Proposition de sujet de thèse 2020

(A remplir par les équipes d'accueil et à retourner à Isabelle HAMMAD : hammad@cerege.fr
**à renseigner obligatoirement pour la validation du sujet, (1) : A remplir lors de la campagne d'attribution des allocations, à l'issue de la session de juin des Masters*

Sujet de doctorat proposé *: ***Diazotroph activity and diversity shaped by fine scale ocean dynamics (DEFINE)***

Encadrant(s) : *BENAVIDES Mar mar.benavides@ird.fr / VAN WAMBEKE France france.van-wambeke@mio.osupytheas.fr*

Laboratoire *: *Mediterranean Institute of Oceanography (MIO)*

Tableau récapitulatif du sujet

Candidat(e)(1)	
Nom - Prénom :	
Date de naissance :	
Licence (origine, années, mention) :	
Mention et classement au Master 1 année (Xème sur Y)	
Mention et classement au S3 du Master 2 (Xème sur Y)	
Mention et classement au S4 du Master 2 (Xème sur Y)	
Mention et classement au M2 (année) (Xème sur Y)	
MASTER (nom, université)	
Sujet de doctorat proposé*	*Diazotroph activity and diversity shaped by fine scale ocean dynamics (DEFINE)*
Encadrants (2 max, indiquer si HDR ou pas)*	• BENAVIDES Mar (deviendra directrice officielle de la these dès le passage de l'HDR d'ici un an) • VAN WAMBEKE France (HDR)
Laboratoire*	*Mediterranean Institute of Oceanography (MIO)*
Programme finançant la recherche (indiqué si obtenu ou envisagé) (1)	• TONGA (ANR, INSU-LEFE-CYBER, obtenus) • DEFINE (LEFE-CYBER, obtenu) • OASIS (Thomas Jefferson Fund, obtenu) • DINDE (CEFIPRA, obtenu) • e-IMPACT (MICINN, obtenu) • SCAN (ERC, déposé) • SWOT (CNES, déposé) • FIGURE (EuroFleets, déposé)

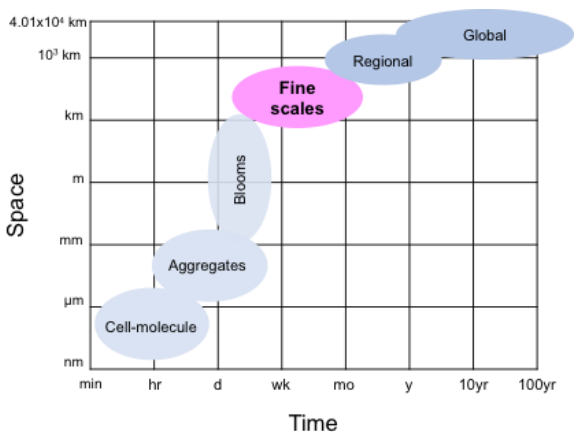
SUJET DE DOCTORAT PROPOSE*

INTITULE* : Diazotroph activity and diversity shaped by fine scale ocean dynamics

DESCRIPTIF *:

I. State-of-the-art and project positioning

Most of the ocean surface is too remote to benefit from land and atmospheric inputs and only receives nitrogen from dinitrogen (N_2) fixation: the reduction of N_2 to ammonia carried out by a single group of plankton called 'diazotrophs'. Diazotrophs play a crucial role in ocean biogeochemistry: they sustain ~50% of marine new primary production[1] and contribute >70% to carbon sequestration in the vast open ocean regions[2,3]. Our current knowledge on diazotroph diversity and activity (diazotrophy) derives from very distant spatiotemporal scales (Fig. 1):



Small scales (light blue): derived from discrete and short duration measurements of diazotrophy obtained by isolating small seawater volumes during individual oceanographic cruises

- Large scales (dark blue): derived from regional extrapolations of discrete measurements and global models of diazotrophy patterns projected over decades to centuries

The **current spatiotemporal gap** between these approaches lies at the **'fine scales'**: dynamic seawater structures <200 km in size and <2 months in duration (pink ellipsoids in Fig. 1).

Fig. 1: *The spatiotemporal scales of diazotrophy in the ocean. Light and dark blue ellipsoids depict scales that have been studied to date. Pink ellipsoids depict the current gap in knowledge: the fine scales.*

Small scale approaches deprive diazotroph communities from interacting with their environment, while large scale approaches cannot reproduce the complexity of such interactions. As a result, the outcomes of these approaches do not match and nitrogen inputs via diazotrophs remain unsolved. Because the ocean is alarmingly losing its ability to absorb CO_2[4], constraining the role of diazotrophs is crucial to understand and predict the ability of the ocean to mitigate climate change. **We hypothesize that nitrogen inputs to the ocean are significantly impacted by the effect of fine scale structures on diazotrophs.**

Fine scale structures are usually studied with satellite remote sensing and numerical modeling[5,6]. These approaches cannot capture the microbial processes that occur in situ. Hence, sampling at sea remains a must, but in situ oceanographic cruises typically sample at sites separated by tens to a few hundreds of km. With a typical navigation speed of ~12 km h^{-1}, the spatiotemporal resolution of these sampling approaches is insufficient to resolve fine scale dynamics.

DEFINE will bridge the gap in the spatiotemporal scales of diazotrophy in the ocean by implementing at-sea high-resolution measurements of diazotroph abundance and activity >10-50 times faster than those available today.

Fine scales structures such as eddies, fronts and filaments drive vertical exchanges between the ocean interior and the surface, uplifting or deepening seawater resources and creating and transforming microbial niches. The changes in the structure and resources of seawater introduced by fine scales may be beneficial to some diazotrophs and detrimental for others, depending on their physiology and metabolism. For example, seawater mass deepening in anticyclonic eddies inhibits the upwelling of deep waters rich in nitrate enhancing diazotroph activity[7,8], promoting the accumulation of floating diazotrophs such as *Trichodesmium*[9,10]. Conversely, accumulations of non-cyanobacterial diazotrophs (such as Gammaproteobacteria) have been detected within cyclonic eddies, presumably due to the alleviation of limitation by other nutrients uplifted from deep waters, such as phosphorus and iron[11]. These previous results stem from conventional oceanographic cruises where the space and time that separated each sampling station was too large and slow to elucidate the effect of fine scales. To improve the spatiotemporal resolution of sampling, in two recent oceanographic cruises we used an automated sampler which allowed retrieving DNA samples every ~3-10 km, and quantified diazotrophs using quantitative PCR (qPCR). During a cruise in 2017 we found a peak of *Trichodesmium* that coincided with the front at the boundary of the Gulf Stream (Fig. 2).

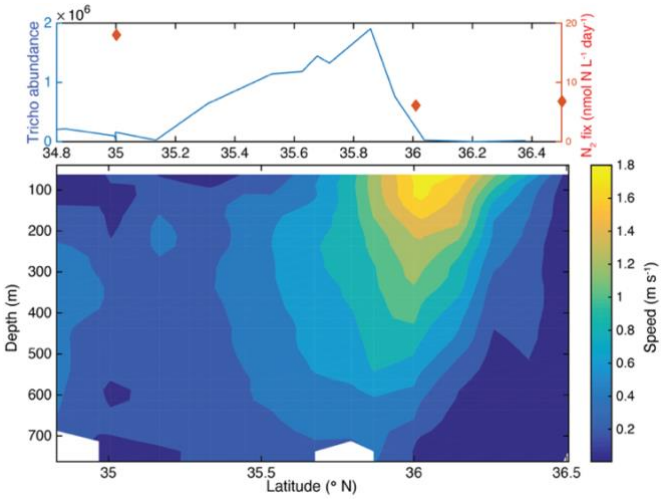


Fig. 2: *Upper panel: abundance of Trichodesmium and diazotroph activity (N_2 fixation rates) across the Gulf Stream. Lower panel: current speed from acoustic doppler current profiler data. Results from Benavides et al., presented at the 2018 Ocean Sciences Meeting and recently accepted for publication*[12].

During another cruise in the South Pacific, we found a drastic change from a dominance of *Trichodesmium* to a dominance of *Crocosphaera* (a group of unicellular diazotrophs) when crossing a front that was <25 km wide[13]. This steep change at the fine scale would have gone unnoticed with a sparser sampling, reinforcing the **urgent need for high-resolution sampling in oceanic diazotrophy research.**

II. Objectives

The **overarching objective of DEFINE** is to study the effect of fine scales on diazotrophy and their ultimate impact on nitrogen inputs to the ocean. To that end, the following **specific objectives** are set:

1) To survey fine scale structures (WP1)
2) To implement high-resolution measurements of diazotrophy (WP2-3)
3) To identify the effect of fine scale structures on diazotrophs and their derived nitrogen inputs (WP4)

III. Approach and methodology

WP1: Oceanographic cruises and physical parameters

The project will be developed thanks to three oceanographic cruises:

1) **e-IMPACT cruise**, Canary Islands (Northeast Atlantic), *R/V Sarmiento de Gamboa* June 2021.
2) **SWOT cruise**[14], New Caledonia (Southwest Pacific), *R/V Alis* February 2022.
3) **FIGURE cruise,** Gulf Stream (Northeast Atlantic) *R/V Atlantic Explorer* or **BIOCAT-IIOE2 cruise** Bay of Bengal (Indian Ocean) *F/S Sonne* June 2022.

Before and during sampling, fine scales will be geolocated and monitored using the SPASSO software to target fine scale structures of interest[15]. Current velocities, surface temperature and salinity will be continuously logged onboard using underway and towed instruments. Day and night samplings will be combined to capture different diazotroph circadian rhythms. Samples for nutrient analyses will be taken from the ship's underway.

WP2: Diazotroph abundance

Plankton biomass will be sampled at high-resolution from the ship's underway using the OCE-5, an automated device that obtains samples as the ship navigates **every ~15 min.** Biomass will be later extracted to obtain DNA samples. Different diazotroph groups will be enumerated using quantitative polymerase chain reaction (qPCR) assays as described in[16]. In addition, we will develop an innovative method capable of quantifying diazotrophs in near real-time in situ. Diazotrophs can be identified using fluorescently-labeled antibodies against their nitrogenase enzyme[17]. The goal is to adapt this method to **automated flow cytometry**[18] using **'nanobodies'**: fluorescent antibodies small enough to penetrate the living cell without prior permeabilization in **<1 h**[19]. Isolated anti-nitrogenase nanobodies will be produced at large scales and fluorescently labeled at the CRCM Nanobody platform (Marseille, France). The nanobodies will be tested for reactivity and specificity on cultured living diazotrophs and non-diazotrophic plankton controls. Immunolabeled diazotrophs will be counted using the automated flow cytometer CytoSense, connected to the ship's underway[18].

WP3: Diazotroph activity

We will measure N_2 fixation using the argon assay developed by our collaborator Sam Wilson (recently presented at the 2020 Ocean Sciences Meeting). The assay has been validated in diazotroph cultures and at sea in the frame of the project OASIS (which Benavides co-leads with Sam Wilson) and provides a rate measurement **every ~15 min.**

WP4: Fine scale effects on diazotrophs and derived nitrogen inputs

The relationship between physical and biological data will be analyzed by multivariate statistical methods[20]. N_2 fixation rates will be extrapolated to the surface of fine scale structures to provide areal nitrogen inputs. As a control, N_2 fixation rates measured outside fine scale structures will be extrapolated to the area of the sampling region covered. These comparisons will reveal the **relative role of fine scale structures in providing fixed nitrogen over background sea surface conditions** dominated by large scale dynamics. Finally, a time-series of satellite data will be used to calculate the total surface covered by fine scale structures in each target region along a whole year. Multiplied by the N_2 fixation rates measured within fine scale structures during our cruises, we will be able to provide diazotroph derived nitrogen inputs on an annual scale.

IV. PhD student thesis planning (starting October 2020)

Competences acquired: Gas chromatography, stable isotope probing, isotope ratio mass spectrometry, membrane inlet mass spectrometry, molecular biology (Illumina sequencing and qPCR), immunolabeling, flow cytometry, microscopy.
Training in Hawaii (December 2020): A visit of Dr Benavides and the PhD student are scheduled for December 2020 in the frame of the Thomas Jefferson Fund project "OASIS" (coPIs Dr Wilson and Dr Benavides) to receive training on the hydrogen-argon assay and test it onboard R/V *Kilo Moana* at station ALOHA.
Cruises: e-IMPACT (June 2021), SWOT (February 2022), FIGURE or BIOCAT-IIOE2 (June 2022).
Participation in conferences: The PhD student will participate in the ASLO meetings of 2021 and 2023. Other participations in national conferences are foreseeable.

Natural year	**2020**	**2021**				**2022**				**2023**			
PhD year	**Year 1**				**Year 2**					**Year 3**			
Trimester	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**
Training & Method development												
Oceanographic cruises			e-IMPACT			SWOT		DINDE/FIGURE				
Sample processing												
Data analysis												
Projects' workshops												
Scientific meetings												
Publications	M2 results											
Final thesis writing												

*V. References (*own in bold)*

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DÉTAIL DU PROGRAMME FINANÇANT LA RECHERCHE* :

The proposed thesis relies on a comprehensive program of research projects, international collaborations and oceanographic cruises devoted to study the effect of fine scales on diazotrophy.

- TONGA (ANR, INSU-LEFE-CYBER, obtenus): Co-Pis Sophie BONNET, Cécile GUIEU. The PhD candidate is currently implementing his M2 work on the data and samples obtained from the TONGA cruise. The exploitation of the results in the form of a scientific article and a congress presentation/poster are planned for late 2020/early 2021.
- DEFINE (LEFE-CYBER, obtenu): PI Mar BENAVIDES.
- OASIS (Thomas Jefferson Fund, obtenu): Co-PIs Mar BENAVIDES, Sam WILSON. Project devoted to the development of the high resolution N_2 fixation method implemented in WP3. This project will cover the mission of the student to Hawaii to receive training on the method.
- DINDE (CEFIPRA, obtenu): Co-PIs Mar BENAVIDES, Arvind SINGH. Project investigating N_2 fixation in the Indian Ocean. Cruise opportunities have been granted to BENAVIDES onboard the German *F/S Sonne*.
- e-IMPACT (MICINN, obtenu): PI Javier ARÍSTEGUI. Project investigating the effect of fine scales on microbial activity and diversity in the Northeast Atlantic Ocean. Cruise opportunity granted to BENAVIDES onboard the Spanish *R/V Sarmiento de Gamboa.*
- SCAN (ERC, déposé): ERC Starting Grant project submitted by BENAVIDES to develop high resolution diazotrophy measurements.
- SWOT (CNES, déposé): PI Lionel GOURDEAU. Project submitted to CNES to study fine scales in the frame of the NASA mission SWOT in New Caledonia. A proposal will also be submitted to the Flotte Océanographique Française to request shiptime onboard the *R/V Alis.*
- FIGURE (EuroFleets, déposé): PI Mar BENAVIDES. Ship time requested to EuroFleets to implement high resolution diazotrophy measurements on the Gulf Stream onboard the *R/V Atlantic Explorer*.

DIRECTEUR(S) DE THÈSE PROPOSÉ(S)*

DIRECTEUR HDR PROPOSÉ*

Nom - Prénom : VAN WAMBEKE France
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Adresse mail : france.van-wambeke@mio.osupytheas.fr

Choix de cinq publications récentes (souligner éventuellement les étudiants dirigés co-signataires) :

Duhamel S., Van Wambeke F., Lefevre, D. Benavides M., Bonnet, S. (2018) Mixotrophic metabolism by natural communities of unicellular cyanobacteria in the western tropical South Pacific Ocean, Environmental Microbiology and Environmental Microbiology Reports, doi: 10.1111/1462-2920.14111.

Van Wambeke, F., Gimenez, A., Duhamel, S., Dupouy, C., Lefevre, D., Pujo-Pay, M., and Moutin, T. (2018) Dynamics and controls of heterotrophic prokaryotic production in the western tropical South Pacific Ocean: links with diazotrophic and photosynthetic activity, Biogeosciences, 15: 2669 – 2689, doi: 10.5194/bg-15-2669-2018

Djaoudi K, Van Wambeke F, Coppola L, D'Ortenzio F, Helias-Nunige S, Raimbault P, Taillandier V, Testor P, Wagener T and Pulido-Villena E (2018) Sensitive Determination of the Dissolved Phosphate Pool for an Improved Resolution of Its Vertical Variability in the Surface Layer: New Views in the P-Depleted Mediterranean Sea. Front. Mar. Sci. 5:234. doi: 10.3389/fmars.2018.00234.

Djaoudi K, Van Wambeke F, Barani A, Hélias-Nunige S, Sempéré R, Pulido-Villena E (2017) Atmospheric fluxes of soluble organic C, N and P to the Mediterranean Sea: potential biogeochemical implications in the surface layer. Progress in Oceanography, doi 10.1016/j.pocean.2017.07.008

Céa B, Lefèvre D, Chirurgien L, Raimbault P, Garcia N, Charrière B, Grégori G, Ghiglione J.F, Barani A, Lafont M, Van Wambeke F (2014). An annual survey of bacterial production, respiration and ectoenzyme activity in coastal NW Mediterranean waters: temperature and resource controls. Environmental Science and Pollution Research. DOI 10.1007/s11356-014-3500-9

THÈSES ENCADRÉES OU CO-ENCADRÉES AU COURS DES QUATRE DERNIÈRES ANNÉES*

Nom : Kahina DJAOUDI
Intitulé : Rôle de l'apport atmosphérique sur les processus de biodégradation et la stœchiométrie de la matière organique dissoute en mer Méditerranée
Type d'allocation : bourse Labex OT-Med
Date de début de l'allocation de doctorat : Novembre 2014
Date de soutenance (si la thèse est soutenue) : 9 mars 2018
Programme finançant la recherche : Projet LEFE BATO, Labex OT-Med
Situation actuelle du docteur (si la thèse est soutenue) : post doc (EIL Lamont Divisions, Lamont-Doherty Earth Observatory, Columbia University, USA.
Pourcentage de participation du directeur à l'encadrement en cas de co-direction : 50% (avec Elvira Pulido)

AUTRE DIRECTEUR PROPOSÉ (*ÉVENTUELLEMENT*)*

Nom - Prénom : BENAVIDES Mar (deviendra directrice officielle de la these dès le passage de l'HDR)
Corps : CRCN IRD
Adresse mail : mar.benavides@ird.fr
Laboratoire : Institut Méditerranéen d'Océanologie, MIO UMR7294, équipe CYBELE.

Choix de cinq publications récentes (souligner éventuellement les étudiants dirigés co-signataires) :

Baños, I., Montero, M.F., Benavides, M., Arístegui, J. INT toxicity over natural bacterial assemblages from surface oligotrophic waters: implications for the assessment of respiratory activity. Accepted. Microbial Ecology.
Benavides, M., Bonnet, S., Berman-Frank, I., Riemann, L., 2018. Deep Into Oceanic N2 Fixation. Front. Mar. Sci. 5:108. doi: 10.3389/fmars.2018.00108
Benavides, M., Berthelot, H., Duhamel, S., Raimbault, P., Bonnet, S. Dissolved organic matter uptake by Trichodesmium in the Southwest Pacific. 2017. Sci Rep 7: 41315
Benavides, M., Moisander, P.H., Bode, A., Arístegui, J., 2016. Longitudinal variability of diazotroph abundances in the subtropical North Atlantic Ocean. J Plankton Res 38 (3): 662-672
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THÈSES ENCADRÉES OU CO-ENCADRÉES AU COURS DES QUATRE DERNIÈRES ANNÉES*

Nom : BERDÚN Javier
Intitulé : Onset and demise of *Trichodesmium* blooms in the Canary Islands
Type d'allocation : Formación Personal Universitario, Universidad de Las Palmas de Gran Canaria (Espagne)
Date de début de l'allocation de doctorat : September 2019
Date de soutenance (si la thèse est soutenue) :
Programme finançant la recherche : Gobierno de Canarias (Espagne), e-IMPACT (Espagne)
Situation actuelle du docteur (si la thèse est soutenue) :
Pourcentage de participation du directeur à l'encadrement en cas de co-direction : 50%

Nom : BAÑOS Isabel
Intitulé : The effect of climate change stressors on marine plankton respiration
Type d'allocation : Formación Personal Investigador, Ministerio de Educación, Ciencia y Universidades (Espagne)
Date de début de l'allocation de doctorat : May 2017
Date de soutenance (si la thèse est soutenue) :
Programme finançant la recherche : HOTMIX (Espagne), FLUXES (Espagne), KOSMOS (Allemagne)
Situation actuelle du docteur (si la thèse est soutenue) :
Pourcentage de participation du directeur à l'encadrement en cas de co-direction : 30%