technique : Alain Lapetite.

Période : 15 novembre- 4 décembre 2002

Lieu : Thio, Nouvelle Calédonie, 21°35S, 166°10^{E}

Figure 1. Emplacement du laboratoire léger sur la côte est de Nouvelle Calédonie

Cette opération s'appuie sur l'utilisation du DARMAD (12 mètres) des Affaires Maritimes de Nouvelle Calédonie qui sera disponible pour l'opération.

Matériel utilisé à bord du DARMAD :
Sonde température salinité. Bouteilles à prélèvements de 5 litres. Prélèvements sans fer. Filets. Bonbonne d'azote liquide. Sondes Hydroscat et Simbada, Ocean Optics.

Matériel utilisé au laboratoire :
Spectrophotomètre Beckman. Spectrofluorimètre Hitachi. Microscopes et loupes binoculaires. Incubateurs.
Requis : FRRF, microscope à épifluorescence + matériel d'analyse d'images et photos, lecteur de microplaque en absorption et fluorescence.

Annexes

1- Taux de fixation d'azote et régénération – *Margaret Mulholland*
2 – Causes et mécanismes de la fin d'un bloom de *Trichodesmium* - *Ilana Berman-Frank*
3 – *Trichodesmium*, carbone organique dissous et TEP - *Xavier Mari, Emma Rochelle-Newall et Cécile Dupouy*

1- Taux de fixation d'azote et régénération– *Margaret Mulholland*

Research Objectives:

New production from N_2 fixation by *Trichodesmium* has been measured in a variety of oceanic regions, yet the fate of new N in many systems is unknown. It is crucial that we address this gap in our knowledge, both in terms of the dominant heterotrophic pathways and in terms of regenerated production fueled by new N inputs into the euphotic zone. The goals of this project are:

1. To determine how relative rates of N_2 fixation and N uptake vary in natural planktonic communities as the abundance and physiological state of *Trichodesmium* changes over the course of bloom initiation, development, and decay.
2. To determine the fate of recently fixed N_2 and assess the importance of regenerated N in supporting the growth of phytoplankton and bacteria associated with or succeeding *Trichodesmium* blooms.

Nitrogen dynamics resulting from N_2 fixation and N release by *Trichodesmium* may profoundly affect the surrounding oligotrophic communities of the SW Pacific Ocean as well. *Trichodesmium* play host to a variety of autotrophic and heterotrophic microorganisms (Paerl et al. 1989, Sellner 1992 & 1997) that appear to get their nutrition either directly or indirectly from *Trichodesmium.* High concentrations of dissolved inorganic and organic nutrients have been observed subsequent to *Trichodesmium* blooms (Devassy et al. 1978, Devassy 1987, Karl et al. 1992, 1997). It has been estimated that *Trichodesmium* releases upwards of 50% of the recently fixed N_2 as DON (Glibert & Bronk 1994, Capone et al. 1994) and NH_4^+ (Mulholland & Capone 2001, Mulholland, Bronk & Capone, unpublished data). These N releases may stimulate phytoplankton and bacterial growth however, direct nutritional links have not been established.

I propose to examine pathways of N release, recycling and uptake in a tropical community where seasonal blooms of *Trichodesmium* spp. occur. **In particular, the following will be examined:**

1) Rates of new production from N_2 fixation,
2) Rates of N regeneration from N_2 fixation relative to other pathways,
3) Uptake of regenerated N by phytoplankton and bacteria

To accomplish these objectives I propose both time series measurements and process studies at a coastal site in the SW Pacific near New Caledonia where *Trichodesmium* occurs seasonally from September through April. At the coastal site, measurements can easily be made over time as a bloom develops and collapses. In addition, experimental manipulations can be undertaken to determine the environmental controls affecting rates of N_2 fixation and N regeneration.

Methods:

Samples of *Trichodesmium* spp. colonies for rate measurements will be collected at the surface and at 15 m by plankton tows using a 1-m mouth plankton net equipped with a flowmeter or in Niskin bottles.

New and regenerated production will be estimated using highly enriched ^{15}N compounds. Nitrogen fixation rates will be measured using incorporation of $^{15}N_2$ into *Trichodesmium* biomass (Montoya et al. 1997). The appearance of label as $DO^{15}N$ and $^{15}NH_4^+$ will be used to quantify release of recently fixed $^{15}N_2$ (Bronk et al. 1994). Uptake of combined N ($^{15}NO_3^-$ $^{15}NH_4^+$, urea and amino acids) will be estimated from tracer incubations using highly-enriched ^{15}N substrates (Glibert & Capone 1993). In addition, NH_4^+ and urea production will be measured by isotope dilution (Glibert et al. 1982). We will conduct time-course incubations to determine the kinetics of N uptake and release by *Trichodesmium.* At the same time we will measure uptake of combined N by *Trichodesmium* and other taxa (including bacteria) isolated on the basis of size (using gentle filtration). From these experiments we will estimate the amount of new production by *Trichodesmium* relative to the amount of regenerated production and heterotrophic activity. Where possible, rates of $^{15}N_2$ uptake will be compared with rates of N_2 fixation estimated using the acetylene reduction technique as an independent measure of N_2 fixation and release of recently fixed N_2 over time.

We will measure the production of DO^{15}N from $^{15}N_2$ fixation using the method of Glibert & Bronk (1994) as modified by Bronk (pers. comm.) and using a SpeedVac system to concentrate DON. $^{15}NH_4^+$ produced from $^{15}N_2$ fixation will be isolated and dried onto a GF/A filter for isotopic determination (Dudek et al. 1986). In addition, we will check for excretion of free amino acids, and ammonium by measuring concentration change during incubations using standard methods (Parsons et al.1984, Solarzano 1969). Estimates of NH_4^+ turnover will also be determined by isotope dilution (Glibert et al. 1982). Rates of N uptake and release will be calculated using the equations of Bronk et al. (1998). Nutrients will be analyzed colorimetrically (Solarzano 1969, Parsons et al. 1984) either manually or using an autoanalyzer. DON will be analyzed by persulfate oxidation (Valderrama 1981). ^{15}N analyses will be done using a Europa 20/20 isotope ratio mass spectrometer with an elemental analyzer at the inlet in the laboratory of M. Mulholland.

To establish direct nutritional links between *Trichodesmium* and other trophic groups, incubations will be conducted in which we physically contain *Trichodesmium* in dialysis bags or in 10 ? m mesh confinements, and then add $^{15}N_2$ label. This will allow released or regenerated ^{15}N compounds derived from N_2 fixation to diffuse to other organisms, which can then be collected to establish nutritional links to *Trichodesmium*. We will also use commercially labeled organic and inorganic N compounds in uptake experiments by diatoms, dinoflagellates and picoplankton in size-fractionated water.

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II - 2 –Causes and Mechanisms of *Trichodesmium* Bloom Demise - Ilana Berman-Frank

Introduction and Statement of Problem:

The factors that control the initiation and growth of phytoplankton blooms in the oceans are well studied. Surprisingly, little is known about the mechanisms of phytoplankton mortality in natural systems, a biogeochemically important process that affects the recycling and export dynamics of carbon and DOM. Determination of the causes and mechanisms of phytoplankton mortality will facilitate quantification of fluxes through the different biogeochemical pathways and the interactions between different trophic levels(grazer-driven food chains, vertical sinking fluxes, and recycling through the microbial loop and DOM pools).

Traditionally, grazing and sinking were considered the main fluxes of mortality for phytoplankton. The recent focus on viruses and bacteria in aquatic systems have invoked these organisms as agents for phytoplankton cell lysis when no grazers were present. In the last few years, an additional process of mortality, autocatalysed cell death, has been documented in a few species of phytoplankton that were subjected to nutrient, light or oxidative stress. (Brussaard 1997, Berges 1998, Vardi et al. 1999, Segovia et al. submitted). The autocatalytic death observed in these cases (a diatom, a chlorophyte, and a fresh-water dinoflagellate) displays features similar to apoptosis, or programmed cell death, an irreversible, suicidal cascade that is an essential feature in the development and function of various systems in multicellular organisms. Whether these systems are homologous to multicellular apoptotic pathways or if they have shared evolutionary pathways remain open questions. Moreover, do not yet understand whether the observed apoptotic cell death in phytoplankton is a shared characteristic of phytoplankton mortality, or limited to a few species. Nor do we know what induces the process and if viral or bacterial agents will confer a similar pathway of mortality as abiotic pressures on the cells.

The diazotrophic cyanobacterium, *Trichodesmium spp*,. blooms extensively in the tropical and subtropical oceans and has been the focus of much field research in its contribution to the oceanic new N and to global N and C cycling. The sources and the mechanisms of mortality for *Trichodesmium* both in the laboratory and for natural populations have not been clearly defined. In some areas, grazing by *Macrosetella gracilis* causes significant mortality (O'neill et al. 1996), while at other sites (and in some laboratory cultures) lytic phages have been implicated as the agents of death (Ohki 1999). We are currently studying the induction and retardation of cell death in laboratory cultures of Trichodesmium IMS101. Preliminary results from our lab show physiological differences between young and old cells and cells grown in high or low iron in response to induction of cell death. Additionally, we demonstrate the presence and activity of caspases (a unique class of intracellular cysteine proteases involved in apoptotic cell death) in *Trichodesmium* IMS 101. These results indicate the existence of autocatalytic cell death in cultures of *Trichodesmium.* Yet we do not know if these are present and are expressed in natural populations. Moreover, the ecological significance of Trichodesmium mortality and bloom demise from different causes in nature have yet to be explored.

Objectives of the proposed research:

1. **To examine the causes of *Trichodesmium* bloom declines and cellular mortality in the natural environment. Are bloom crashes due to grazing, natural viral phages (as indicated previously), or abiotic pressures such as reactive oxidative stress?**
2. **To determine the mechanism/s of cell death. Is there an autocatalytic apoptotic-like cascade regulating cell mortality during the natural cycle of a bloom and is the same mechanism manifested for different causes of death.**

Proposed Research:

The framework of the DIAP'ATHIO project provides a unique opportunity to study these questions. The abundance and frequency of near-shore *Trichodesmium* blooms enable close monitoring and analysis of populations without the limitations of large sea-going cruises. In

conjunction with the other investigators of the project (examining changes in n2 fixation and uptake, remote sensing data, biomass and pigments, elemental cellular composition, etc.), we will follow *Trichodesmium* blooms in semi-enclosed lagoons in New Caledonia (South Western Tropical Pacific) from bloom- initiation, development, and through their demise. Measurements will be made throughout the blooms to determine oxidative stress (ambient H_2O_2 concentrations, internal reactive oxygen species (ROS) concentrations, ascorbate peroxidase and superoxide dismutase activity), changes in the cellular photosynthetic capacity due to stress (using fast repetition rate fluorometry FRRF), mortality and the presence of viral phages (live-dead stains,TEM microscopy), existence of an apoptotic autocatalytic cell death cascade by protease and caspase presence (western blots with available caspase antibodies, *in-situ* immunolocalization) and activity (using commercially available substrates).

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II-3. TEP et CDOM au cours d'une efflorescence de *Trichodesmium*. *Xavier Mari, Emma Rochelle-Newall et Cécile Dupouy,*

Projet scientifique :

Une des hypothèses émises au sujet du métabolisme des *Trichodesmium* est qu'une grande partie du carbone est excrétée sous forme dissoute. Une partie de ce carbone excrété pourrait constituer le CDOM (Colored Dissolved Organic Matter) associé aux colonies (Jones et al., 1986; Prufert-Bebout et al., 1993 ; Gordon et al., 2002, nos propres mesures). Une autre fraction du carbone organique relargué pourrait coaguler pour former des colloïdes, et ensuite des TEP (Transparent Exopolymeric Particles). Une question nouvelle serait de caractériser ces TEP (Mari et al., 2001) issues de *Trichodesmium* au cours d'une efflorescence et d'en connaître le devenir dans le milieu naturel. Les particules exopolymériques transparentes (TEP) constituent une classe de particules récemment mises en évidence. À cause de leurs propriétés physico-chimiques et de leur forte abondance, les TEP jouent un rôle majeur dans les différents processus biogéochimiques de la zone pélagique. Elles pourraient agir comme des sites d'adsorption pour de nombreux composés organiques et inorganiques dissous, comme source de nourriture pour de nombreux animaux filtreurs ou brouteurs. De plus, elles sont à l'origine de phénomènes de coagulation avec d'autres particules pour former les agrégats de la neige marine et ainsi augmenter le transport de substances par sédimentation. Les TEP possèdent des propriétés physico-chimiques très différentes des organismes dont elles sont issues et par conséquent, il est nécessaire de distinguer optiquement ces deux pools de matière organique (vivant et non-vivant) qui ont des implications distinctes au niveau des flux de matière et de la structure de la chaîne trophique.

Les mesures sont destinées à connaître les variations de concentrations de TEP et de CDOM au cours du développement d'une efflorescence de *Trichodesmium*.

Méthodes

Afin d'étudier la relation TEP-CDOM au cours d'un bloom de *Trichodesmium*, une solution de TEP qui aura été purifiée et concentrée à partir de matière organique dissoute relarguée par les colonies de *Trichodesmium* et bactéries associées se développant en incubateur, sera examinée. Les TEP et le CDOM seront caractérisés au laboratoire à partir d'échantillons récupérés et congelés.

Les mesures optiques seront effectuées dans les domaines UV et visible. L'absorption par les colonies (particules sur filtres) et le CDOM (filtrat 0.2 µm, cuve de 10 cm) seront mesurés entre 200 et 800 nm au spectrophotomètre. La rétrodiffusion des colonies sera mesurée grâce à l'Hydroscat-6 à 6 canaux dans les bacs, sans lumière parasite. Surtout, une information spectrale continue dans le spectre visible sera obtenue sur les *Trichodesmium* grâce à un spectroradiomètre hyperspectral portable miniaturisé Ocean Optics SD2000 350-880, 1024 canaux. L'utilisation de fibres optiques orientables (en parallèle aux mesures d'assimilation des différentes formes d'azote) dans des conditions d'éclairement contrôlées permettra ce type de mesures. Ces mesures complèteront celles du SIMBADA à 13 canaux, faites en lumière polarisée, donc évitant la réflexion spéculaire.

Cette approche devrait nous permettre de découpler les signatures spectrales des colonies de *Trichodesmium* et celles des TEP qui leur sont associées in situ.

Matériel utilisé:

Spectrophotomètre UV-Vis DU-600 + sphère intégrante
Hydroscat-6 (442-488-510-555-620-676 nm)
Ocean Optics SD2000 (350-850nm, 1024 canaux) + fibre optique
SIMBADA-13 canaux (380-750nm)
Microscope, conteneur azote liquide.

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