

OUTPACE, Appel à projets génériques 2014 ‘Gestion sobre des ressources et adaptation au changement climatique’

TITRE DE LA PROPOSITION: OCEANIC CO₂ SEQUESTRATION AND ATMOSPHERIC DINITROGEN FIXATION IN THE OLIGOTROPHIC OCEAN (OUTPACE OCEANOGRAPHIC CRUISE)

Contenu

Executive summary of the proposal	2
1. Context, position and objectives of the proposal.....	5
1.1. Context, state of the art, scientific bottlenecks	5
1.2. Objectives, Originality and Novelty of the project.....	9
2. Scientific and technical programme, Project organisation	10
2.1. Scientific programme and project structure.....	10
2.2. Description by task	11
2.3. Task schedule	23
2.4. Team description	23
2.5. Justification of requested resources (398.9 k€)	25
3. Dissemination, exploitation. Intellectual property.....	27
4. References cited.....	27

EXECUTIVE SUMMARY OF THE PROPOSAL

The functioning and productivity of oligotrophic systems, and the balance between production and mineralization in these oceanic areas, is still the subject of much debate. Once thought to be biological deserts, recent research has shown that these systems could largely contribute to the global ocean organic carbon (C) export. It is important to thoroughly understand the biogeochemical functioning of these vast ecosystems in order to characterize their evolution in the context of climate alteration. This project aims to give a zonal characterization of the biogeochemical functioning and biological diversity of the South West (SW) Pacific toward a gradient of macro- and micro-nutrients availability, and produces a detailed study of the biological production and its subsequent fate in 3 contrasting sites, with a specific emphasis on the production sustained by dinitrogen (N₂) fixation. We will determine whether organic matter production, re-mineralization and export processes are different at these sites, and if so, determine how these differences are related with the diversity/functioning of N₂-fixing organisms in the planktonic community. Comparing different sites, along a zonal gradient of variable nutrient availability, should provide us with a new insight for identifying and understanding the fundamental interactions between marine biogeochemistry and ecosystems. We will focus on several current issues of interest regarding the coupling/uncoupling between carbon and nutrient (N,P,Si,Fe) delivery and removal processes in the surface layer. These processes control the planktonic community structure and functioning and, ultimately, the ability of the ocean to biologically sequester C. We shall describe the functioning of each distinct ecosystem under physical conditions with new approaches combining satellite localization and drifters for Lagrangian strategy, and, finally, examine the biogeochemical role of diazotrophs in each system, using experimental and modelling approaches.

The scientific objectives will be achieved through the realization of 6 complementary Tasks (including promotion of Science, Task 6) involving international collaborations between physicists, biologists and biogeochemists with expertises ranging from marine physics, chemistry, optics, biogeochemistry, microbiology, molecular ecology, genetics, and modelling. The overall strategy of the project will combine experimental and modelling approaches. The experimental part is based on the realization of a 45-days oceanographic cruise onboard the R/V L'Atalante in the SW Pacific in 2015. The cruise will consist in 18 short duration (8 h) stations (SD stations) and 3 long duration (7 d) stations (LD stations) distributed along a zonal transect (19° S) in the SW Pacific ocean. During the SD stations, a characterization of the biogeochemistry and biological diversity from the North of New Caledonia to the western border of the South Pacific gyre will be performed (Task 1). The LD stations will allow to perform a process studies in contrasting oligotrophic and N₂ fixation conditions following a Lagrangian approach for a 3D characterization of the upper water column (Task 2). During the LD stations, high frequency acquisition of physical, optical, biogeochemical and biological variables will be assessed. We will quantify primary / secondary production and their fate (Task 3), as well as mineralization and export of organic matter (Task 4) by measuring biogenic element (C,N,P,Si) stocks/fluxes and organisms responsables for these fluxes ("Who does what?"). Moreover, a multi-scale modelling approach is proposed that we plan to start from the beginning of the OUTPACE project (Task 5). Its objective is to complement the observations with the analyzed data in order to better understand the interactions between the biogeochemical cycles of the biogenic elements (predominantly the C cycle) and the dynamics of the planktonic trophic network in oligotrophic marine areas.

People involved in the project:

All the participants are listed in the tables below. Partner 1: Institut Méditerranéen d'Océanologie (M I O), Partner 2: Laboratoire d'Océanographie et du Climat (LOCEAN), Partner 3: Laboratoire d'Océanographie de Villefranche (LOV), Partner 4 : Woods Hole Oceanographic Institution (WHOI, USA) and Partner 5: Bar Ilan University, Israel. The last table is for participants not from ANR partners laboratories and therefore not funded by the ANR.

Partner 1 : M I O				Role and responsibility in the project				
Last name	First name	Position	Country	On board/shore	Field	Specialty	Responsability	p.m
Moutin	Thierry	Pr1	FR	On board	Biogeochemistry	Photoc zone C,N,P cycles	Chief scientist, PI WP1	25
Baklouti	Melika	MCF	FR	On shore	Physics	Modeling	PI WP5	6
Barani	Aude	IE	FR	On shore	Biology	Flow cytometry		1,8
Bellomo	Lucio	MCF	FR	On board	Physics	MVP200, Moorings, CTD		3,6

Benavides	Mar	Post-Doc	FR	On board	Biogeochemistry	N ₂ fix. and DOM control	M I O	9,6
Berthelot	Hugo	PhD	FR	On board	Biogeochemistry	Fate of N ₂ fix. (nanoSIMS)		4,8
Bonnet	Sophie	CR1	FR	On board	Biogeochemistry	Euphotic N ₂ fix., fate (nanoSIMS)	PI WP3	11
Carlotti	François	DR1	FR	On board	Biogeochemistry	Zooplankton, MPV		6
Cornet	Véronique	IE1	FR	On board	Biology	Phytoplankton, Pigments, TEP		7,2
Delmont	Anne	IE	FR	On board	Biogeochemistry	DOC, Optical measurements		4,8
Doglioli	Andrea	MCF	FR	On shore	Physics	Lagrangian strategy	PI WP2	7,2
Dupouy	Cécile	CR1	FR	On board	Optics	Optical measurements		4,8
Goutx	Madeleine	DR2	FR	On shore	Biogeochemistry	FDOM		1,8
Gregori	Gérald	CR1	FR	On shore	Biology	Flow cytometry		3
Grosso	Olivier	IE2	FR	On board	Biogeochemistry	Nutrients & N ₂ fix. mesurments		4,8
Hélias	Sandra	IE	FR	On board	Biogeochemistry	DOM, POM		4,8
Leblanc	Karine	CR1	FR	On board	Biogeochemistry	Si cycle		7,2
Lefevre	Dominique	CR1	FR	On board	Biogeochemistry	O ₂ -Argon		7,2
Pagano	Marc	DR1	FR	On shore	Biology	Zooplankton		2,4
Petrenko	Anne	MCF	FR	On shore	Physics	Lagrangian trajectories		6
Rougier	Gilles	IR1	FR	On board	Physics	MVP200, Moorings, CTD		4,8
Sempere	Richard	DR1	FR	On shore	Biogeochemistry	DOC, Optics		1,8
Tedetti	Marc	CR2	FR	On shore	Biogeochemistry	UV, optics		1,8
Thyssen	Melilotus	CR2	FR	On shore	Biogeochemistry	Flow cytometry		4,8
Van Wambeke	France	DR2	FR	On board	Biogeochemistry	HB (BP, single-cell analyses)		4,8
Wagener	Thibaut	MCF	FR	On shore	Biogeochemistry	DIC/Alkalinity		3
-	-	PhD	FR	On board	Biogeochemistry	N ₂ fix. N cycle		36
-	-	Post-Doc	FR	On shore	Physics	Lagrangian strategy		24

Partner 2 : LOCEAN				Role and responsibility in the project				
Last name	First name	Position	Country	On board/shore	Field	Specialty	Responsability	p.m
Bouruet Aubertot	Pascale	Pr2	FR	On board	Physics	VMP250, ADCP, CTD	Co-PI WP2	9,6
Cuyppers	Yannis	MCF	FR	On shore	Physics	Turbulence		3
Deboissieu	Florian	Post-Doc	FR	On shore	Optics	PHYSAT algorithm		2,4
D'ovidio	Francesco	CR	FR	On shore	Physics	Lagrangian trajectories		1,8
Lo Monaco	Claire	CNAP	FR	On shore	Biogeochemistry	DIC/Alkalinity		1,8
Lourenço	Antoine	IE	FR	On board	Physics	MVP250, CTD		4,8
Menkes	Christophe	CR1	FR	On shore	Physics	ADCP /Zooplankton		2,4
Metzl	Nicolas	CR	FR	On shore	Biogeochemistry	DIC/Alkalinity		2,4

Partner 3 : LOV				Role and responsibility in the project				
Last name	First name	Position	Country	On board/shore	Field	Specialty	Responsability	p.m
Claustre	Hervé	DR1	FR	On shore	Biogeochemistry	PROVBIO floats	Co-PI WP1	1,8
Guidi	Lionel	CR2	FR	On board	Biogeochemistry	Particle size spectra, UVP		4,8
Guieu	Cécile	DR2	FR	On board	Biogeochemistry	Aerosols, dissolved iron		4,8

Leblond	Nathalie	IE	FR	On shore	Biogeochemistry	Sediment trap analyses		2,4
Louis	Justine	PhD	FR	On board	Biogeochemistry	Aerosols, low level nutrient		3,6
Picheral	Marc	IR2	FR	On shore	Biogeochemistry	Instrumentation Zooplankton		1,8
Ras	Josephine	IE	FR	On shore	Biogeochemistry	Pigments HPLC		1,8
Schmechtig	Catherine	IE	FR	On shore	Physics Biogeochemistry	LEFE CyBER database		1,8
Uitz	Julia	CR2	FR	On shore	Biogeochemistry	PROVBIO floats		1,8

FOREIGN PARTNERS (Funds not requested to ANR)

Partner 4 : WHOI (US)				Role and responsibility in the project				
Last name	First name	Position	Country	On board/shore	Field	Specialty	Responsability	p.m
Van Mooy	Benjamin	DR2	US	On board	Biogeochemistry	Cell-cell signaling, POM	PI WP4	4,8
Dyhrman	Sonya	DR2	US	On shore	Biogeochemistry	Cell-cell signaling, POM		3
Krupke	Andrea	Post-Doc	US	On shore	Biogeochemistry	Cell-cell signaling, POM		
Ossolinski	Justin	Tech.	US	On shore	Biogeochemistry	Cell-cell signaling, POM		

Ben Van Mooy (<https://www.whoi.edu/bvanmooy/>, PI of the OUTPACE WP4) and Sonya Dyhrman (<http://www.whoi.edu/profile.do?id=sdyrman>) are associate scientists from the WHOI (see <https://www.whoi.edu/>). The participation of the Van Mooy and Dyhrman labs will be supported by funds recently awarded by the National Science Foundation, Chemical Oceanography Program (\$1,163,722, Oct 1, 2013 to Sep 30, 2016). Contact at the NSF: Donald L. Rice, PhD, MPH, Director Chemical Oceanography Program, Division of Ocean Sciences, National Science Foundation, 4201 Wilson Boulevard Arlington, Virginia USA 22230 (Tel: 703-292-8582, Fax: 703-292-8590, Email: drice@nsf.gov).

Partner 5 : Bar Ilan University				Role and responsibility in the project				
Last name	First name	Position	Country	On board/shore	Field	Specialty	Responsability	p.m
Berman-Frank	Illana	Pr1	IS	On board	Biogeochemistry	PCD and role on export	Co-PI WP3	4,8
Elifantz	Hila	Lab. PI	IS	On shore	Biology	PCD and molecular assays		
Spungin	Dina	PhD	IS	On shore	Biology	PCD		

Ilana Berman-Frank (IBF) (<http://life-sciences.biu.ac.il/en/berman-frankilana/>, co-PI of OUTPACE WP3) is a biological oceanographer from the Mina and Everard Goodman, Faculty of Life Sciences, Bar Ilan University (Israel) working on phytoplankton for the last 25 years with a focus on marine N₂ fixers for the past 15 years. Funding for travel and analyses from the OUTPACE expedition for IBF is already available via this collaboration and a German-Israeli collaborative GIF Project to IBF No. 1133/2011 "Diazotrophs in face of global changes".

OTHER PARTICIPANTS (Funds not requested at ANR)

Participants (Not ANR funded)				Role in the project			Laboratory /University
Last name	First name	Position	Country	On board/shore	Field	Specialty	
Duhamel	Solange	CR2	US	On board	Biology	FC, single cell analyses	Univ. Columbia
Foster	Rachel	CR2	SW	On board	Biology	MB, onboard qPCR, SIMS	Univ. Stockholm
Knapp	Angela	CR1	US	On board	Biogeochemistry	Delta ¹⁵ N budget	Florida St University
De St Léger	Emmanuel	IE	FR	On board	Physics	CTD, sediment traps	DT INSU
Alvain	Séverine	CR1	FR	On shore	Optics	PHYSAT algorithm	LOG (LU)
Bouffard	Jerôme	CR	IT	On shore	Physics	Altimetry	RHEA (ESA/ESRIN)
Capone	Douglas	Pr1	US	On shore	Biogeochemistry	N ₂ fix. expert	University of S. California

Cravatte	Sophie	CR1	FR	On shore	Physics	Circulation in the SW Pacific	LEGOS (UPS)
Dekaezemacker	Julien	Post-Doc	DE	On shore	Biogeochemistry	N ₂ fix. microcosms	MPI
Jacot des Combes	Hélène	CR	FJ	On shore	Geochemistry	Radiolarians	USP
L'Helguen	Stéphane	CR1	FR	On shore	Biogeochemistry	N cycle	LEMAR (UBO)
Maes	Christophe	CR1	FR	On shore	Physics	Argo floats	LEGOS (UPS)
Martinez	Elodie	CR2	FR	On shore	Optics	Ocean-colours	EIO Papeete
N'Yeurt	Antoine	CR2	FJ	On shore	Biology	Taxonomy algae	USP
Rodier	Martine	CR1	FR	On shore	Biogeochemistry	SW Pacific expert	EIO Papeete
Sanudo-Wihelmy	Sergio	Pr	US	On shore	Chemistry	Trace metals other than Fe	USC Los Angeles
Zehr	Johnatan	Pr1	US	On shore	Biology	Diazotrophs diversity	Univ. of California SC

Preamble

No change in the scientific questions / strategy and composition of the consortium has been performed since the pre-proposal.

Since the submission of the pre-proposal, the OUTPACE proposal has been evaluated by the French INSU-LEFE programme (CyBER action) and obtained a co-funding of 51 k€ for 2014. We obtained 17 k€ from the leading partner MIO Institute (Action for the South) for 2014. The project has been evaluated by the French fleet commission and the OUTPACE cruise has been ranked in priority number 1 for a scheduling in 2015 (February 14th - March 30th most probably according to the person in charge of the R/V programming). We have obtained 10 Argo floats profilers by the GMMC (Groupe Mission Mercator/Coriolis, the French organization for Argo-floats deployments) in order to better characterize the general circulation in the SW Pacific. Finally, the OUTPACE project received a letter of support from the international IMBER programme.

1. CONTEXT, POSITION AND OBJECTIVES OF THE PROPOSAL

1.1. CONTEXT, STATE OF THE ART, SCIENTIFIC BOTTLENECKS

1. Societal and environmental context

The ocean provides regulating services to humanity. One of the most important is **the regulation of climate**. *Climate change* is now widely recognized as the major environmental problem affecting the globe, and costs annually 200-300 billion dollars (UNEP 2011, Towards a Green Economy - A Synthesis for Policy Makers, www.unep.org/greeneconomy). On a global scale, the fixation of atmospheric C by oceanic micro-algae (phytoplankton) and its transfer to deep waters represents an important part of the global C cycle and influence climate trends. In the context of global warming, the ocean plays a key role **by sequestering 30-40% of human CO₂ emissions every year**. Global warming is at the core of **human, environmental and economical** issues.

Human survival and well-being depend on ecosystem services, and therefore on the conservation and the best management of the ecosystems that provide them. Research on ecosystem services has grown exponentially during the last decade, particularly after the Millennium Ecosystem Assessment. Data and methods to assess the provision of marine ecosystem services are much more limited when compared to terrestrial assessments despite the oceans contribute for more than 60% of the total economic value of the biosphere. In the context of global warming, the ocean is changing quickly and initiating large oceanographic programmes is of major importance if we want to understand how the ecosystem services provided by the ocean will evolve in the future.

OUTPACE (Oligotrophy from Ultra-oligoTrophy PACific Experiment) is a multidisciplinary project dedicated to this issue involving 50 scientists from several French and international institutions; it is based on an original combination of the most up to date techniques in oceanography, from genes to satellites. The proposed team wants to benefit from the rare presence of the French Research/Vessel "L'Atalante" in the South Pacific in 2015 to perform a 45-days oceanographic cruise.

2. National and international context

The OUTPACE project is a multidisciplinary oceanographic project operating at various scales (from local to global), and fits perfectly with the first societal challenge "sober management of resources and

adaptation to climate change” of the present ANR call. In particular, it fits with the first axis which aims to “*understand and forecast environmental changes*”. It appears essential to “*consolidate the knowledge-base on dynamical, physical, chemical and biological processes of the different compartments of the Earth system*” and in particular of “*the Atmosphere and the Ocean*”. The biological C pump functioning in the oligotrophic ocean, and particularly its link with atmospheric dinitrogen fixation, appears as a “*scientific bottleneck*” that needs to be resolved for a better understanding and representation of the ocean/atmosphere C fluxes. There is 60 times more C in the ocean than in the atmosphere and the biological carbon pump significantly contributes to the control of the atmospheric CO₂ concentrations. The increasing atmospheric CO₂ concentrations is the main cause of climate alteration (recently confirmed by the new IPCC report, 2013). It is therefore essential to study the “*interactions between the C and N cycles*” to “*reduce the bias and uncertainties of the models*” on a large scale and be able to better forecast the earth climate evolution. By involving experts in experimental and modelling work, the OUTPACE project will actively contribute to this subject.

Our project also echoes the second ANR axis of the first societal challenge concerning “*functioning and adaptation of species, agro-ecosystems and continental and marine ecosystems*”. Indeed, this axis concerns “*the climate changes, the CO₂ increase and changes in organism’s diversity*”. The shift of the first control of primary production observed in the North Pacific at the end of the eighties (Karl et al., 1997) has been recently related to a higher frequency of diazotrophs blooms probably linked with climate change (Karl et al., 2008). At a shorter time scale (decadal), N₂ fixation and upper ocean nutrient availability are closely linked requiring the study of organisms responsible of this function. In the framework of OUTPACE, we will improve the “*fundamental knowledge of marine ecosystems populations*” in the SW Pacific by studying 1/ the biodiversity of the first levels of the marine food chain, but also 2/ the links between biodiversity and function at the single-cell level in relation to nutrient availability in contrasting trophic situations. It will therefore be possible to “*estimate the adaptation capacity or the evolution of the ecosystems as a function of their functional biodiversity*”. The marine production (up to fishes) mainly depends on nutrient availability in the upper ocean photic layer, and the input of new N by N₂ fixation is the main external source to the ocean (largely higher than by rivers). The SW Pacific exhibits some of the highest N₂ fixation rates in the world ocean (Bonnet et al., 2009) and it is thus important to focus on this region. France is at the second rank in the world in terms of maritime domain extension thanks to its territory in the Pacific Ocean where important fisheries are organized. It is therefore necessary to increase the fundamental knowledge of diazotrophs in order to predict the probable evolution of marine production in the SW Pacific at a decadal time scale, and subsequently the changes in higher trophic levels. Our project has therefore “*issues in fisheries resources and stocks conservation*”, research that is particularly encouraged by the ANR.

The OUTPACE project also fits with the scientific priorities of the national LEFE programme described in the INSU Prospective 2011-2016. It fits in particular with Topic 1 of the LEFE/CYBER action “*Study of the marine production at different scales*”. The OUTPACE will allow to (1) better identify and understand the vertical structuration of production at the day scale (microlayer formation, turbulent diffusion...), (2) better quantify marine productivity and its fate through higher trophic levels or C sequestration at the basin scale thanks to the consideration of meso- and submesoscale physical features, and (3) better understand the evolution and fate of marine production. The project also fits with Topic 4 “*Functional biodiversity, ecosystems functioning and biogeochemical cycles*” in particular the subtopic “*Role of marine ecosystems diversity on biogeochemical functions and trophic transfer*”. To answer these questions, we will use specific methodologies particularly encouraged by LEFE/CYBER, which are “*to examine the modifications of ecosystems structures through trophic interactions, characterize and quantify biogeochemical stocks and fluxes at the individual/specific level..., develop budget models of organisms including their stoichiometry*” (see section 2.2 “Description by Task”).

At the international level, the OUTPACE project fits into the scientific priorities of the large international programmes from IGBP and SCOR (IMBER, SOLAS, GLOBEC) as it aims at acquiring knowledge to understand, quantify and model/forecast interactions between biogeochemical cycles and marine ecosystems. **The OUTPACE project received the support of the international IMBER (Integrated Marine Biogeochemistry and Ecosystems Research) programme.** The letter of support from Eileen E. Hofmann (Chair, IMBER SSC) states that OUTPACE will efficiently contribute to the overall goal of IMBER, which is to investigate the sensitivity of marine biogeochemical cycles and ecosystems to global change, on time scales ranging from years to decades. The main objective of IMBER is to provide a comprehensive understanding of, and an accurate predictive capacity for ocean responses to accelerating global change and the consequent effects on the Earth System and human society. OUTPACE fits particularly in IMBER Topic 1 “*Interactions between marine biogeochemical cycles and marine food webs*” in which the main scientific question that needs to be addressed is “*Which marine biogeochemical cycles and ecosystems*

will be impacted by climate change”? The OUTPACE project will contribute to answer this question, in particular it will answer the underlying secondary questions of the IMBER topic 1.1 “What controls the production, transformation and degradation of organic matter in marine trophic chains”? and topic 1.3 “How key functional groups, species and genes affect biogeochemical cycles”.

At the regional scale (South Pacific), the OUTPACE project fits within at least 2 scientific priorities of the GOPS (South Pacific Observatory) “Marine ecosystems” and “Climate change and natural hazard”. It also fits within one of the transversal axis ‘Technologic innovation’ as this project is a medium for methodological developments and application of innovative technologies (see Section 2.2).

3. Scientific context - State of the Art

The additional CO₂ in the atmosphere, mainly resulting from fossil fuel emissions linked with human activities (anthropogenic CO₂), is the main cause of global warming. The ocean has acted as a major sink of anthropogenic CO₂ (Sabine et al., 2004) preventing a greater accumulation in the atmosphere and therefore a greater increase in the earth’s surface temperature. The biological pump (**Fig. 1**) provides the main explanation for the vertical gradient of C in the ocean. Its strength and efficiency depends upon the complex balance between organic matter production in the euphotic zone and its remineralisation in both the epipelagic and mesopelagic zones. The biological pump was thought to be in an equilibrium state with an associated near-zero net exchange of CO₂ with the atmosphere (Broecker 1991, Murnane et al., 1999). Climate alterations are beginning to disrupt this equilibrium and the expected modification of the biological pump will probably considerably influence oceanic C sequestration (and therefore global warming) over a decadal time scale (Sarmiento and Grüber, 2006). The long term decrease of phosphate availability and the shift from previously N to phosphorus (P) limited production associated with increasing inputs of N by N₂ fixation was one of the first evidence of the biological pump alteration observed at the HOT time series station in the north Pacific gyre (Karl et al., 1997; 2008).

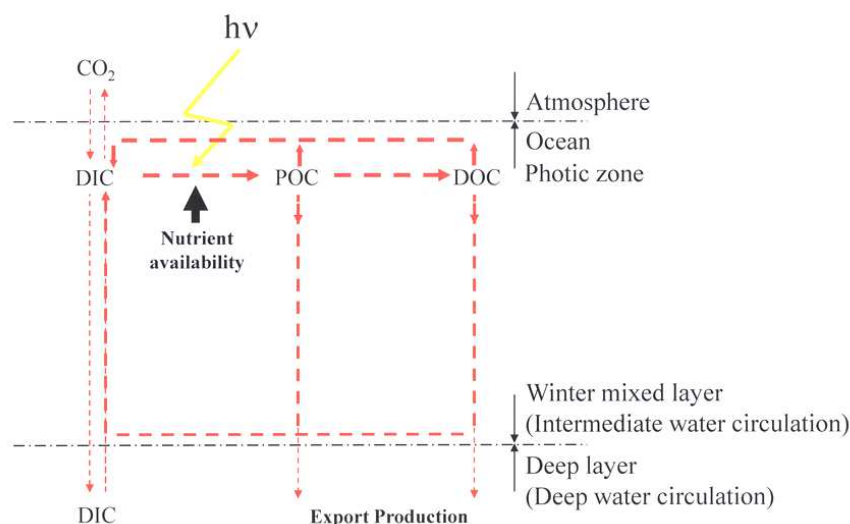


Fig. 1. Major C fluxes for a biological pump budget. Biological pump: C transfer by biological processes into the ocean interior. DIC: Dissolved Inorganic C, POC: Particulate Organic C, DOC: Dissolved Organic C. See Moutin et al., (2012) for a detailed description.

The input of new N to the surface ocean through biological N₂ fixation represents a major link between the C and N biogeochemical cycles, i.e between the upper ocean nutrient availability and the biological pump, i.e between ocean and climate. This link was recently shown to play a central role in previous natural climate changes over long time scales (Galbraith et al., 2013). It is nevertheless clear that expected climate changes followed by anthropogenic atmospheric CO₂ changes may concern shorter time scales: the increase in atmospheric CO₂ over the past 200-y is equal to the increase in atmospheric CO₂ between glacial to interglacial conditions, which took place over several thousand years (Sarmiento and Grüber, 2006). **It is therefore necessary to obtain a precise representation of the N₂ fixation process in global biogeochemical models.** Even if considerable scientific progresses have been made over the last decades (see reviews from Sohm et al., 2011; Zehr & Kudela 2011), the impact of this process on biogeochemical cycles and C export in ocean deserts is still poorly understood, mainly due to technical limitations. Monitoring properly the structure/function of the first levels of the marine food web, production, mineralisation and export of organic matter in a given water mass from the surface illuminated ocean to deeper depths requires a tight coupling between disciplines and is difficult to gather on a single scientific project.

The role of N₂ fixation in the oligotrophic ocean and overview of previous cruises in the SW Pacific

The efficiency of oceanic C sequestration depends upon many factors, among which is the availability of nutrients to support phytoplankton growth in the photic zone of the surface ocean. Large amounts of N are required for phytoplankton growth, as it is an essential component of proteins, nucleic acids and other cellular constituents. Fixed N in the form of nitrate (NO₃⁻) or ammonium (NH₄⁺) is directly usable for growth, but concentrations of fixed N are low (<0.1 μmol L⁻¹) in the oligotrophic ocean and often growth-limiting in most of the open ocean euphotic zone (Falkowski et al., 1998). Dissolved N₂ gas in seawater, on the other hand, is very abundant in the euphotic zone (ca. 450 μmol L⁻¹) and could constitute a nearly inexhaustible N source for the marine biota. Some prokaryotic organisms (Bacteria, Cyanobacteria, Archaea) called 'N₂-fixers' (or diazotrophs) are able to use this gaseous N source since they can break the triple bond between the two N atoms of the N₂ molecule, and convert it into a usable form (i.e. NH₃) for assimilation thanks to the nitrogenase enzyme system (Zehr et al., 1998; 2001). By doing this conversion, they release dissolved N in surface waters. At the global scale, they provide 100-200.10¹² g of N to the surface ocean every year (Mahaffey et al., 2005). It is the major external source of N for the ocean, significantly larger than atmospheric and riverine sources (Codispoti et al., 2001; Gruber and Sarmiento, 2002; Gruber, 2004). N₂-fixing organisms act thus as 'natural fertilizers', and contribute to sustain life and C export in coastal and oceanic environments (e.g. Karl et al., 2012).

Most of the ocean is comprised by low-nutrient, low-biomass oligotrophic ecosystems (Longhurst et al., 1995), which constitute the largest coherent biomes on our planet. They support a large part (40%) of the photosynthetic C fixation in the ocean (Antoine et al., 1996). This C fixation is mainly performed by picoplankton (smaller than 2-3 μm in diameter) that are generally thought to represent a negligible fraction of the total particulate organic C (POC) export flux due to their small size, slow individual sinking rates, and tight grazer control that leads to high rates of recycling in the euphotic zone. Consequently, the efficiency of the biological C pump in these oligotrophic systems has long been considered to be low as the greatest proportion of fixed C is thought to be recycled in the surface layer and rapidly re-exchanged with the atmosphere.

Recent studies have challenged this view and indicate that all primary producers, including picoplanktonic cells, contribute to export from the surface layer of the ocean at rates proportional to their production rates (Richardson & Jackson, 2007). Export mechanisms differ compared to larger cells as picoplanktonic export is mainly due to packaging into larger particles and via grazing and/or aggregation processes (Jackson et al., 1990; 2001; Lomas et al., 2010). More recently, Close et al. (2013) pointed out that 40-70% of picoplanktonic cells are small enough to escape detection under the most common definition of suspended particulate organic matter (POM). Thanks to a coupling between lipid profiles, radiocarbon and stable isotopic signatures of lipids from the North Pacific Subtropical gyre (NPSG), Hawaii Ocean Times-series station), they showed that the transfer of submicron POM from the surface to mesopelagic waters is an important contributor to the global biological pump. Besides submicron POM export, Karl et al. (2012) have reported an efficient summer export flux of C (3 times greater than the mean wintertime particle flux) at the same HOT station thanks to a 13-year sediment trap experiment. This summer export flux has been attributed to an increase in biomass and productivity of symbiotic N₂-fixing cyanobacteria associated with diatoms, which have high sinking and low remineralization rates during downward transit. This efficient POM export was thus not related to submicron particles but rather to large-size phytoplankton that could grow in the oligotrophic waters of the NPSG thanks to new N provided by N₂ fixation (Karl et al., 2012). **Consequently, the conventional view of low POM export in oligotrophic areas should be revised to include alternative pathways for picoplankton export and the role of N₂ fixation in potentially promoting C export.**

The SW Pacific is an ideal location to study this question, as it is considered to be one of the highest areas of global N₂ fixation (Capone et al., 1997; Sohm et al., 2011). It exhibits among the highest abundances, diversity of N₂-fixing organisms (Moisander et al., 2010) and activity (Montoya et al., 2004; Bonnet et al., 2009) in the world ocean. While average N₂ fixation rates range from 50-200 μmol N.m⁻².d⁻¹ in the tropical North Atlantic (Capone et al., 2005) and Pacific (Dore et al., 2002 ; Karl et al., 2012), they reach 126-4000 μmol N.m⁻².d⁻¹ (Montoya et al., 2004), 50-11900 μmol N.m⁻².d⁻¹ (Bonnet et al., 2009) and 151 to 703 μmol N.m⁻².d⁻¹ (Garcia et al., 2007) in the SW Pacific, in the Arafura Sea (off Australia, TRICHONESIA cruise), the western warm pool (EUC-Fe cruise) and the loyalty channel (DIAPALIS cruises), respectively. During the seasonal DIAPALIS cruises the highest rates have been measured in austral summer have been directly linked to phosphate availability (Van den Broeck et al., 2004; Moutin et al., 2005). The strong link between N₂ fixation and phosphate availability has recently been confirmed in the whole SW Pacific area (Shiozaki et al. 2014). Satellite observations (TRICHOSAT algorithm, Dupouy et al., 2011) corroborate recurrent large *Trichodesmium* blooms during the austral summer (October-March) during the 1998-2010 period in the Melanesian archipelagos (New Caledonia, Vanuatu, Fiji Islands). In addition to *Trichodesmium*, high

abundances of unicellular diazotrophic cyanobacteria from Group B (UCYN-B) and Group A (UCYN-A) have been reported in the SW Pacific (Campbell et al., 2005; Bonnet et al., 2009; Hewson et al., 2007; Moisander et al., 2010).

When going eastward towards the South Pacific gyre, Halm et al., (2011) have reported rates of $12\text{--}190 \mu\text{mol N m}^{-2} \text{d}^{-1}$ on the western border of the gyre and Raimbault and Garcia, (2008) and Moutin et al., (2008) reported rates of $60 \pm 30 \mu\text{mol N m}^{-2} \text{d}^{-1}$ in the central gyre during the BIOSOPE cruise, **indicating a decreasing gradient of N_2 fixation from W to E** and low N_2 fixation rates relative to other ocean gyre ecosystems. The organisms responsible for these fluxes are different from common autotrophic diazotrophs such as *Trichodesmium* or UCYN-B, and are mainly affiliated with heterotrophic proteobacteria and low abundances of UCYN-A (Bonnet et al., 2008, BIOSOPE cruise; Halm et al., 2011).

The W to E zonal gradient of N_2 fixation and the distinct diversity of N_2 -fixing organisms along this gradient provide a unique opportunity to study how production, mineralisation and export of organic matter depends upon N_2 fixation in contrasting nutrient conditions. Comparisons between different systems along a zonal gradient of trophic status and N_2 fixation will provide new insights for identifying and understanding fundamental interactions between marine biogeochemical C, N, P, Si, Fe-cycles and oligotrophic ecosystems.

1.2. OBJECTIVES, ORIGINALITY AND NOVELTY OF THE PROJECT

1. Scientific objectives

The OUTPACE project is organized on **three main objectives**. The final goal is to obtain a precise representation of the complex interactions between planktonic organisms and the cycle of biogenic elements (C,N,P,Si,Metals), considering a variety of scales, **from single-cell processes to the whole SW Pacific ocean**. The main objectives of this research are the following:

- 1) *To perform a zonal characterization of the biogeochemistry and biological diversity of the SW Pacific during the strongest stratified period (austral summer)*
- 2) *To study the production and fate of organic matter (including C export) of 3 contrasting oligotrophic environments with a particular emphasis on N_2 fixation*
- 3) *To obtain a satisfactory representation of the main biogeochemical fluxes (C, N, P, Si) and the dynamics of the planktonic trophic network, both in situ and by using microcosm experiments*

2. Originality and Novelty of the proposed project

The OUTPACE project is original because **1/** it focuses specifically on oceanic deserts (oligotrophic ocean). They represent 80% of the global surface ocean and their role in CO_2 sequestration has long been considered to be low, but this view is currently challenged by new studies highlighting alternative pathways for C export to the deep ocean, **2/** it focuses on the tropical South Pacific ocean, which is critically undersampled despite it represents a strategic marine territory for France as the second Economic Exclusive Zone in the world, **3/** the scientific objectives of OUTPACE are at the core of the concerns of contemporary Oceanography and closely connected with top priorities of international structuring science programmes (IMBER, SOLAS) regarding science and societal issues. It aims at better understanding the role of the ocean in the C fluxes exchanged with the atmosphere and its participation in the control of the earth climate.

At the technological level, this project is the culmination of a long process that will allow scientists of different fields (from physicists specialized in Lagrangian navigation to biologists specialized on genes and biodiversity/function at the single-cell level) to work together in the same project. **The original coupling between satellite imagery, Lagrangians floats, turbulence profilers, biogeochemical sensors and molecular tools which allow real time acquisition of physical, chemical en biological parameters to answer the scientific questions of the project, is one of the major originality and novelty of this project.** The OUTPACE project benefits from a very favorable international dynamics and regroups some of the better specialists in the field.

In addition to the transdisciplinary field work approaches, the modelling work (Task 5) performed in this project will deliver a final end-product useful for the international modelling community. Several mathematical formulations of the diazotrophy process are currently implemented in the numerical library of biogeochemical processes of the Ecological Mechanistic and Modular Modelling platform model (Eco3M). They will contribute to answer the scientific questions of the project and will provide simulations for the future regarding the ability of oligotrophic system to sequester C. They will finally become available for modellers once validated for integration in climate models.

2. SCIENTIFIC AND TECHNICAL PROGRAMME, PROJECT ORGANISATION

2.1. SCIENTIFIC PROGRAMME AND PROJECT STRUCTURE

The scientific program will be achieved through the realization of 5 complementary Tasks (+ a ‘Promotion’ task) involving **international collaborations** between physicists, biologists and biogeochemists with expertises ranging **from physics, chemistry, optics, marine biogeochemistry, marine microbiology, molecular ecology, genetics, and modelling** (Fig. 2).

The overall strategy of the project will combine experimental and modelling approaches. The experimental part is based on the realization of a **45-days oceanographic cruise** onboard the R/V L’Atalante in the SW Pacific in 2015 (Feb.-March). The cruise will consist in 18 SD and 3 LD stations distributed along trophic and N_2 fixation gradients (**Fig.3**). During the SD stations, we will perform a zonal characterization of the biogeochemistry and biological diversity from the North of New Caledonia to the western border of the South Pacific gyre (**Task 1**). The LD stations will allow to perform a process study in contrasting oligotrophic and N_2 fixation conditions following a Lagrangian approach for a 3D characterization of the upper water column (**Task 2**). During the LD stations, high frequency acquisition of physical, optical, biogeochemical and biological variables will be assessed. We will quantify primary / secondary production and their fate (**Task 3**), as well as mineralization and export of organic matter (**Task 4**) by measuring biogenic element (C,N,P,Si) stock/fluxes and organisms responsible for these fluxes (“Who does what?”). Finally, a multi-scale modelling approach is proposed from the beginning of the OUTPACE project (**Task 5**). Its objective is to complement the observations with the analyzed data in order to understand the interactions between the biogeochemical cycles of the biogenic elements and the dynamics of the planktonic trophic network. Modelling will also be used in combination with observation and measurements at several scales and at several points of the project in order to optimize the potential of each of these tools. Final end-products (model, new techniques) will be available to the scientific community (database, publications, international conferences), to students (training/courses), and to a broad audience through the realization of a blog, a website and a documentary film (**Task 6**).

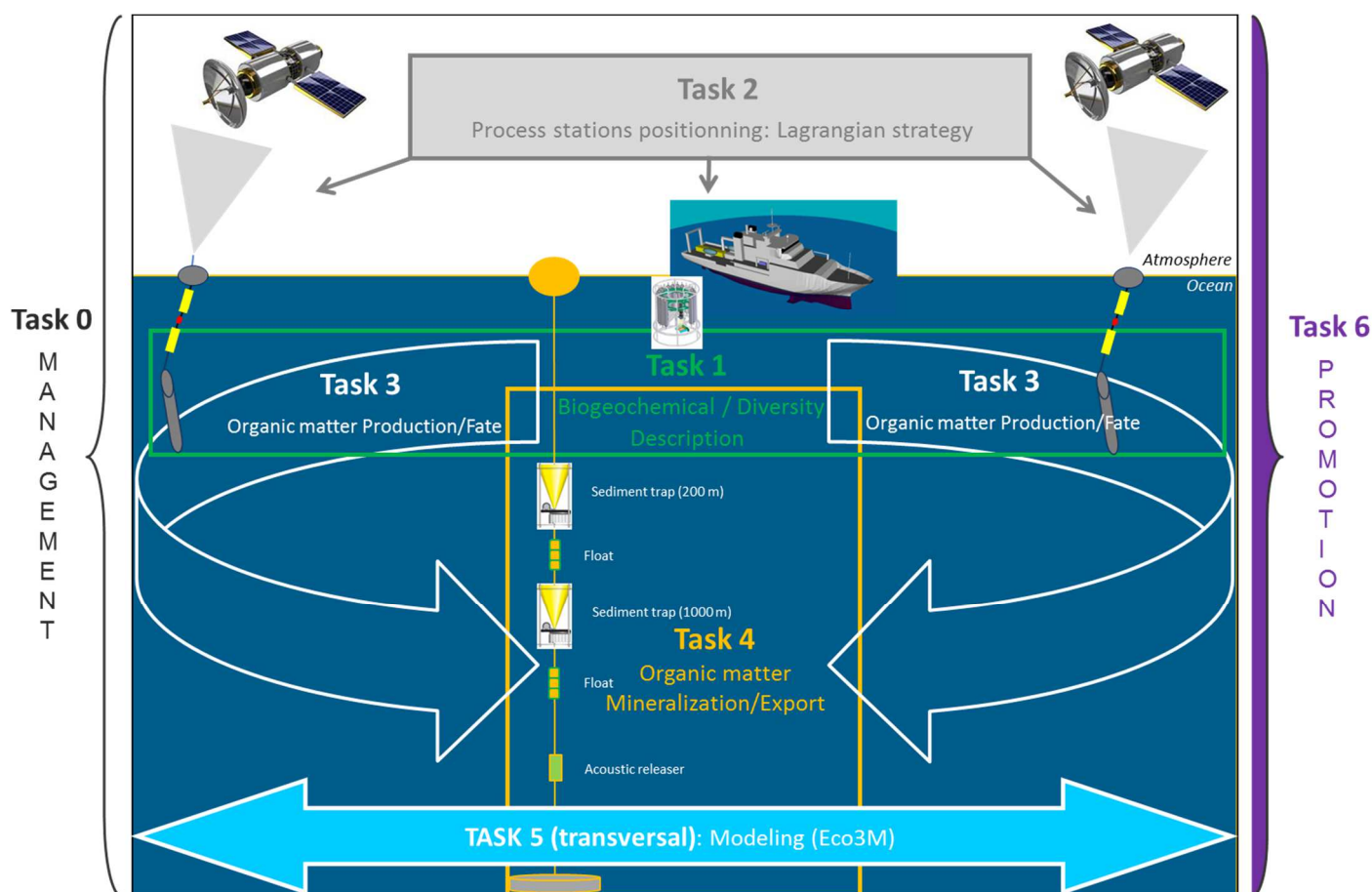


Fig. 2. Diagram summarizing the different tasks of the project and their interrelations.

General schedule of the cruise:

The general schedule of the cruise will follow the provisional timetable presented below. The boarding of scientists and equipment will be performed in Noumea (New Caledonia) and will benefit from the logistical and administrative support of the IRD Center of Noumea (200 people). Calculations of ship time were done considering an average of 24-h for characterizing the hydrological conditions at each LD station and a boat speed of 10 nautical miles as recommended for the R/V L'Atalante in the 2014 call for proposal.

Table 1: Provisionnal schedule of the work at sea

Day	Transit and station	Latitude Longitude	Type of works
J1	Nouméa	22°14S ; 166°28E	Boarding and loading
J2-J4	Transit		
J5-J11	LD station A	19°00S ; 155°00E	See Task 2, 3, 4
J12	SD station 2 + transit toward SD station 3		See Task 1
J13	SD station 3 + transit toward SD station 4		See Task 1
	Idem until SD station 10		
J20-J26	LD station B	19°00S ; 176°19E	See Task 2, 3, 4
J27	SD station 11 + transit toward SD station 12		See Task 1
J28	SD station 11 + transit toward SD station 12		See Task 1
	Idem until SD station 19		
J36-J42	LD station C	19°00S ; 161°00W	See Task 2,3, 4
J43-J44	Transit		
J45	Papeete	17°34S ; 149°36W	Deboarding of people and material

2.2. DESCRIPTION BY TASK

1. Task 1: zonal characterization of the biogeochemistry and biological diversity of the SW Pacific during the strongest stratified period (austral summer)

PIs: Thierry MOUTIN and Hervé CLAUSTRE

This task will specifically answer Objective 1 of the project, which aims at characterizing the biogeochemical parameters and biological diversity/function along a W-E gradient (**Fig. 3**) from the North of New Caledonia, an area exhibiting high N_2 fixation rates, to the central South Pacific gyre, which has been characterized as the most oligotrophic oceanic area of the world ocean (Claustre et al., 2008), and exhibiting low N_2 fixation rates (Moutin et al., 2008).

SD stations positioning and detailed operations onboard

Figure 3 presents the position of the stations that will be sampled during the cruise. The 19°S transect has been chosen as it is located in the southern branch of the South Equatorial Current (SEC) i.e. in a relatively homogeneous water mass (Fig. 3A) coming from the East (South Pacific Gyre). Moreover, this area is relatively homogeneous in terms of physics as it is located in the northern part of the very active zone with regards to mesoscale structures (Qiu et al., 2009). However, the studied area presents an interesting contrast in terms of tides with a barotrope tide more intense in the western part (Station B in particular) compared to the eastern part of the transect. This transect has also been chosen because SST are always higher than 25°C (Fig. 3B), which are the optimal temperatures for the development of diazotrophs and constitute the area in the SW Pacific where they are the most abundant (Moisander et al., 2010; Campbell et al., 2005). Finally, this transect has been chosen in order to sample and perform process studies along a trophic gradient, from oligotrophic waters in the western part of the transect to ultra-oligotrophic waters in the eastern part (Fig. 3C).

During the transect, we will perform 18 SD (8 h) “descriptive” stations. The systematic operations at every station will be:

- 0-500 m profiles with the Vertical Microstructure Profiler VMP250 to evaluate the turbulence on the surface layer. The time necessary is 1h.

- 2 CTD profiles (0-2000 m and 0-200 m) with the CTD rosette (24 niskins) (3h30) to sample for the following parameters:

(1) core parameters: temperature, salinity, dissolved oxygen O₂, DIC, alkalinity, nutrients, chlorophyll, particulate organic C, N, P (POC, PON, POP), particulate Si, dissolved organic C, N, P (DOC, DON, DOP)
 (2) specific variables allowing to evaluate the planktonic composition (diversity), with a specific emphasis on diazotrophs

(3) at selected depths, specific biogeochemical fluxes (Primary production, N₂ fixation) with incubations under controlled conditions onboard

-Between the 2 CTD casts, in order to provide enough time to sample from the rosette, we will perform 3 net tows for phytoplankton, zooplankton and diazotrophs (*Trichodesmium* spp.) (1h30)

-1 Trace Metal Clean Rosette 0-500 m CTD cast (12 go-Flo bottles rosette) for trace metals sampling and specific flux measurements requiring ‘ultra-clean’ conditions (2 h)

The distance between SD stations is 130 nautical miles, corresponding to 13 h of transit (calculation based on a ship speed of 10 knots).

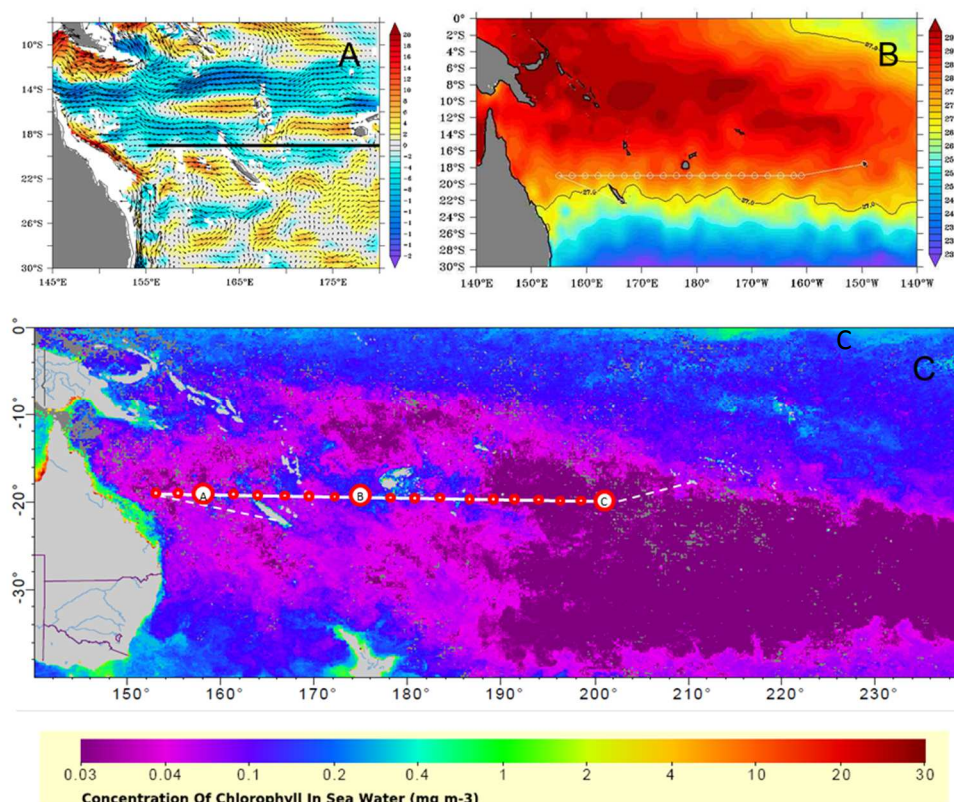


Fig. 3. Transect of the OUTPACE cruise superimposed on several maps (A) Absolute geostrophic currents (from CARS climatology ref to 1000 m, plus currents 0-200 m deduced from Argo floats drift, Kessler and Cravatte, 2013, JGR). Colorbar: zonal currents; Arrows: horizontal currents), (B) CARS2009 Sea Surface Temperature (SST) climatology (Ridgway and Dunn, 2003) (Dec-March), and (C) Surface chlorophyll concentrations (MODIS, composite Dec-March 2011-2012).

Subtask 1.1. Biogeochemical characterization

The classic variables temperature, salinity, dissolved oxygen concentrations, along with alkalinity, pigments, organic and mineral C and other biogenic elements (N, P, Si) pools, and trace metals of biogeochemical importance (Fe, Zn, Cu, Co, Ni), will be measured, as well as other specific variables at selected depths (N₂ fixation, diazotroph diversity, recycling times for dissolved phosphate...) at the 18 SD stations across the 3000 nautic miles transect. The aim is to describe the variations in the biogeochemical characteristics (nutrient and trace metals availability in the photic layer, depth of the nutriclines, ratios of dissolved/particulate and organic/mineral in the biogenic elements pools, drawdown of CO₂ of anthropogenic origin, phytoplankton biomass and diversity, N₂ fixation rates...). This biogeochemical characterization will extend to the gyre in the central South Pacific where oligotrophic conditions are the most extreme (Claustre and Maritorena, 2003).

Our recently acquired techniques for biogeochemical measurements in ultra-oligotrophic waters should enable us to significantly improve our understanding of the biogeochemistry of the SW Pacific. We do not have a good insight into the spatial distribution of certain biogeochemical variables, such as N₂ fixation, or absolute nutrients concentrations in surface waters, which have a predominant role in controlling planktonic production. Recent studies infer the importance of oligotrophic areas in the drawdown of anthropogenic C (e.g.

Close et al., 2013). **It is necessary to quantify the C fluxes in the upper water of the SW Pacific** and their potential links with N₂ fixation.

Specific question: *What is the actual zonal distribution of the main biogenic elements stocks/fluxes and trace metals in the SW Pacific?*

Subtask 1.2. Biological diversity

A major objective will be to produce an overall description of the planktonic community composition in the SW Pacific pelagic ecosystems, providing a “functional” structure for specific analysis. A detailed description of the biological diversity is essential as it is hypothesised that biodiversity increases the functional redundancy of marine ecosystems. Such redundancy may play an important role in an ecosystems ability to withstand natural and anthropogenic disturbances (Fonseca and Ganade, 2001). The need to describe a more “operational” functional structure should not replace having a “complete” description of the species composition. Variation in species composition probably remains the most effective tool in identifying natural and / or anthropogenic perturbations. A coupling of complementary techniques will be used along the transect, including surface continuous flow cytometry (CYTOSUB) and discrete flow cytometry for bacteria, pico-, nano- and micro-phytoplankton (Marie et al., 1999), microscope determination of taxonomy for microphyto-, microzooplankton (Hasle et al., 1978) and heterotrophic nanoflagellates, and imagery (zooscan, flowcam) for metazooplankton taxonomy (Grosjean et al., 2004), associated with particle counters (UVP, LISST, LOPC) for spatial distribution and size-structure of particules and plankton. The UVP will also assess the *Trichodesmium* spp. colony abundance in the water column (Guidi et al., 2012).

A special attention will be given to studying the diversity of diazotrophs. The scientific community made intense progress over the last decade thanks to the emergence of molecular methods, which allowed the discovery of an increasing diversity of N₂-fixing organisms (Zehr et al., 2001; 2008), active in previously unsuspected ecological niches such as N-rich areas (e.g. Short and Zehr, 2007; Needoba et al., 2007; Moutin et al., 2008; Bonnet et al., 2011; Fernandez et al., 2011; Dekaezemacker et al., 2013). The discovery of heterotrophic and photo-heterotrophic (UCYN-A) diazotrophs implies that N₂ fixation is not necessarily linked with C fixation in the euphotic zone, and N₂ fixation has recently been reported in aphotic waters (Hamersley et al., 2011; Rahav et al., 2013) and controlled by DOM availability (Bonnet et al., 2013b). However, despite the increasing recognition of their abundance in the global ocean (Luo et al., 2012), very few studies have examined in details the precise role and regulation of N₂ fixation in photo-heterotrophic and heterotrophic (cyano) bacteria in marine waters. In the North Pacific Subtropical Gyre (NPSG) at station ALOHA, Dore et al. (2008) estimated that the highest integrated N₂ fixation rates occurred during summer while either *Trichodesmium* spp. or diatom blooms (mainly constituted by *Rhizosolenia* spp. or *Hemiaulus* spp. with *Richelia intracellularis*) were observed. However, the Southern Pacific exhibits a negative Si* (SiOH₄-NO₃) whereas it is strongly positive in the Northern Pacific (Sarmiento and Gruber, 2006) and the relative importance of diatoms in N₂ fixation remains unknown in the proposed study area. We should not dismiss the role of diazotrophs living in association with diatoms as possible key players in N₂ fixation and C export (Karl et al., 2012), and offering a link between the N and silicon biogeochemical cycles. **There is a clear need to study all diazotrophs if we want to know how N₂ fixation and therefore the oceanic N budget will respond to global change in the ocean.**

The cruise transect will cross a gradient of phytoplanktonic biomass identified from satellite data, providing *in situ* observations to compare with such satellite-based analysis. It will be used to calibrate PHYSAT, which allows determining large organism's classes from space (Alvain et al. 2005; 2008), toward a better representation of N₂ fixing organisms. A comparison with vertical distribution of phytoplankton communities based on surface chlorophyll determination (Uitz et al., 2006) will also be undertaken. Ocean color remote sensing will also give a synoptic overview of the chlorophyll concentration in the South Pacific during the cruise. In parallel, synoptic observations of satellite SST and Sea Level anomaly will provide insights about the physical context of the upper water column at basin scale: i.e. variability of ocean stratification and thermocline depth (which can be used as a proxy of the nutricline depth) as well as horizontal and vertical advection from the surrounding waters of the transect (Wilson and Coles, 2005; Martinez et al., 2009). These physical observations will also be compared to *in situ* measurements to validate the use of this proxy.

Specific questions: *What species currently characterize the SW Pacific's pelagic ecosystems during austral summer conditions? Which species or group is responsible for specific functions (N₂ fixation, dissolved organic phosphate utilization, bacterial production, silification rates)?*

Subtask 1.3. Biogeochemical process from optical measurements

Integrated measurements of bio-optical properties and pigments will be made using instruments measuring hyperspectral radiometry in the UV-Visible domain, with UV-VIS Trios spectroradiometers (Murakami and Dupouy, 2013). Two consecutive profiles of downward irradiance [Ed(Z,λ)] will be conducted at solar noon

with a MicroPro free-fall profiler (Satlantic) equipped with pressure, temperature and tilt sensors and OCR-504 downward irradiance sensors for the UVR-B (305 nm), UVR-A (325, 340 and 380 nm) and PAR (412, 443, 490 and 565 nm) spectral domains, according to the protocol described in Tedetti et al. (2007) and Para et al. (2013). Surface (atmospheric) irradiance [$E_s(\lambda)$], which is equivalent to the downward irradiance just above the sea surface [$E_d(0^+, \lambda)$], will be continuously measured in the same channels from the ship deck with other OCR-504 downward irradiance sensors (surface reference). These measurements will be made to account for the variations in cloud conditions occurring during measurements. Backscattering at the satellite channels will be obtained with the Hydrosat-6 (Dupouy et al., 2008; Loisel et al., 2011). Hyperspectral light absorption of particles and CDOM and FDOM characterization (with the identification of markers of bloom degradation in the surface, above and below the euphotic depth, etc...) will be performed in relation with pigment concentrations (Task 1.1) (Coble, 1996; Tedetti et al., 2011; 2012). Additionally, the characterization of optical properties of dominant species (Dupouy et al., 2008) encountered along the transect will be realized. In addition, inorganic aerosol quantification and atmospheric radiance measurements will be continuously done along the transect, especially around the Vanuatu archipelago. Simulations reveal that in Northern Atlantic atmosphere, dusts are able to induce a significant decrease of primary productivity due to the attenuation of light by about 15-25% for dust optical depth (DOD) larger than 0.6-0.7 (at 550 nm).

Subtask 1.4. Physical and biogeochemical characterization using Argo float profilers

We have obtained 10 Argo floats profilers and will probably obtain 3 additional profilers PROVIO (specific Argo floats equipped with new biogeochemical sensors) by the GMMC (Groupe Mission Mercator/Coriolis, the French organization for Argo-floats deployments). These floats will be used for several objectives and mainly to obtain data at a larger time scale (until several years) than the time scale of the cruise. The physical data obtained from the Argo-floats will be used mainly to better characterize the general circulation in the SW Pacific. It was shown that changes in the thermohaline properties of the South Equatorial Current may influence the cycle of the ENSO oscillation (El Niño Southern Oscillation) at a decadal time scale and then lead to climatic feedback at a basin scale. The OUTPACE cruise offers the opportunity to extend studies on the incoming jets in the Coral Sea and specially to focus on the South of Fiji jet. Secondly, the biogeochemical data obtained from the 3 PROVIO deployed at each LD station will allow to study the production and fate of organic matter on a longer term. We will focus on net community production estimation on an annual basis. The question of whether the plankton communities in low nutrient regions of the ocean, comprising 80% of the global ocean surface area, are net producers or consumers of oxygen and fixed C is a key uncertainty in the global C cycle (Riser and Johnson, 2008). The NCP estimates need to add recent oxygen optodes on the PROVIO floats which equipment is asked in the present request. The PROVIO will allow to estimate organic matter export from fluorescence and backscattering sensors (Briggs et al., 2011) that will permit to extend the measurements after the occupation of the station to eventually catch the ends of the blooms.

2. Task 2: Process stations positioning – Lagrangian strategy

PIs: Andrea DOGLIOLI and Pascale BOURUET-AUBERTOT

The second major objective of this project is to study, at the 3 LD stations representing contrasting oligotrophic areas, the production, mineralisation and export of organic matter and how it depends on the N_2 fixation process. We will quantify the flow of material (biogenic elements) through each ecosystem and particularly focus on the role of N_2 fixation in the production and fate of organic C. **The fate of newly fixed N by diazotrophs in the ocean has been poorly studied so far, despite the fact that N_2 fixation provides the major external source of N for the global ocean.** In most studies, the input of N brought to surface waters by N_2 fixation is converted using Redfield ratios in order to ‘theoretically’ evaluate the C production potentially sustained by this source of N. It is not clear whether this N fuels photo-autotrophic phytoplankton and can be transferred along the trophic chain and rapidly exported out from the euphotic zone, and/or if it fuels heterotrophic growth and thereby contributes to remineralisation in surface waters. In addition, the plankton diversity and its evolution after the development of a diazotroph bloom (classical food web versus microbial loop) have been very poorly studied, mainly due to the difficulty of observing and monitoring a bloom in the natural environment.

Subtask 2.1. Positioning of the LD stations

A real added value for the project will be to sample 3 water masses along the trophic gradient characterized by different populations of N_2 -fixing organisms. We expect to locate the first LD station (A) in the Coral Sea (north of New Caledonia) in an area dominated by the photo-heterotrophic diazotrophs UCYN-A present at very high abundances (10^5 - 10^6 *nifH* copies.L⁻¹) (Moisander et al., 2010) using **diazotroph abundance**,

diversity and gene expression assessed onboard using qPCR (Foster et al., 2009). The intensive use of satellite data will provide a key contribution for the identification of the 2 other LD stations. We expect to locate the second LD station (B) close to the Fiji Islands in an area dominated by the autotrophic diazotrophs *Trichodesmium* spp. (Dupouy et al., 2011) and/or UCYN-B, which often appear spatially associated in the SW Pacific (Moisander et al., 2010). The previously developed algorithms **TRICHOSAT** (Dupouy et al., 2011) and **PHYSAT** (Alvain et al. 2005; 2008; the *Trichodesmium* algorithm for PHYSAT is currently being developed in the framework of the SAVALEFER project (PI: C. Menkès) will help to locate *Trichodesmium* spp. blooms from space. The Third LD station (C) will be located in the western part of the South Pacific gyre, a low N_2 fixation region (Moutin et al., 2008) where heterotrophic diazotrophs dominate (Bonnet et al., 2008; Halm et al., 2011).

Subtask 2.2. Lagrangian strategy (including detailed operations onboard)

To achieve our process study at the LD stations, a detailed knowledge of the local dynamics is needed. The regions along the vessel route will be first characterized at large scale through the analysis of satellite (SST, Altimetry, Ocean color) and operational model data. These will be automatically retrieved and processed to derive Eulerian and Lagrangian diagnostics of ocean circulation and biogeochemistry: Okubo-Weiss parameter maps, Lagrangian Coherent Structures, Chlorophyll maps, Phytoplankton group maps. This information will be transmitted onboard by a dedicated server on land. The data will be analyzed in near realtime both on board and on land in order to optimize the sampling strategy according to the local physical and biogeochemical characteristics. This approach has already been successfully implemented by our team and employed during several previous cruises: LATEX (Nencioli et al. 2011), KEOPS2 (d'Ovidio et al., 2012) and STRASSE. Then, the selected area will be sampled by performing a cross of multisensor MVP transects of about 40 km at a speed of 10 knots (maximum depth of the MVP200 around 230 m). The MVP data (CTD, Fluorescence and LOPC) will be immediately processed onboard to identify a second smaller area (around $25 \times 25 \text{ km}^2$) where a high resolution mapping will be performed. Moreover, the phytoplanktonic assemblages will also be assessed onboard by using surface continuous flow cytometry (Cytosub) and discrete flow cytometry for bacteria, pico-, nano- and micro-phytoplankton. In the smaller area local meso and submesoscale dynamics (eddy, filaments and frontal processes and their impact on regulating vertical fluxes and structuring the planktonic community) will be characterized at very high resolution through MVP200 zig-zag mapping at the beginning and at the end of each LD station survey (**Fig.4**). This will allow the accurate identification of the homogeneous and coherent water masses which will be the focus of each LD station. For this purpose the MVP200 represents an extremely useful instrument to sample a large zone in a short time to preserve synopticity.

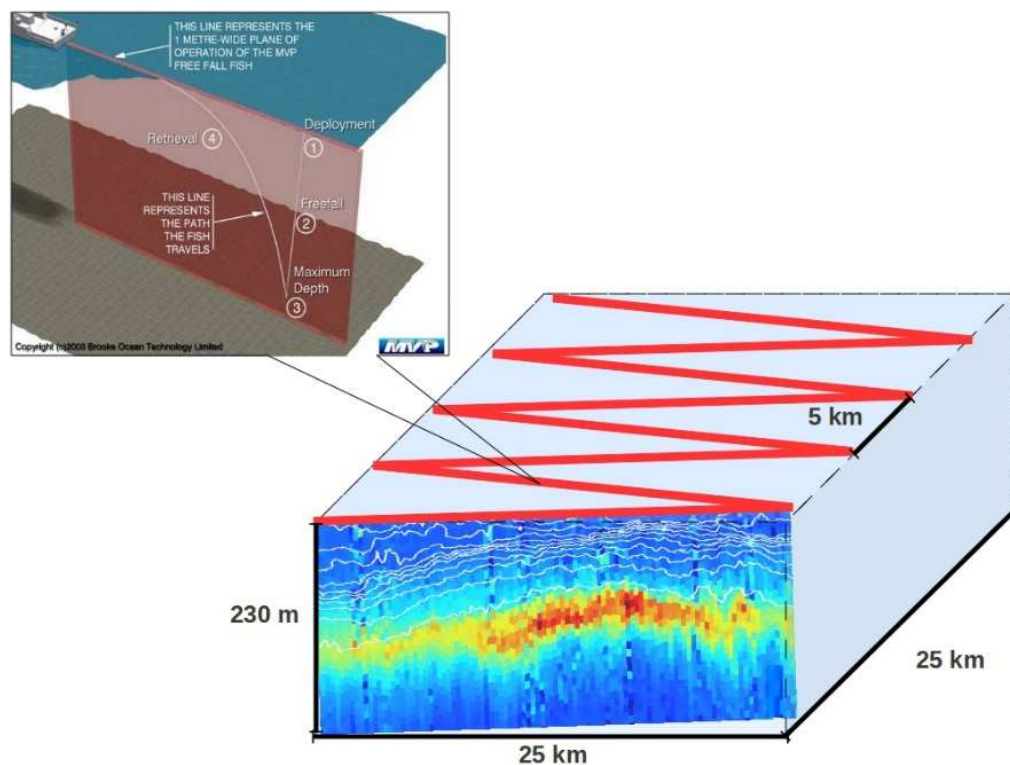


Fig.4. Sketch of the MVP200 zig-zag mapping, with a zoom on the events for a typical cast are: Deployment, Free-fall, Maximum depth, and Retrieval. Credit: ©Brooke Ocean Technology, Ltd.

This strategy will allow the investigation of the impact of high-frequency small-scale physical phenomena on biomasses and biogeochemical fluxes and the identification of the initial position of the Lagrangian reference frame for the Lagrangian navigation. Once the Lagrangian reference frame is defined, 3 drifters (1 central and 2 annex for each site) will be immediately dropped. A recently improved version of the Lagrangian navigation software developed during the LATEX project (Doglioli et al., 2013; **Fig.5**) will be adopted in order to take into account the translation of the studied water masses, as well as their rotation and deformation all along the 6-day process study.

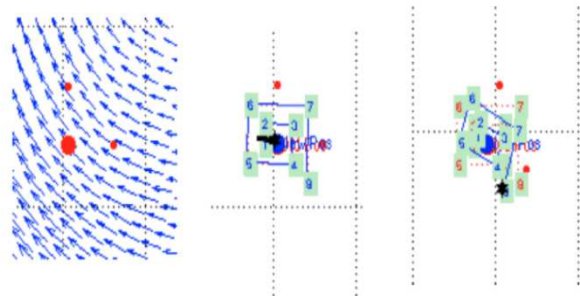


Fig.5. Example of Lagrangian navigation in a reference frame defined on the basis of the positions of three buoys (red dots). The square spiral route performed by the vessel (black star) is translated, rotated and deformed (red to blue lines) to follow the studied water mass.

Then, CTD and micro-structure profiles, water samplings and deployments of a drifting mooring line (equipped with a primary production line, O₂ productivity autosampler and sediment traps) will all be performed close to the central drifter all along the *in situ* process study. The drifting mooring line will be deployed for 24-h then recovered and then deployed again the following day until the end of the 6-days process study.

The sequence of operations at LD stations will be as follows, for a sun rise at 6:00

DAY 1

- 1:00-1:30 CTD 0-200 m : sampling for oxygen production
- 1:30-2:00 Plankton nets
- 2:00-3:00 CTD 0-200 m : sampling for production and molecular biology measurements
- 3:00-4:00 CTD 0-500m Physics + Cycle sampling + optical grape
- 4:00-5:00 OPC + Plankton nets
- 5:00-6:00 Drifting mooring deployment : *in situ* production line (C,N,P,Si,ΔCO₂,ΔO₂) + O₂ Productivity Autosampler (IODA) + sediment traps
- 6:00-7:00 CTD 0-500m Physics + Cycle sampling + optical grape + VMP250 deployment and recovery
- 7:00-8:00 CTD 0-500m Physics + large volume sampling at one depth
- 8:00-9:00 *In situ* pumping
- 9:00-10:00 CTD 0-500m Physics + Cycle sampling + optical grape + VMP250 deployment and recovery
- 10:00-11:00 OPC + Plankton nets
- 11:00-12:00 CTD 0-500m Physics + large volume sampling at one depth
- 12:00-13:00 CTD 0-500m Physics + Cycle sampling + optical grape + VMP250 deployment and recovery
- 13:00-15:00 Radiometric profile, plankton nets
- 15:00-16:00 CTD 0-500m Physics + Cycle sampling + optical grape + VMP250 deployment and recovery
- 16:00-18:00 Radiometric profile
- 18:00-19:00 CTD 0-500m Physics + Cycle sampling + optical grape + VMP250 deployment and recovery
- 19:00-21:00 *In situ* pumping
- 21:00-22:00 CTD 0-500m Physics + Cycle sampling + optical grape
- 22:00-24:00 *In situ* pumping
- 24:00-1:00 CTD 0-500m Physics + Cycle sampling + optical grape,

DAY 2

- 1:00-2:00 Plankton nets
- 3:00-4:00 CTD 0-500m Physics + Cycle sampling + optical grape
- 4:00-5:00 Plankton nets
- 5:00-6:00 Drifting mooring recovery
- 6:00-7:00 CTD 0-500m Physics + Cycle sampling + optical grape + VMP250 deployment and recovery
- 7:00-9:00 Sediment traps and IODA lines recovery
- 9:00-10:00 CTD 0-500m Physics + Cycle sampling + optical grape
- 10:00-11:00 OPC + Plankton nets
- 12:00-13:00 CTD 0-500m Physics + Cycle sampling + optical grape + VMP250 deployment and recovery
- 13:00-15:00 Radiometric profile, plankton nets
- 15:00-16:00 CTD 0-500m Physics + Cycle sampling + optical grape
- 16:00-18:00 Radiometric profile

- 18:00-19:00 CTD 0-500m Physics + Cycle sampling + optical grape + VMP250 deployment and recovery
- 19:00-21:00 CTD rosette TMC (Trace Metal Clean) 0-2000 m
- 21:00-22:00 CTD 0-500m Physics + Cycle sampling + optical grape
- 22:00-24:00 *In situ* pumping
- 24:00-1:00 CTD 0-500m Physics + Cycle sampling + optical grape + VMP250 deployment and recovery

The same sequence of operations will be performed on days 3 & 4, and on days 5 & 6. A deep CTD (surface-bottom) cast will be undertaken before the departure to the next station.

Remark: This time schedule will provide a continuous 147-h of CTD casts every 3 hours (50 CTD casts), 3 daily measurements (24-h dusk-to-dusk) of *in situ* production and particulate export.

Niskin Bottles (12 L) available for sampling: Rosette 24 Niskin bottles (1 miniPVM which do not need any bottle space); Rosette TMC (Trace Metal Clean): 12 Go Flo bottles.

Finally, the atmosphere will be continuously sampled by a system installed at the front of the boat and enslaved by a wind vane to collect only when the boat is in motion, and when the wind arrives effectively from forward as detailed in Wagener et al., (2008). 4 samples will be sampled simultaneously in order to quantify the fluxes of nutrients and micronutrients brought by the atmosphere, and also to evaluate the soluble fraction (bioavailable) by dissolution experiments performed on board as detailed in Ternon et al., (2008). In this way, water, heat and momentum surface fluxes, will be calculated with considering the bulk method in order to evaluate the atmospheric forcing on the mixed layer activity and its temporal variability.

Subtask 2.3. Estimation of the turbulent activity

For each CTD profile, each variable (T, S, O₂) will be measured at 24 Hz and the vertical profile of the horizontal current (LADCP) calculated with a vertical resolution of 4 m (LADCP). The data treatment will give average profiles and T-S anomalies with a 1 m vertical resolution, the amplitudes of internal waves and in some cases density inversions, which are indicators of turbulent activity.

The microstructure profiles will give the rate of dissipation of the turbulent kinetic energy, the eddy diffusion coefficient will be deducted and used to calculate the turbulent fluxes. These direct measurements will also allow to validate the fine scale parameterization which provides dissipation as a function of fine scale shear and stratification. These parameterizations depends on the level of turbulence and on the processes leading to turbulence so it is crucial to collect more joint CTD/LADCP and turbulence measurements to refine these relations. The temporal repetition of profiles will be done days 1, 3 and 5 with profiles performed every 3 h with VMP250 (a light microstructure profiler able to profile down to 500 m). These measurements will be performed just after the CTD-Physics so that the question of the impact of turbulence on phytoplankton distribution could be addressed and computations of biogeochemical fluxes performed.

3. Task 3: Production and fate of organic matter in contrasted environments

PIs: Sophie BONNET and Ilana BERMAN-FRANK

Subtask 3.1. Measurements of nutrient concentrations and/or availabilities in the photic zone, and identification and quantification of the major external fluxes.

Nutrient availability (N, P, Si, Fe) in the photic zone controls primary production of organic matter, and thus exerts a strong influence on the species composition of the food web. It is dependent on several input, recycling and export fluxes. Nutrient concentrations in the upper layer represent the equilibrium state between all those fluxes and is the first indicator of nutrient availability. **Nevertheless, as nutrient concentrations are very low in marine oligotrophic habitats, these measurements represent a major observational challenge.** Nanomolar methods developed by the proposed team will be used for nutrient concentrations measurements when necessary (Rimmelin & Moutin, 2005, Rimmelin et al., 2007, Pascoa et al., 2012).

New nutrient inputs in the upper mixed layer include fluxes induced by ocean dynamics (meso- and submesoscale pumping; vertical turbulent mixing), biological sources (N₂ fixation) and surface fluxes (atmospheric deposition). The dynamics of meso- and submesoscale structures are often associated with strong vertical motion that can induce intense upwelling of deeper, nutrient-rich waters into the euphotic zone (Levy et al., 2012). The impact of those structures in regulating local nutrient availability and primary production will be investigated through the observations described in Task 2.

Vertical turbulent mixing is a key process driving physical and biogeochemical fluxes between the mixed layer and the stratified ocean (Cuyppers et al., 2012) and can strongly impact primary production (Levy et al., 2012). While mixing in the mixed layer is mostly driven by direct atmospheric forcing, mixing in the stratified water column is mainly driven by internal wave breaking. Internal waves can be generated from inertial currents at

the surface or tidal forcing at the bottom. The study area encompasses a large variety of environments regarding the internal waves (Internal tides notably vary from strong to negligible over the cruise area). As well we expect significant mixing resulting from instabilities of the strong currents of the western part. Therefore the measurements would provide a unique data set characterizing turbulent mixing in a poorly sampled area and under a variety of internal wave dynamical regimes and different current structures. Microstructure measurements that resolve the dissipative scales will be performed using a tethered microstructure profiler, VMP250. This profiler, which can profile down to 500 m is equipped with microsensors for temperature and shear that enable two independent accurate estimates of the eddy diffusion coefficient K_z . These two estimates will be used to test the relevance of the different indirect methods based on classical CTD and ADCP measurements. This validation will allow to estimate K_z also when microstructure measurements are not performed. The same strategy has been used by the proposed team during the BOUM cruise (Cuypers et al., 2012).

In addition to the input of nutrients by turbulent mixing, we will quantify the N input through biological N_2 fixation, as it may be a major source in the SW Pacific. Gross and net N_2 fixation rates will be measured at every station (SD and LD) between 0 and 2000 m depth using both the acetylene reduction method (Capone, 1993) and the $^{15}N_2$ labelling procedure, incubation (24 h) and mass spectrometry analyses (MIO Marseille) as described in Montoya et al., (1996) and Bonnet et al., (2011). The $^{15}N_2$ enriched seawater method (Mohr et al., 2010; Wilson et al., 2012; Grosskopf et al., 2012) will be used during the cruise and several method inter-comparisons will be performed between the $^{15}N_2$ bubble method and the $^{15}N_2$ seawater enriched method, both performed under trace metal and DOM-clean (Bonnet et al., 2009) and non-trace metal clean and DOM-clean procedures.

In order to better understand the relationship between (photo)-heterotrophic diazotrophs and DOM, we will perform DOM characterization through FT-ICR MS (allows the detection of individual DOM compounds) simultaneously to N_2 fixation measurement and diazotrophs diversity.

Aeolian dust transport represents, on a global scale, the dominant source of iron, an essential micronutrient for phytoplankton growth, to the ocean (Jickells et al., 2005). Some of the Melanesian archipelagos crossed during the transect (Vanuatu, Fiji) exhibit active volcanoes, which have been seen (in the North Pacific) to provide sources of very soluble and bioavailable trace metals such as iron (Olgun et al., 2011) and potentially N and P. Consequently, dust inputs could stimulate *in situ* biological productivity in oligotrophic ecosystems and alter community composition, distribution of nutrients and finally the net sequestration of atmospheric CO_2 .

We will quantify the input of nutrients by the atmosphere and study the influence of dust deposit on the food web and their potential impact on N_2 fixation, using microcosm experiments.

Specific questions: *Is N_2 fixation a major source of new N for primary production in the photic layer? Do direct measurements of eddy diffusion coefficients reasonably agree with previous estimations? What could be the role of dust?*

Subtask 3.2. Organic matter production and food web structure

Because of light/energy requirements, marine organic matter is almost entirely produced in the upper photic zone of the ocean. Variable primary production fuelled by physical processes acting over a wide range of scales interacts with predation to define the species composition of planktonic populations (Gargett & Marra, 2002). The basic principle of the conceptual food web structure, which has not been critically challenged since its original description (Johannes, 1965; Thingstad et al. 1999), is to consider two trophic strategies. Osmotrophy referring to organisms that feed by taking up dissolved nutrients and phagotrophy referring to organisms that feed by eating particulate matter. Osmotrophs include both heterotrophic bacteria and autotrophic phytoplankton, while the predatory food chain includes protozoa, mesozooplankton and higher predators (all heterotrophs).

Biogeochemical fluxes in relation to osmotrophic production

We will measure fluxes of biogenic elements (including C) and determine parameters that will help to represent these fluxes (P vs I parameters, K_s , V_{max} , affinity constants...). Classical approaches using stable (^{15}N , ^{13}C) and unstable (^{14}C , ^{33}P , ^{32}Si) isotopes for measuring biogeochemical fluxes will be used for bulk measurements, along with new techniques at the single-cell level, which address by which species (or group of species) a specific element has been taken up. **New methods concerning both dominant species separation as cell sorting by flow cytometry developed by the proposed team** (Duhamel et al., 2009, Gregori et al., 2011; Talarmin et al., 2011) **and low detection limits of chemical analyses will allow us to define species-specific uptake parameters.** The contribution of mixotrophy and photoheterotrophy to the biogeochemical fluxes of C and N will be studied together with the environmental factors that control mixotrophy and symbiosis by microbial eukaryotes. **The methods proposed will allow us to significantly improve our understanding of**

the factors controlling the distribution and activity of different groups of plankton functional types and to link their phylogenetic and functional diversity.

Regarding N₂-fixing organisms, recent major development in SIMS (Secondary Ion Mass Spectrometry) instrumentation (NanoSIMS 50TM) now allows for the measurement of isotopic ratios with a spatial resolution better than 100 nm (Guerquin-Kern et al., 2005; Slodzian et al., 1992). The capability of such instrument to measure isotopic composition makes it suitable to study environmental microbiology at the single-cell level (Lechene et al., 2006). Most of these recently discovered N₂-fixing micro-organisms are uncultured and many unresolved questions remain about their physiology and their importance in term of input of new N in the ocean. The nanoSIMS technology associated with cell sorting (Thompson et al., 2012; Bonnet et al., In Rev.) or FISH (HISM-SIMS, Thompson et al., 2012; Krupke et al., 2013) will be applied to assess the contribution of every diazotrophic group to overall fluxes, and determine organic/inorganic nutrients controlling their activity. We will focus on the recently described UCYN (Moisander et al. 2010, Thompson et al. 2012) group whose role in C and N cycling is not well understood thanks to recent methods developments realized by the proposed team (see references above).

The silicification process in marine diatoms will be investigated at the single-cell level using the PDMPO labelling technique (Leblanc & Hutchins, 2005).

Phagotroph production (secondary production)

The planktonic food web structure influences the fate of primary production in the ocean and has consequences for the CO₂ transfer process. It is generally understood that when small eukaryotes or prokaryotes dominate the microbial community, the grazers of picoplankton are small protozoa which do not produce rapidly sinking faecal pellets. **Despite their omnipresence and their pivotal role in the energy flow in marine waters on a global scale, the physiological ecology of these organisms is poorly understood.** Several grazing steps are necessary to enable primary production to be incorporated into the upper trophic levels. Therefore, most of the C fixed by phytoplankton is respired and remineralised by the microbial community in the surface mixed layer and there is little or no net uptake of CO₂ from the atmosphere to the sea. In contrast, when large phytoplankton cells dominate, they can either sink to depth or be grazed by copepods and other mesozooplankton which produce rapidly sinking faecal pellets. However, mesozooplankton, i.e. copepods, have recently been coupled to lower trophic levels in the eastern Mediterranean Sea (Thingstad et al. 2005) and their contribution to the C cycle of oligotrophic areas needs to be reconsidered. **The upper meso- and macrozooplankton, largely under-sampled by classical techniques, may also significantly contribute to the vertical transport of organic matter.**

An apparently high contribution of diazotroph N to zooplankton directly east of New Caledonia was recently evidenced (Hunt et al., 2013), supporting similar findings in diazotroph rich areas of the Atlantic (Hauss et al. 2013). It is possible to trace the diazotroph signature through the macrozooplankton to the micronekton component of the food web. These are the first detailed stable isotope measurements of the pelagic community presented for the region. We will now increase the geographic coverage of zooplankton and stable isotope sampling across the SW Pacific diazotroph gradient, **specifically to investigate the relative contributions of diazotrophs to food web N and the their role in structuring community composition. We will apply bulk stable isotope analysis in combination with amino acid specific N isotope analysis (CSIA).** The latter method provides the added resolving power of direct estimate of source N isotope signature for any organism measured (McClelland et al. 2003). **A key question remains what are the pathways that fixed N takes to reach primary consumers (microzooplankton and zooplankton grazers) and upwards.** In part we can contribute to answering this question by detailed species level stable isotope analysis of all lower trophic level community components. Additional insights will be gained by experimental studies using labelled N tracers (Loick-Wilde et al. 2012).

Specific questions: *What are the characteristics of the dominant species concerning photosynthesis, N₂ fixation, nutrient uptake, osmotrophy and phagotrophy?*

Subtask 3.3. Fate of N₂ fixation

Although direct evidence of trophic transfer from diazotrophs through planktonic food webs is lacking, studies based on natural abundance of ¹⁵N in suspended particles and zooplankton suggest that N from diazotrophy would support a part of primary and secondary production in the subtropical Atlantic Ocean (Montoya et al., 2002; Landrum et al., 2011). In addition, a low delta ¹⁵N signature of exported material at the time series station HOT (Karl et al., 1997; 2002; Casciotti et al., 2008), and of nitrate accumulating in the upper thermocline of the Sargasso Sea (Knapp et al., 2008) would indicate that recently fixed N is transferred out of the euphotic zone. As fragments of *Trichodesmium* spp. are rarely recovered in sediment traps (Walsby, 1992) and are mostly ungrazed (O'Neil, 1999), transfer of fixed N is probably done through the dissolved pool (Mulholland,

2007). Indeed, *Trichodesmium* spp. has been seen in the natural environment and in cultures to release up to 50-80% of recently fixed N_2 as dissolved organic N (DON) and ammonium (NH_4^+) (Capone et al., 1994; Mulholland et al., 2005) potentially available for surrounding planktonic communities. Alternatively, Brandes et al., (1998) suggested that some material derived from N_2 fixation can be remineralized in surface waters, lightening the isotopic nitrate signal, which may be propagated along the trophic chain.

The lack of knowledge regarding the fate of N_2 fixation is essentially due to the lack of techniques allowing to trace the N transfer through the different compartments of the food web. We propose here to overcome these difficulties by using a combination of techniques previously used by the proposed team in the framework of the VAHINE project, including: 1/ high-resolution nanometer scale secondary ion mass spectrometry (NanoSIMS) coupled to 2/ flow cytometry cell sorting and 3/ $^{15}N_2$ isotopic labelling (Bonnet et al., Sub.).

Previous studies have shown that increased TEP production by *Trichodesmium* was coupled with caspase activation and programmed Cell Death (PCD, Berman-Frank et al., 2004, 2007). The concentration of TEP will be correlated to PCD of diazotrophs using diagnostic genetic markers currently developed by I. Berman-Frank (Bar Ilan Univ., Israel).

In order to quantify the part of export production that has been sustained by N_2 fixation, a $\delta^{15}N$ budget will be performed at LD stations, based on ^{15}N natural abundance (collaboration with A. Knapp, Florida State University, see similar calculations in Knapp et al., 2005). Using this $\delta^{15}N$ budget, we will determine what fraction of export production is supported by N_2 fixation. By multiplying the fraction of export production supported by N_2 fixation by the particulate nitrogen sinking flux, we can calculate an absolute N_2 fixation rate for the duration of the sediment trap deployment and compare them with direct measurements.

Specific questions: *What is the fate of N_2 fixation? Does programmed cell death (PCD), induced in some of the dominant diazotrophs – *Trichodesmium* induce enhanced vertical export as we see from lab experiments? Can we identify changes in physiological status of the populations using PCD markers (Bar-Zeev et al. 2013)?*

4. Task 4: Mineralization and export of organic matter

PI: Benjamin VAN MOOY

Subtask 4.1. Organic matter mineralization and food web structure

The study of organic matter degradation will focus on the relationship between mineralization and bacterial diversity. **Hence, examining the relationships between diversity and functionality within the bacterial community (ectoenzymatic activity, uptake of specific organic compounds representative of a chemical family) is a major challenge for understanding the impact of prokaryotic heterotrophic processes on mineralization of organic matter along the water column.** We will also study the factors controlling bacterial production and the consequences of such controls on heterotrophic activity, and the hydrolysis rate of Dissolved Organic N and P.

The wide geographical and vertical (in the oceanic water column) distribution of photo-heterotrophic diazotrophs (UCYN-A) (Luo et al., 2012) suggests they are **bound to have a key role in global N_2 fixation.** However, failure to isolate and maintain these organisms in culture limits our knowledge on their activity and nutritional needs (Zehr, 2011). While the genetic information of these organisms reveals their dependence on organic matter (Riemann et al., 2010; Tripp et al., 2010), this relationship has not been studied so far. We will perform microcosm experiments at LD stations to test whether different sources of DOM (C-enriched, N-enriched or P-enriched commercially available sources, and natural *in situ* DOM) trigger N_2 fixation and/or the expression of *nifH* by different groups of heterotrophic diazotrophs. Additional nutrient amendment experiments will be performed using ^{13}C - and ^{15}N -labelled organic molecule and the **HISH-SIMS** (Thompson et al., 2012) method in order to identify which diazotrophs assimilate which organic molecule and in which quantity.

Specific questions: *What are the changes in prokaryotic heterotrophic activity and community composition in relation with horizontal (west-east) and vertical (surface to depth) nutrient gradients and the composition/lability of the dissolved organic matter?*

Subtask 4.2. Organic matter export (particulate and dissolved matter)

The structure of pelagic ecosystems influences the size and type of downward flux of biogenic C in the sea (Legendre and Rassoulzadegan, 1996). This vertical flux is composed mainly of large particles such as fecal pellets, hard parts of zooplankton, amorphous aggregates, marine snow (Fowler and Knauer, 1986; Silver and Gowing, 1991), and senescent diatoms, particularly in the aftermath of blooms (Billet et al., 1983). Fecal pellets can sink quickly: 20-900 m d⁻¹ for copepods (Welschmeyer and Lorenzen, 1985), and up to 2700 m d⁻¹ for large gelatinous zooplankton (Bruland and Silver, 1981; Madin and Purcell, 1992). High sinking velocities can lead to the efficient export of biogenic C. The downward flux of biogenic C can therefore be largely dominated by C of zooplankton origin (Thibault et al., 1999). We will deploy sediment traps (see Task 2 for

detailed strategy) and **will study the C/N/P/Si stoichiometry of settling particulate matter because despite its biogeochemical relevance, there are few direct measurements available** (Geider & La Roche, 2002). We will also examine the role of larger particulate matter responsible for the marine snow rarely observed in oligotrophic areas and quantify instantaneous DOC export. A special attention will be given on the direct export of diazotrophs. We will use complementary measurements of export using the Underwater Vision Profiler (UVP; Picheral et al. 2010) to assess vertical POC flux from particle size distribution (PSD) (Guidi et al. 2008) and vertical distribution of zooplankton (Stemmann et al., 2008) that are directly linked to the vertical flux. We will also complement the zooplankton distribution by an indirect estimate of zooplankton vertical profiles through acoustics from the ship borne S-ADCP and Lowered ADCP on stations as well as from ER 60 (200 kHz) echosounder. In addition, using images from the UVP, we will describe vertical, horizontal, and temporal evolution of the *Trichodesmium* spp. colonies similar to what has been done recently in the North Pacific (Guidi et al. 2012). ***Trichodesmium* spp. colonies will be spatially and temporally correlated to vertical particle flux and their potential contribution will be estimated.**

It has been recently reported that bacteria living within colonies of the N₂-fixing cyanobacterium *Trichodesmium* spp. use cell-cell signaling to regulate the degradation of organic phosphorus compounds (Van Mooy et al., 2012). In a separate study, it has been found that bacteria living on sinking particles may also use cell-cell signaling to regulate organic matter degradation (Hmelo et al., 2011). Finally, rates of N₂-fixation were also found to be affected by cell-cell signaling (Van Mooy and Dyhrman, unpublished). We will combine these three findings and ask the next question: **How does cell-cell signaling via acylated homoserine lactone (AHL) and autoinducer-2 (AI-2) molecules in senescent *Trichodesmium* spp. colonies affect the export of new organic N in sinking particles?**

Specific questions: *What is the ratio of particulate vs dissolved organic matter export? What is the role of *Trichodesmium* spp., and of cell-cell signalling in the export?*

5. Task 5: Modelling - PI : Melika BAKLOUTI

The multidisciplinary field data gathered during the OUTPACE cruise will be available for the international community as soon as possible (CYBER data base) in order to help in representing the interactions between climate, biogeochemical cycles and marine ecosystems. Associated with this data set, **a multi-scale modelling approach is proposed from the beginning of the OUTPACE project** in order to understand the interactions between the biogeochemical cycles of the biogenic elements and the dynamics of the planktonic trophic network. Modelling will be used in interaction with observation and measurements at several scales and at several moments of the project in order to optimize the potential of each of these tools. This modelling strategy relies on the use of a realistic non-Redfieldian biogeochemical model which will benefit from several previous work (Baklouti et al., 2006a, Mauriac et al., 2011, Baklouti et al., 2011, Alekseenko et al., 2013) and which is fully relevant to study the complex interactions between the C, N and P cycles.

Subtask 5.1. Process scale studies

We first plan to focus on some physical and biogeochemical processes through (i) the zero-dimensional (0D) modelling of microcosm experiments performed during the field work and through (ii) the simplified 1D vertical modelling of the physical and biogeochemical processes at the LD stations. These stations will be positioned in regions where the horizontal variability of the hydrological and biogeochemical properties is supposed to be low over the duration of sampling making it possible to consider a 1DV modelling approach. Moreover, the majority of the model's needs, in terms of state variables and parameters, will be acquired through in situ measurements or by controlled activities i.e. sampling and microcosms during the cruises.

Microcosm experiments and modelling (0D) are important for several reasons, the main one being that they provide a vision of the biogeochemical and trophic web functioning deconvoluted from hydrodynamic aspects. This is a critical step towards the understanding of the ecosystem. Microcosms are also useful to acquire certain model parameters (characteristics of nutrient assimilation, photosynthetic parameters, respiration rates, mineralisation...) and to test or develop new mathematical formulations for processes where current formula are not validated or represented. It is also important that these microcosm experiments are undertaken during the cruises (or field studies) with in situ water samples in order to obtain parameters which are representative of the studied ecosystem.

In practice, a biogeochemical model relevant for SW Pacific waters and developed during the VAHINE project will be used to model the 0D experiments. It is based on a previous version of a biogeochemical model developed for the Mediterranean Sea which included 6 functional types, namely bacteria, two phytoplankton size-classes, HNF, ciliates and mesozooplankton and which was already implemented in the Eco3M modelling tool (Baklouti et al., 2006b). In this model, organisms have flexible stoichiometry and are represented in terms

of C, N, P biomasses as well as in abundances; the diazotroph *Trichodesmium* spp. and other unicellular diazotrophs have been added. This model successfully compared with available data can already be used as an a priori model to help in designing the microcosm experiments that will be held on board. The measurements will in turn be useful to refine the a priori model.

Subtask 5.2. *In situ* experiments at the LD stations (1DV)

The major objective of 1DV modelling is to represent fluxes of biogenic elements in the surface layer and provide an accurate budget on the fate of the organic C produced (Re-mineralisation, export). The use of a flexible stoichiometry model (non Redfieldian) will potentially make it possible to illustrate the decoupling of the biogenic element cycles in the oligotrophic ocean. The objective is to understand the production and fate of organic matter in the oligotrophic food web, and their role in the global C cycle. To achieve this, we will proceed to the extraction of those criteria which appear essential in providing a satisfactory representation of the marine pelagic ecosystem and biogenic element fluxes. The complexity of the trophic relationships will be simplified by focusing on the groups of species having similar biogeochemical functions (i.e. functional groups). The relevance of the 1DV modelling will depend on in situ measurements carried out at the LD stations. Indeed, a certain number of parameters which will be measured are essential for achieving a realistic representation of the dynamics of the pelagic ecosystem and thus a precise estimate of the C budget. These parameters include all the parameters related to phytoplankton photosynthesis and to nutrient uptake by the plankton. In coupled physical-biogeochemical models, the parameterization of turbulence is frequently a source of error, because it is based on empirical laws, and because these laws are not then re-calibrated for each studied site (typical values found in the literature are used to parameterize these laws). Here, it will be possible to use realistic vertical profiles in the hydrodynamic 1DV model to accurately estimate the coefficients of turbulent vertical diffusion - K_z - (cf Task 2.3). The use of such realistic profiles is an essential step in representing the mixing processes in the surface layer and the nutrient fluxes at the base of the euphotic layer. The biogeochemical model that will be used for the 1DV configuration is the same as the 0D one except that it will benefit from the refining that will have been done thanks to the microcosm experiments. This type of model enables us to obtain a representation of the biogeochemical cycles of the principal biogenic elements (C, N, P) as well as representing organisms ("functional" groups) that play a key role in the dynamics of organic C production and transfer. The whole data set collected from the LD stations, and in particular the data obtained in a quasi-continuous manner such as nitrate fluxes and stocks, will offer the unique possibility of validating the coupled model which should in turn provide the lacking information to fully understand the biogeochemical functioning of the oligotrophic waters of SW Pacific.

Specific questions: *Is it possible to reproduce, with only one uniquely structured model, the principal physical and biogeochemical characteristics of the different oligotrophic areas studied?*

6. Task 6: Scientific communication – Promotion of Science

The results from the OUTPACE project will be disseminated to the whole national and international scientific community through publications in peer-review journals and participation to international scientific conferences (see Section 3.). They will also be available to a broad audience at several steps of the project:

- A dedicated OUTPACE website will be set up to exchange informations regarding the project. All broad audience communication operations will be available on this website as well as the database of the project which will be in restricted access
- During the cruise, we will implement a blog describing the daily life and science onboard for a broad audience, as previously done in the past on the BOUM cruise (same PI as OUTPACE, http://www.mio.univ-amu.fr/BOUM/IMG/pdf/Carnet_de_bord_BOUM.pdf) to communicate to the public the role of scientists working in oceanography.
- A documentary film will be realized by CANAL IRD by J.M Boré and H. Bataille as we have previously done in 2010 and 2013 (see movie <http://www.ird.fr/la-mediatheque/videos-en-ligne-canal-ird/des-trichos-s-il-fait-trop-chaud> and <http://www.ird.fr/la-mediatheque/videos-en-ligne-canal-ird/mission-vahine>) and will be available on the OUTPACE dedicated website
- Before the cruise starting from Noumea, New Caledonia, a 'communiqué de presse' will be sent by Mina Vilayleck (Director of Communication at IRD Noumea) as usually done by IRD Center Noumea and the event will be covered by local and national TV and radios,
- During and after the data exploitation, results from the project will be disseminated through the dedicated website, press releases in the local press (région PACA and New Caledonia), vulgarization scientific articles such as 'Sciences au Sud', or other vulgarization operations as previously done in the past with France Inter and France Culture. The OUTPACE team is already in contact with journalists from these medias. We will undertake

communication operations, such as the ‘La fête de la Science’ (<http://www.drrt-paca.fr/Culture-scientifique-technique-et-industrielle/La-Fete-de-la-Science-2013-en-PACA>), as performed in 2012 for the TV Broadcast (24h sur la Terre’ (http://www.terre.tv/fr/5133_24h-sur-la-terre-2012---3h). This will be a good opportunity to expose the main results obtained in the OUTPACE project to the public and increase its awareness of the role of the marine ecosystems into our daily life, and the necessity to protect marine resources. It is also a good opportunity to communicate/explain to a young public the job of researcher.

All these communication operations will be done in consultation with the Communication department of Aix-Marseille University and MIO (E. Lamadie, C. Walch), IRD (C. Duos, attachée de presse), M. Carrard (‘rédacteur en chef’ ‘Science au Sud’), and V. Arnaud, in charge of the external communication), and Mina Vilayleck (director of communication at IRD Noumea).

- Finally, the OUTPACE project Teaching-mentoring will be used as a support for training/mentoring students (see Section 3)

A specific budget will be dedicated to these communication operations (see Section 2.5).

2.3. TASK SCHEDULE

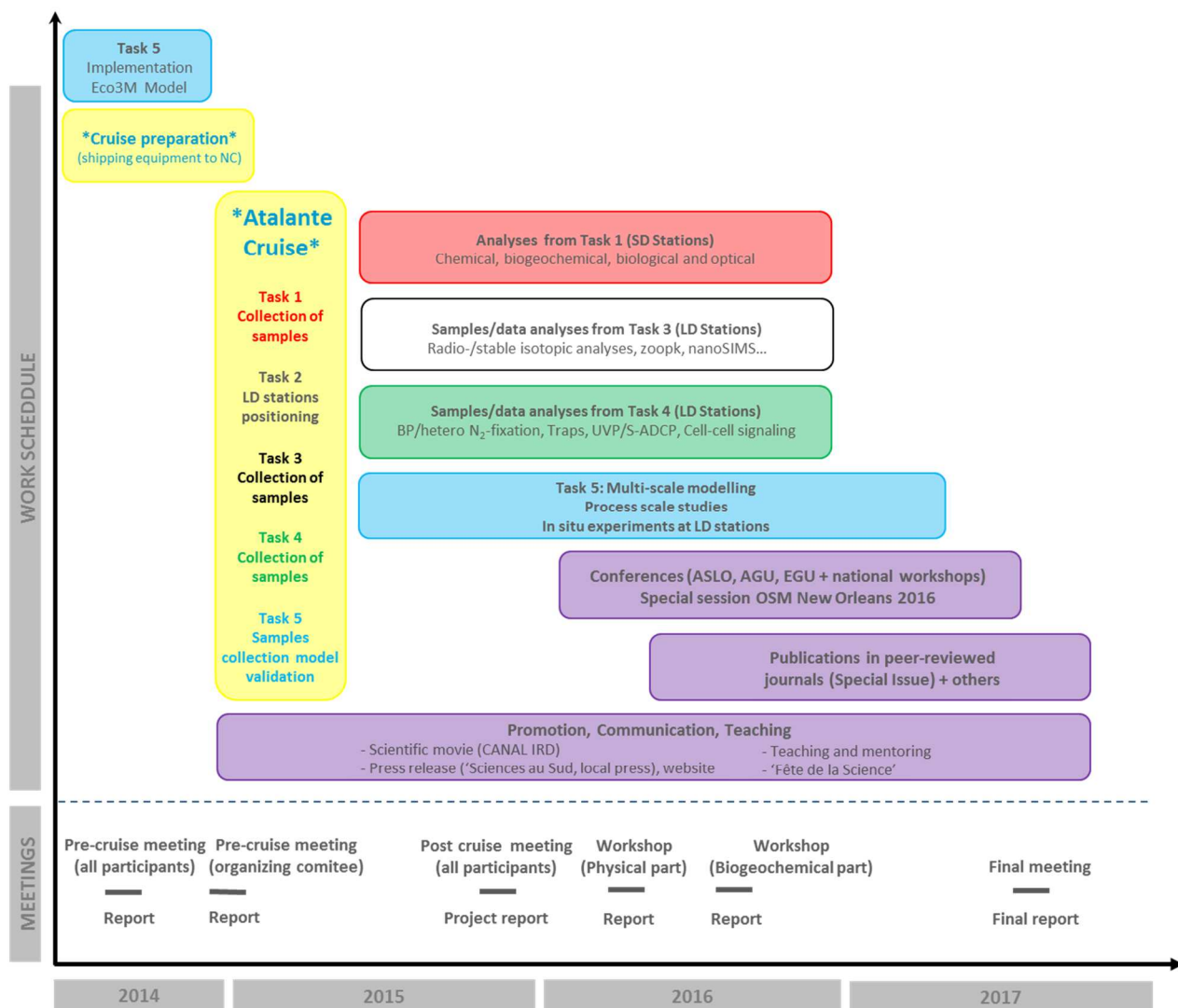


Fig. 6. Diagram showing the work schedule of the project by Task and date of project meetings and reports. The dependency between tasks is indicated by the arrows (except for Task 6 and publications, for which all arrows cannot be drawn, as they are in relation with all the other Tasks).

2.4. TEAM DESCRIPTION

This collaborative project leaded by a French PI (T. Moutin) will confirm the expertise of the French scientific community in oceanography, and will offer the opportunity to reinforce the French collaborations with international teams. The project **brings together around 50 scientists from 7 French research**

laboratories and universities in 5 foreign countries (USA, Partner WHOI), Germany, Israel (Partner Bar Ilan University), Sweden, Fidji) and illustrates well the attractiveness of France in Oceanography. Fundings have already been obtained by the foreign participants of OUTPACE, and co-funding has been obtained by French participants through the LEFE programme (INSU, 51 k€) and the MIO Institute (17 k€) to set up the cruise: **the ANR funding is necessary for the completion and valorisation of the project.**

The OUTPACE project is based on the strong expertise of each partner in their complementary research fields: **chemistry, optics, marine biogeochemistry, marine microbiology, molecular ecology, genetics, and modelling.** Every partner and member is fully qualified to meet the objectives they are in charge of (See publication list of participants and below).

PARTNER 1, AMU-MIO. The lead research institute is the [Mediterranean Institute of Oceanography \(MIO\)](#), which is part of [Aix-Marseille University \(AMU\)](#). With roots dating back to 1409, the AMU was formed by the [merger of 3 Universities](#) in 2012, resulting in the creation of the largest university in France and the French-speaking world, with about 70,000 students. AMU has the largest financial endowment of any academic institution in France, standing at €650 million. AMU has produced many notable alumni, as 4 Nobel Laureates. In the 2012 [Academic Ranking of World Universities \(ARWU\)](#), the University was ranked 101-150th in the world, making it the 4th highest ranked University in France. The MIO, within the AMU, is one of the oldest, largest, and most important centers for oceanographic research and training in France. Currently, MIO staff numbers approximately 250, including about 90 Faculty, and some 50 graduate students. Oceanographic research at MIO covers many aspects of the field, including physical, biological, and chemical oceanography and the MIO is particularly qualified in the physical/biogeochemical coupling and N₂ fixation, two majors domains necessary to complete the OUTPACE project. The MIO promotes prolific international collaborations with European and non-European countries, through involvement in programs such as SESAME, EUROCEANS, and many international research projects/oceanographic successful expeditions led by scientists from the MIO such as MINOS, KEOPS, BOUM (PI: T. Moutin), which involved, like the OUTPACE project a strong coupling between Physics/Biogeochemistry/Microbiology. The MIO is a member of the [European network of excellence for Ocean Ecosystems Analysis](#), and stands out in integrating marine ecosystems, foodweb, climate change and marine resources research. The MIO has been ranked A+ by the Evaluation Agency for Research and Higher education (AERES) in 2012, thanks to its high levels of high-ranking publications and the scientists from MIO involved in OUTPACE are perfectly qualified to answer the scientific questions of the project.

PARTNER 2, LOCEAN is an UMR between the UPMC, the CNRS, the IRD and the MNHN. LOCEAN has a core research component on ocean dynamics and biogeochemistry with a strong implication in instrumental development and *in situ* measurements. The team involved in the project gather specialists of turbulence and internal waves, an engineer in marine technics involved in instrumental development, three chemists and biogeochemists and 2 specialists of submeso-scale dynamics.

PARTNER 3, LOV is an UMR, between the UPMC and the CNRS. LOV, which hosts about 50 permanent staff (roughly equally divided into researchers, students/postdocs, and technical staff), is a laboratory which prime activities are in optical, biogeochemical and environmental oceanography. It is actively involved in the development of *in situ* experimentation and the implementation of novel instruments to monitor and study the ocean, techniques that will be used in the framework of OUTPACE.

PARTNER 4, The Woods Hole Oceanographic Institution (WHOI) is a private, independent, not-for-profit corporation dedicated to research and higher education at the frontiers of ocean science. Its primary mission is to develop and effectively communicate a fundamental understanding of the processes and characteristics governing how the oceans function and how they interact with the Earth as a whole. With over 1,000 employees, WHOI is the largest institution of its kind in the US. WHOI has granted PhD degrees through an agreement with the Massachusetts Institute of Technology for over 40 years. Furthermore, WHOI is routinely ranked as one of the top ten institutions for postdoc among all universities and research institutions in the US. WHOI is host to the Ocean Observing Initiative, and maintains collaborative studies on all seven continents. The facilities available for this project are with the laboratory of Associate Scientist Benjamin Van Mooy, and include mass spectrometers for analysis of biochemical within *Trichodesmium* and field equipment for sampling the sinking particulate export flux. Instrumentation and equipment for more routine analysis and sampling are also dedicated for use by Van Mooy's group.

PARTNER 5, Bar-Ilan University (BIU) in Ramat Gan, Israel was established in 1955. Bar-Ilan is currently Israel's largest academic institution, with 33,000 students in the main campus and regional colleges around the country. Marine research at BIU includes the fields of physical oceanography, biological oceanography, Marine biology and ecology, and chemical oceanography. Presently Bar Ilan University has joined the newly founded Mediterranean Sea Research Center of Israel (MERC), a consortium of six universities and two governmental research institutes. IBF is an active member of this center and the head of the educational committee for undergraduate and graduate programs within the Center. The facilities at the I. B-F Laboratory at Bar-Ilan University are well equipped for the molecular, physiological and biochemical analyses required for the resulting OUTPACE samples including spectrophotometers, epifluorescent microscopes, a FID-GC, PCR thermocycler, nucleic acid and protein electrophoresis. IBF will also bring aboard the OUTPACE cruise a new next-generation submersible FIRE fluorometer capable of measuring the variable fluorescence of photosynthetic organisms *in situ*.

In summary, the proposed team integrates various complementary disciplinary fields required to meet the objective of OUTPACE. At the international level, one objective of our project is to reinforce existing collaborations inside and outside Europe. An important point is that most of the partners have already collaborated together in the framework of recent international cruises (BIOCOPE, BOUM, ETSP), and have thus already an experience of working and publishing together, which is a gage of success for this project. We counted 125 recent (since 2008) publications (rank A) of the members of the OUTPACE community directly related with the main scientific questions of OUTPACE (including several ones in the prestigious magazine Nature and Science, including the PIs of OUTPACE).

Quick presentation of the PI:

Thierry MOUTIN, 1st class Professor at the « Institut Méditerranéen d'Océanologie (MIO) of Aix-Marseille University (AMU) is co-responsible of the « Physical and Biogeochemical Oceanography » specialty of the Oceanographic Master. He works on the biogeochemical cycles of biogenic elements in the photic layer of the ocean, and particularly on the biological C pump. He has a long term expertise in oceanography, attended numerous oceanographic cruises and published his results in 52 A rank papers (**H-index: 20; 5.3 papers/year over the last 3 years**). **Thierry Moutin is also fully experienced in managing international projects such as OUTPACE. He has been the leader and chief scientist of the BOUM project between 2008-2012 (European and national funding, 50 international scientists), which has been a real success (cited as an example in the evaluation report by the French Oceanographic Fleet Commission in June 2013) and led to more than 30 rank A international publications (24 in a Biogeosciences special issue co-organized by Thierry Moutin; http://www.biogeosciences.net/special_issue82.html). 17 of these international publications were written in collaboration with members of the OUTPACE community.**

2.5. JUSTIFICATION OF REQUESTED RESOURCES (398.9 k€)

PARTNER 1: M I O (334.9 k€, including management fees: 12.9 k€)

Staff (194.9 k€)

- **Post-Doc** (2 years, 107.8 k€): The main scientific goal of the post-doc will be the study of the impact of the coupled physical-biogeochemical submesoscale processes on the carbon export in the three contrasting sites sampled during the LD stations. The postdoctoral fellow will help in implementation of the software tools for the adaptive strategy that will be used for the *in situ* biogeochemical process studies. She/He will be in charge of the treatment and analysis of the data that will be obtained during the high frequency and high resolution mappings performed in the areas identified for the Lagrangian LD stations. She/He will interpret the measurements in the context of the large-scale physical and biogeochemical environment observed by satellites and simulated by numerical models. The candidate should have a PhD in Physical Oceanography with a background in in-situ observation and/or remote sensing and excellent skills in computer programming.

- **PhD student** (3 years, 87.1 k€): The doctoral fellow requested for the MIO will be dedicated to the study of the role of N₂ fixation on export production. He/she will study the direct versus indirect export of primary production sustained by diazotrophy during the 3 process stations of the OUTPACE cruise. He/she will specifically compare the ability of the different diazotrophs targeted during the project (autotrophs/heterotrophs, symbiotic/non symbiotic) 1/ to be directly exported, and 2/ to sustain the bacterial/phytoplankton production and its subsequent export. He/she will use a combination of isotopic methods (¹⁵N₂ labelling) associated with mass spectrometry on bulk and at the single-cell level and microscopy. He will also closely collaborate with the scientists in charge of the delta ¹⁵N budget during the project as well

as microbial ecologists for the molecular part. He will study both the water column and the exported material in sediment traps. He/she will be hired at the beginning of the project for 3 years.

Missions (28.0 k€)

- Communications and special session dedicated at the Ocean Sciences Meeting (18.0 k€): Organization of a dedicated session at the Ocean Science Meeting. Participation (1.8 k€) for 10 searchers as previously done for the BOUM project by T. Moutin (see below).

BOUM special session at the OSM (Portland, US): http://www.mio.univ-amu.fr/BOUM/IMG/pdf/Special_session_program.pdf

- Trip expenses-Meetings for targeted workshops (10.0 k€): Beside of the post-cruise meeting with the whole OUTPACE community, we plan to organize 2 specific workshops targeted on the physical and biogeochemical oceanographic aspects (see work schedule).

Other expenses (99.1 k€)

- Gratifications for 2 Master 2 student internships (4.4 k€): (1) Physical oceanography: Satellite data (altimetry, SST, ocean color) data analysis and implementation of a climatological circulation model based on the ROMS code. Computer simulations of the *in situ* sampling during the Lagrangian LD stations. (2) Biogeochemistry: Biogeochemical impact of N₂ fixation along the OUTPACE transect (isotopic methods/mass spectrometry).

- Chemical and biological analyses of samples collected during the cruise. 61.7 k€ are requested to cover the analytic costs associated with the project (see Subtasks 1.1, 1.2, 1.3, 2.2, 3.1 and 3.2). The detailed table of costs per parameter is available on demand. NB: co-funding is already available to cover analytical expenses performed by foreign scientists.

- Publications of results (30.0 k€): As previously done for the BOUM project led by T. Moutin (see below), we plan to publish the major part of our results in a special issue of Biogeosciences. The impact factor of this European journal is high (3.75) in the oceanographic field and constantly growing.

Please see details on the BOUM special issue (25 articles available online) in Biogeosciences: http://www.biogeosciences.net/special_issue82.html.

- Communication operations: 3 k€ are requested to cover costs associated with communication around the project (Task 6), including posters printing and logistics for 'La fête de la Science', the blog and website, costs associated with the realization of the documentary film and courses.

PARTNER 2: LOCEAN (34.3 k€, including management fees: 1.3 k€)

Equipment (28.3 k€)

Microstructure profiler: VMP250 (velocity microstructure profiler) is a tethered microstructure profiler which performs measurements in the first 500 m. It is equipped with shear and temperature microstructure sensors as well as classical conductivity temperature sensors and a fluorometer. These measurements made at each LD station in between CTD/LADCP measurements with the rosette and at SD stations will enable us to characterize vertical mixing processes under a variety of dynamical regimes (internal tides may notably vary from strong to negligible over the cruise area) and their impact on primary production. Note that there are few vertical microstructure profilers in France: a few tethered profilers (e.g. MIO, LOCEAN), SCAMP, that are 100 m depth limited, and one deep un-tethered vertical microstructure profiler (LPO/LOCEAN) that can profile down to 6000 m. VMP6000 is already scheduled for cruises in 2015 and SCAMP does not profile deep enough for our purposes. VMP250 (requested here) is the ideal tool for OUTPACE and will respond to the requirement for turbulence measurements in the French community in forthcoming cruises.

Missions (2.5 k€)

5 missions (Paris/Marseille) required for cruise preparation and post meetings.

Other expenses (2.2 k€)

Gratification of a M2 student internship (2.2 k€). The Master student will participate to the analysis of microstructure measurements. The main goals of the project will be to characterize spatial distribution of dissipation rate during the cruise and see how it is correlated to larger scale dynamics as well to infer biogeochemical fluxes in collaboration with specialists from MIO and LOCEAN.

PARTNER 3: LOV (29.7 k€, including management fees: 1.1 k€)

Equipment (13.5 k€)

Acquisition of 3 oxygen optodes that will be implemented on each standard Bio-Argo profiling floats equipped with a bio-optical package (Chlorophyll fluorescence, CDOM fluorescence, backscattering and radiometry).

Missions (2.4 k€)

8 missions Villefranche/Marseille are requested for optode calibrations and implementation on Argo float profilers, as well as for cruise preparation and post-cruise meetings.

Other expenses (12.7 k€)

Consumable: All optical biological and chemical analyses performed by the LOV (HPLC, Dissolved-Fe, aerosols total Fe,N,P and total dissolved-Fe, sediment trap analyses, Marine Video Profiler treatment)

3. DISSEMINATION, EXPLOITATION. INTELLECTUAL PROPERTY

Scientific valorisation, protection and exploitation. The scientific benefits, on the basis of the experience of the team, of the originality of the project and of its current interest for the international community, will be major and at several levels. At the first level, the data acquired will enrich the international databases and help to improve the representation of the interactions between biogeochemical cycles and ecosystems in the context of a changing world. At a second level, we expect to publish around 50 “first rank” articles and at least one in the very famous magazine “Nature” or “Science” as already done in the past by the proposed team, as well as a similar number of international communications. At a third level, we will federate a multidisciplinary community of French, European and international scientists around a unique and major oceanographic challenge. Finally, on a global aspect, there is tremendous societal sustainability relevance for research focusing on climate impacts on economically and ecologically important marine organisms, and on maintaining the biodiversity of marine ecosystems that support millions of people around the world in the face of a rapidly changing ocean.

Scientific results will be protected until they are published in peer-review journals by the main participants, who all agree on the respect of intellectual property when undertaking collaborative work. All results/data will be shared on a database hosted by LEFE-CYBER (see example on <http://mio.pytheas.univ-amu.fr/BOUM/spip.php?rubrique26>) to maximize collaborative work and ideas exchange during the project. After two years, they will become public. In addition, final end-products will be available to the whole national and international scientific community through publications in peer-review journals, participation to international scientific conferences (Organisation of a Special issue, as Pis of the project did for past projects) and national seminars and courses.

Teaching-mentoring. The OUTPACE project involves **17 Professors or Assistant Professors from 10 universities and therefore the results of this research will rapidly be integrated in the training of students.** They will communicate to students the up to date techniques used in oceanography developed/adapted in the framework of OUTPACE, as well as the main results of the OUTPACE project. Moreover the PI of the project, Professor T. Moutin, is the co-PI of the ‘Physical and Biogeochemical Oceanography speciality of the Master “Oceanography” at AMU and promotes teaching in direct relation with research and will hire master and PhD students to work on OUTPACE. In addition, OUTPACE is the research support for 3 already funded PhD students (+ 1 requested in the present proposal), 3 funded postdocs (+ 1 requested in the present proposal) and 8 master students. Finally, our project also aims to strengthen relationships between the IRD (Institut de Recherche pour le Développement) and the University of South Pacific (USP), through training of students from ‘southern’ countries working on ‘Climate Change’ in the Pacific islands, which are vulnerable to global warming and sea level rise (USP federates 12 Pacific countries (Fidji, Tonga, Iles Cook, Tuvalu, Micronesie, Iles Marshall, Samoa, Salomon, Kiribati, Nauru, Tokelau, et Vanuatu). The OUTPACE cruise will cross the territorial waters of 4 of these States and will involve the supervision of students from USP who will work on the OUTPACE data.

Scientific communication, promotion of science (see Task 6).

4. REFERENCES CITED

References cited (from the OUTPACE community)

- Alekseenko, E., Raybaud, V., Espinasse, B., Carlotti, F., Quéguiner, B., Thouvenin, B., Garreau, P., Baklouti, M. Ocean Dynamics, 64:179-207 (2014)
- Alvain, S., Moulin, C., Dandonneau, Y., & Bréon, F. M. Deep Sea Research Part I, 52(11), 1989-2004 (2005)
- Alvain, S., Moulin, C., Dandonneau, Y., & Loisel, H. Global Biogeochemical Cycles, 22(3) (2008)
- Baklouti, M., Diaz, F., Pinazo, C., Faure, V., Queguiner, B. Progress in Oceanography, 71:1-33 (2006a)
- Baklouti, M., Faure, V., Pawlowski, L., Sciandra, A. Progress in Oceanography, 71:34-58 (2006b)
- Baklouti, M., Chevalier, C., Bouvy, M., Corbin, D., Pagano, M., Trousselier, M. and Arfi, R. Ecological Modelling 222: 2704-2721 (2011)
- Bar-Zeev, E., E., Avishay I., Bidle K.D., and Berman-Frank I. The ISME Journal, 1–9 (2013)
- Berman-Frank, I., Quigg, A., Finkel, Z.V., Irwin, A.J., Haramaty L. Limnology and Oceanography 52, 2260 (2007)

- Berman-Frank, I., Bidle, K.D., Haramaty, L., Falkowski, P.G. *Limnology & Oceanography* 49, 997 (2004)
- Bonnet, S., Biegala, I., Dutrieux, P., Slemmons, L., Capone, D.G., *Global Biogeochemical Cycles*, 23, GB3012, doi:10.1029/2008GB003439 (2009)
- Bonnet, S., Gregori, G., Barani, A., S. Mostefaoui, S., Cornet-Barthaux, V., Dekeazemacker, J., Charrière, B. *Sub. The ISME Journal*.
- Bonnet, S., Grosso, O., Moutin, T., *Biogeosciences* 8, 2257–2267 (2011)
- Bonnet, S., Guieu, C., Bruyant, F., Prasil, O., Raimbault, P., Gorbunov, M.Y., Zehr, J.P., Grob, C., Masquelier, S.M., Garczareck, L., Moutin, T., Van Wambeke, F., Claustre, H., *Biogeosciences*, 5, 1-11 (2008)
- Bonnet, S., Dekaezackemacker, J., Turk-Kubo, K., Moutin, T., Grosso, O., Zehr, J., Capone, D.G. *Plos One*. DOI: 10.1371/journal.pone.0081265 (2013)
- Bonnet, S., Webb, E., Panzeca, C., Karl, D.M., Capone, D.G., and Sanudo-Wilhelmy. *Limnology and Oceanography*, 55(5), 1959-1964 (2010)
- Capone, D.G. in *Handbook of Methods in Aquatic Microbial Ecology*. (ed. J.J. Cole) 621–631 Lewis Press, Boca Raton (1993)
- Capone, D. G., Burns, J.A., Montoya, J.P., Subramaniam, A., Mahaffey, C., Gunderson, T., Michaels, A.F. & Carpenter, E.J. *Global Biogeochemical Cycles*, 19, GB2024, doi:10.1029/2004GB002331 (2005)
- Capone D. G., M. D. Ferrier and E. J. Carpenter. *Applied & Environmental Microbiology*, 60, 3989-3995 (1994)
- Capone DG, Zehr JP, Paerl HW, Bergman B, Carpenter EJ. *Science* 276: 1221-1229 (1997)
- Carpenter, E.J., H.R. Harvey, B.F Ry, and D.G. Capone. *Deep-Sea Res.* 44: 27–38 (1997)
- Claustre, H., & Maritorena, S. *Science*, 302(5650), 1514-1515 (2003)
- Claustre, H., Sciandra, A., & Vaultot, D. *Biogeosciences* 605-640 (2008)
- Cuypers, Y., Bouruet-Aubertot, P., Marec, C. and Fuda, J.L. *Biogeosciences*, 9, 3131–3149 (2012)
- Dekaezackemacker, J., Bonnet, S., Moutin, T., Grosso, O., Bressac, M., Capone D.G. *Global Biogeochemical Cycles* DOI: 10.1002/gbc.20063 (2013)
- Doglioli, A.M., Nencioli, F., Petrenko, A.A., Fuda, J.-L., Rougier, G., Grima, N. *Journal of Atmos. Ocean. Tech.* doi: 10.1175/JTECH-D-12-00183.1 (2013)
- d'Ovidio, F., Zhou, M., Park, Y.H., Nencioli, F., Resplandy, L., Doglioli, A., Petrenko, A., Queguiner, B., Blain, S. Guiding biogeochemical campaigns with high resolution altimetry: waiting for the SWOT mission. "20 years of progress in radar altimetry" Symposium, Venice, Italy. Abstract Poster (2012)
- Duhamel, S., and Moutin, T. *Marine Ecology Progress Series*, 375, 53–64. DOI: 10.3354/meps07765 (2009)
- Duhamel, S., Björkman K.M., Karl, D.M.. *Aquatic Microbial Ecology* 67:225-238 (2012)
- Duhamel, S., Gregori, G., Van Wambeke, F., and Nedoma, J.. *Current Protocols in Cytometry*, 49:11.18.1–11.18.8. DOI: 10.1002/0471142956.cy1118s49 (2009).
- Dupouy, C., Neveux, J., Dirberg, G., Röttgers, R., Tenório, M. M. B., S. Ouillon. *Journal of Applied Remote Sensing*, 2, 1-17. doi:10.1117/1.2839036 (2008)
- Dupouy, C., Benielli-Gary, D., Neveux, J., Dandonneau, Y, T. Westberry. *Biogeosciences*, 8, 1-17, doi:10.5194/bg-8-1-2011 (2011)
- Grégori, G., M. Denis, S. Duhamel, and F. Wambeke. 2011. In H.-J. Ceccaldi, I. Dekeyser, M. Girault and G. Stora [eds.], *Global Change: Mankind-Marine Environment Interactions*. Springer Netherlands. DOI: 10.1007/978-90-481-8630-3_44
- Guidi, L., Calil P.H.R., Duhamel S., Björkman K.M., Doney S.C., Jackson G.A., Li B., Church M.J., Tozzi S., Kolber Z.S., Richards K.J., Fong A.A., Letelier R.M., Gorsky G., Stemman L., and D. M. Karl. *Journal of Geophysical Research*, 117, G02024, doi:10.1029/2012JG001984 (2012)
- Guidi, L., Jackson, G.A., Stemmann, L., Miquel, J.C., Picheral, M. and G. Gorsky. *Deep-Sea Res. I*. 55: 1364-1374 (2008)
- Hmelo L.R., Mincer T. J., and Van Mooy B.A.S. *Environ. Microbiol. Reports* 3: 682-688 (2011)
- Hunt, B.P.V., Allain, V., Menkes, C., Lorrain, A., Rodier, M., Pagano, M., Carlotti, F., Graham, B.S. *Deep Sea Res. Part II Top. Stud. Oceanogr.* (In rev.)
- Hamersley, M.R., Turk, K.A., Leinweber, A., Gruber, N, Zehr, J.P., et al. *Aquatic Microbial Ecology* 63: 193-205 (2011)
- Hewson I, Moisaner PH, Achilles KM, Carlson CA, Jenkins B. *Aquatic Microbial Ecology* 46: 15-30 (2007)
- Kessler, W.S. and S. Cravatte. *Journal of Geophysical Research* 118: 6385-6410 (2013)
- Hmelo, L. R., Mincer, T. J., & Van Mooy, B. A. *Environmental microbiology reports*, 3(6), 682-688 (2011)
- Knapp, A.N., D.M. Sigman, and F. Lipschultz. *Global Biogeochemical Cycles*, 19, GB1018, doi:10.1029/2004GB002320 (2005)
- Knapp, A.N., P.J. DiFiore, C. Deutsch, D.M. Sigman, and F. Lipschultz. *Global Biogeochem. Cycles*, 22, GB3014 (2008)
- Leblanc, K., and Hutchins, D.A.: *Limnology and Oceanography-Methods*, 3, 462-476 (2005)
- Loisel, H., Vantrepotte, V., Norkvist, K., Mériaux, X., et al., *Biogeosciences*, 8, 3295-3317. doi:10.5194/bg-8-3295-2011 (2011)
- Luo, Y.-W., Doney, S. C., Anderson, L. A., Benavides, M., Berman-Frank, I., Bode, A., Bonnet, S., et al. *Earth System and Science Data*, 4, 47-73, doi:10.5194/essd-4-47-2012 (2012)
- Martinez, E., Antoine, D., D'Ortenzio, F., B. Gentili. *Science*, 36, 1253-1256 (2009)
- Mauriac, R., Moutin, T., Baklouti, M. *Biogeosciences*, 8:933-950 (2011)
- Moisaner, P.H., Beinart, R.A., Hewson, I., White, A.E., Johnson, K.S., Carlson, C.A., Montoya, J.P., Zehr, J.P. *Science* 327:1512-1514 (2010)
- Moutin, T., Karl, D.M., Duhamel, S., Rimmelin, P., Raimbault, P., Van Mooy, B., and H. Claustre. *Biogeosciences*, 5, 95–10 (2008)
- Moutin, T. and L. Prieur. *Biogeosciences*, 9, 3827-3855, doi:10.5194/bg-9-3827-2012 (2012)
- Moutin, T., Van Wambeke, F., and L. Prieur *Biogeosciences*, 9, 3817-3825, doi:10.5194/bg-9-3817-2012 (2012)
- Moutin, T. Van Den Broeck, N., Beker, B., Dupouy, C., Rimmelin, P., LeBouteiller, A. *Marine Ecology-Progress Series* 297, 15-21 (2005)
- Murakami, H. and Dupouy C. *Applied Optics* 52, Iss. 2, pp. 182–198 (2013)
- Nencioli, F., d'Ovidio, F., Doglioli, A.M., Petrenko, A.A. *Geophysical Research. Letters*, Vol.38, L17604, doi:10.1029/2011GL048815 (2011)
- Nencioli, F., d'Ovidio, F., Doglioli A.M. and A.A. Petrenko. *Geophysical Research Letters*, Vol 38, L17604. doi: 10.1029/2011GL048815 (2011)
- Picheral, M., L. Guidi, L. Stemmann, D.M. Karl, G. Iddaoud, and G. Gorsky. *Limnology and Oceanography: Methods*. 8: 462–473 (2010)
- Rahav E., Herut B., Levi A., Mulholland R.M. and Berman-Frank I. *Ocean Sciences*, 9, 1-10, doi: 10.5194/os-9-1-2013 (2013)

- Rimmelin, P. & T. Moutin. *Analytica Chimica Acta*, 548(1-2), 174-182 (2005)
- Rimmelin-Maury, P., T. Moutin, B. Quéguiner, *Analytica Chimica Acta* doi:10.1016/j.aca.2007.01.055 (2007)
- Short, S.M., & Zehr, J.P. *Environmental Microbiology*, 9(6), 1591-1596 (2007)
- Sohm, J.A., Webb, E.A. Capone, D.G. *Nature Reviews Microbiology* 9 (7), 499-508 (2011)
- Talarmin A., Van Wambeke F., Duhamel S., Catala P., Moutin T., and P. Lebaron. *Limnology and Oceanography: Methods*, 9:443-453. DOI: 10.4319/lo.2011.56.4.1244 (2011)
- Tedetti, M., Sempéré, R., Vasilkov, A., Charrière, B., Nérini, D., Miller, W.L., Kawamura, K. and Raimbault, P. *Geophysical Research Letters* 34: doi: 10.1029/2007GL029823. issn: 0094-8276 (2007)
- Thompson, A.W., Foster, R.A., Krupke, A., Carter, B.J., Musat, N., Vulot, D., Kuypers, M.M.M., Zehr, J.P.. *Science* 337:1546-1550 (2012)
- Tedetti, M., Longhitano R., Garcia N., Guigue G., Ferretto N., Goutx M. *Environmental Chemistry*, 9 (5), 438-449 (2012)
- Tedetti, M., Cuét, P., Guigue, C., Goutx, M. *Science of The Total Environment*, 409, 2198-2210 (2011)
- Thompson, A. W., Foster, R. A., Krupke, A., Carter, B. J., et al. (2012). *Science*, 337(6101), 1546-1550 (2012)
- Tripp, H. J., Bench, S. R., Turk, K. A., Foster, R. A., Desany, B. A., Niazi, F., & Zehr, J. P. *Nature*, 464(7285), 90-94 (2010).
- Uitz, J., Claustre, H., Morel, A., & Hooker, S. B. *Journal of Geophysical Research: Oceans* (1978–2012), 111(C8) (2006)
- Van Den Broeck, N., T. Moutin, M. Rodier & A. Le Bouteiller. *Marine Ecology Progress Series*. 268, 1-12 (2004)
- Wagener, T., Guieu C., Losno R., Bonnet S., Mahowald N. *Global Biogeochemical Cycles*, 22, GB2006, doi:10.1029/2007GB002984 (2008)
- Van Mooy, B.A.S., H.F. Fredricks, B.E. Pedler, S.T. Dyhrman, D.M. Karl, M. Kobal, M.W. Lomas, T.J. Mincer, L.R. Moore, T. Moutin, M.S. Rappe, and E.A. Webb. *Nature*, 458, 69-72. doi:10.1038/nature07659 (2009)
- Van Mooy, B.A.S., Hmel, L.R., Sofen, L.E., Campagna, S.R., May, A.L., Dyhrman, S.T., Heithoff, A., Webb, E.A., Mincer, L. and Mincer, T.J. *The ISME Journal* 6:422-429 (2012)
- Zehr, J.P., Mellon, M.T., Zani, S. *Applied and Environmental Microbiology* 64: 3444-3450 (1998)
- Zehr, J., Waterbury, J.B., Turner, P.J., Montoya, J.P. et al. *Nature*, 412, 635-638 (2001)
- Zehr, J.P., Bench, S.R., Carter, B.J., Hewson, I., Niazi, F., et al. *Science* 322: 1110-1112 (2008)
- Zehr, J. P. and R. M. Kudela. *Annual Review of Marine Sciences* 3:197-225 (2011)

References cited (outside the OUTPACE community)

- Antoine, D., André, J.M. and A. Morel. *Global Biogeochemical Cycles*, 10, 57-69 (1996)
- Billet, D.S.M., Lampitt, R.S., Rice, A.L., Mantoura, R.F.C. *Nature* 302, 520-522 (1983)
- Brandes, J.A., A. H. Devol, T. Yoshinari, D.A. Jayakumar, and S.W.A. *Global Biogeochemical Cycles* 1, 15–29 (1998)
- Broecker, W. S. *Global Biogeochemical Cycles* 1, 15–29 (1991)
- Bruland, K.L.W., Silver, M.W. *Marine Biology* 63, 295-300 (1981)
- Campbell, L., Carpenter, E. J., Montoya, J. P., Kustka, A. B., & Capone, D. G. *Vie et Milieu*, 55(3-4), 185-195 (2005)
- Casciotti, K.L., Trull, T.W., Glover, D.M., & Davies, D. *Deep Sea Res. Part II*, 55(14), 1661-1672 (2008)
- Close, H. G., Shah, S. R., Ingalls, A. E., Diefendorf, A. F., Brodie, E. L., Hansman, R. L., & Pearson, A. *Proceedings of the National Academy of Sciences*, 110(31), 12565-12570 (2013)
- Coble PG. *Marine Chemistry* 51:325–46 (1996)
- Codispoti, L.A., Brandes, J.A., Christensen, J.P., Devol, A.H., Naqvi, S.W. A., Paerl, H.W., & Yoshinari, T. *Scientia Marina*, 65(S2), 85-105 (2001)
- Dore, J.E., Brum, J.R., Tupas, L.M., Karl, D.M. *Limnology and Oceanography*, Vol. 47, No. 6 1595-1607 (2002)
- Dore, J.E., Letelier, R.M., Church, M.J., Lukas, R., and Karl, D.M. *Progress in Oceanography*, 76, 2-38 (2008)
- Falkowski, P.G., Barber, R.T. & Smetacek, V. *Science* 281, 200-206 (1998)
- Fernandez, C., Farias, L., Ulloa O. *PLoS ONE* 6: e20539 (2003)
- Fonseca, C. R., G. Ganade. *Journal of Ecology*, 89, 118-125 (2001)
- Fowler, S.W., Knauer, G.A.. *Progress in Oceanography* 16, 147-194 (1986)
- Galbraith, E.D., Markus Kienast and the NICOPP working group members. *Nature Geoscience*, 2 june 2013 | DOI: 10.1038/NGEO1832 (2013)
- Garcia, N., Raimbault, P., & Sandroni, V. *Marine Ecology Progress Series*, 343, 25-33 (2007)
- Gargett, A. E, and J. Marra. *The Sea*, Vol.12, ed. A. R. Robinson, J. J. McCarthy, and B. J. Rothschild, John Wiley & Sons, NY, 19-49. (copyright John Wiley and Sons) (2002)
- Geider, R., & La Roche, J. *European Journal of Phycology*, 37(1), 1-17 (2002)
- Grosjean, P., Picheral, P., Warembourg, C. and G Gorsky. *ICES Journal of Marine Science*. 61 (4): 518-525 (2004)
- Großkopf, T., Mohr, W., Baustian, T., Schunck, H., Gill, D., Kuypers, M., & LaRoche, J. *Nature*, 488(7411), 361-364 (2012)
- Gruber, N. In *The ocean carbon cycle and climate* (pp. 97-148). Springer Netherlands (2004)
- Gruber N., Sarmiento J.L. *Global Biogeochemical Cycles* 11: 235-266 (1997)
- Guerquin-Kern, J.L., Wu, T.D., Quintana, C., & Croisy, A. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1724(3), 228-238 (2005)
- Halm, H., Lam, P., Ferdelman, T.G., Lavik, G., Dittmar, T. *The ISME Journal* 6: 1238-1249 (2011)
- Hasle, G.R. In *Phytoplankton Manual*, Vol. 6. (ed. A. Sournia) (UNESCO Monographs on oceanographic methodology, Paris (1978)
- Haus, H., Franz, J.M.S., Hansen, T., Struck, U., Sommer, U. *Deep Sea Res. Part I*: 75: 135 (2013)
- Jackson, G.A. *Deep Sea Res. Part I*, 37(8), 1197-1211 (1990)
- Jackson, G.A. *Deep Sea Res. Part I*, 48(1), 95-123 (2001)
- Jickells, T., et al. *Science*, 308, 67– 71 (2005)
- Karl, D.M. *Nature*, 415(6872), 590-591 (2002)
- Karl, D.M., Letelier, R., Tupas, L., Dore J., Christian, J. et al. *Nature* 388: 533-538 (1997)
- Karl, D.M. and Letelier, R.M. *Marine Ecology Progress Series* 364, 257-268 (2008)
- Karl, D.M., M.J. Church, J.E. Dore, R.M. Letelier and C. Mahaffey. *Proceedings of the National Academy of Sciences*, 109: 1842-1849 (2012)

- Krupke, A., Musat, N., LaRoche, J., Mohr, W., Fuchs, B.M., Amann, R.I., & Foster, R.A. *Systematic and applied microbiology* 36 259-271 (2013)
- Landrum, J.P., Altabet, M.A., & Montoya, J.P. *Deep Sea Res. Part I*: 58(5), 615-625 (2011)
- Lechene, C., Hillion, F., McMahon, G., Benson, D., Kleinfeld, AM, Kampf, JP, Distel, D, Luyten, Y, Bonventre, J, Hentsche, D, Park, KM, Ito, S, Schwartz, M, Benichou, G, Slodzian, G. *Journal of Biology* 5, 20 (2006)
- Lechene, C. P., Luyten, Y., McMahon, G., & Distel, D. L. *Science*, 317(5844), 1563-1566 (2007)
- Legendre, L., Rassoulzadegan, F. *Marine Ecology Progress Series* 145, 179-193 (1996)
- Lévy, M.; Ferrari, R.; Franks, P. J. S.; Martin, A. P. & Rivière, P. *Geophysical Research Letters*, 39, L14602 (2012)
- Loick-Wilde, L.N., Dutz, J., Miltner, A., Gehre, M., Montoya, J.P., Voss, M. *Limnology and Oceanography* 57: 199-210 (2012)
- Lomas, M.W., Steinberg, D.K., Dickey, T., Carlson, C. A., Nelson, N.B., Condon, R.H., & Bates, N.R. *Biogeosciences*, 7(1), 57-70 (2010)
- Longhurst, A., Sathyendranath, S., Platt, T., Caverhill, C. 1995. *Journal of Plankton Research* 17 (1995)
- Madin, L.P., Purcell, J.E. *Limnology and Oceanography* 37, 1236-1251 (1992)
- Mahaffey, C., Michaels, A.F., Capone, D.G. *American Journal of Science* 305: 546-595 (2005)
- Marie, D., Partensky, F., Vaulot, D., Brussaard, C. in *Current Protocols in Cytometry*, Robinson, Ed. (John Wiley & Sons, Inc., NY, 1999), pp. 11.15 (1999)
- McClelland, J.W., Holl, C.M., Montoya, J.P. *Deep Sea Res. (Part I)* 50: 849-861 (2003)
- Mohr, W., Grosskopf, T., Wallace, D. W., & LaRoche, J. (2010). *PloS one*, 5(9), e12583
- Montoya, J.P., Voss, M., Kahler, P. & Capone, D.G. *Applied and Environmental Microbiology* 62, 986-993 (1996)
- Montoya, J. P., Carpenter, E. J., & Capone, D. G. *Limnology and Oceanography*, 47(6), 1617-1628 (2002)
- Montoya JP, Holl CM, Zehr JP, Hansen A, Villareal TA, et al. *Nature* 430: 1027-1031 (2004)
- Mulholland, M. R., Bernhardt, P. W., Heil, C. A., Bronk, D. A., & O'Neil, J. M. *Limnology and Oceanography*, 51(4), 1762-1776 (2005)
- Mulholland, M. R. *Biogeosciences*, 4(1), 37-51 (2007)
- Murnane, R., Sarmiento, J. L. and Le Quere, C. L. *Global Biogeochemical Cycles* 13, 287–305 (1999)
- Needoba, J.A., Foster, R.A., Sakamoto, C., Zehr, J.P., Johnson, K.S. (2007). *Limnology and Oceanography* 52: 1317-1327.
- Olgun, N., Duggen, S., Croot, P.L., Delmelle, P., Dietze, H., Schacht, U. & Garbe-Schönberg, D. *Global Biogeochemical Cycles*, 25(4), GB4001 (2011)
- O'Neil, J. M. *Bull. Inst. Oceanogr. Monaco*, 19, 293–317 (1999)
- O'Neil, J.M., Metzler, P. & Glibert, P.M. *Marine Biology* 125, 89-96 (1996)
- Para, J., Charrière, B., Matsuoka, A., Miller, WL., Rontani, JFR., Sempéré, R. *Biogeosciences* 10, 2761-2774 (2013)
- Páscoa, R.N., Tóth, I.V., & Rangel, A.O. *Analytica chimica acta*, 739, 1-13 (2012)
- Qiu, B., S. Chen, and W.S. Kessler. *J. Phys. Oceanogr.*, 39, 404-420 (2009)
- Rabouille, S., M. Staal, L.J. Stal, et K. Soetaert. *Applied and Environmental Microbiology*. 72(5) :3217-3227 (2006)
- Raimbault, P., Garcia, N., & Cerutti, F. *Biogeosciences*, 5(2), 281-298 (2008)
- Richardson, T. L., & Jackson, G. A. *Science*, 315(5813), 838-840 (2007)
- Ridgway, K. R., and J. R. Dunn. *Progress in Oceanography*, 56(2), 188-222 (2003)
- Riemann L., Farnelid H., Steward G.F. *Aquatic Microbial Ecology* 61: 235-247 (2010)
- Riser, S.C., & K.S. Johnson. *Nature* 451:323–326, doi:10.1038/nature06441 (2008)
- Sabine, C. L., Feely, R. A., Gruber, N., Key, R. M., Lee, K., and Bullister, J. L. *Science*, 305, 367–371 (2004)
- Sarmiento, J. L., and Gruber, N. *Ocean Biogeochemical Dynamics*, Princeton University Press, Princeton. 503 pp (2006)
- Silver, M.W., Gowing, M.M. *Progress in Oceanography* 26, 75-113 (1991)
- Shiozaki, T., T. Kodama, K. Furuya. *Geophysical Research Letters* DOI: 10.1002/2014GL059835 (2014)
- Slodzian, G., Daigne, B., Girard, F., Boust, F., & Hillion, F. *Biology of the Cell*, 74, 43-50 (1992)
- Stemmann, L., Eloire, D., Sciandra, A., Jackson, G. A., Guidi, L., Picheral, M., & Gorsky, G. *Biogeosciences*, 5(2), 299-310 (2008)
- Stephens, N., K.J. Flynn, et J.R. Gallon. *New Phytologist* 160: 545-555 (2003)
- Ternon E. C. Guieu, C. Ridame, L'Helguen, S, Catala, P. *Biogeosciences*, 8, 1067–1080 (2011)
- Thibault D., Head E., and Wheeler A. *Deep Sea Res. I* 46:1391-1415 (1999)
- Thingstad, T.F., Rassoulzadegan, F. *Progress in Oceanography* 44: 271–286 (1999)
- Thingstad, T. F., Krom, M. D., Mantoura, R. F. C., Flaten, G. A. F., Groom, S., Herut, B., Kress, N., Law, C., Pasternak, A., Pitta, P., Psarra, S., Rassoulzadegan, F., Tanaka, T., Tselipides, A., Wassmann, P., Woodward, E. M. S., Wexels Riser, C., Zodiatis, G., and Zohary, T. *Science* 309, 1068–1071 (2005).
- Walsby, A.E. In *Marine Pelagic Cyanobacteria: Trichodesmium and other Diazotrophs*. (eds. E.J. Carpenter, D.G. Capone & J.G. Rueter) 141-161 (1992)
- Welschmeyer, N.A., Lorenzen, C.J. *Marine Biology* 90: 75-86 (1985).
- Wilson, S. T., Böttjer, D., Church, M. J., & Karl, D. M. *Applied and environmental microbiology*, 78(18), 6516-6523 (2012)
- Wilson, C., and V.J. Coles. *Journal of Geophysical Research* 110 (C10) (2005)