AO INSU 2021
Section « Océan-Atmosphère »
Dossier scientifique LEFE

Nom du porteur du projet: Regaudie de Gioux Aurore (chargé de recherche)
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Titre et acronyme du projet : Co-Limitation of light and nutrients on the mIxotrophy of nanophytoplanktonic communities in a coaStal ecosystem with strong environment gradient, the French Guiana coastal waters. **LINKS-2**
Durée totale du projet: 2 ans
Demande budgétaire pour les années à venir: 2021 : 13840 euros
2022 : 9875 euros

Visa *obligatoire* du Directeur de laboratoire porteur :
(préciser nom et prénom)

Description du projet

1. Prénom, Nom, et laboratoire de rattachement du porteur du projet
Aurore REGAUDIE DE GIOUX – Laboratoire DYNECO/PELAGOS du Centre Bretagne d'Ifremer, Plouzané

2. Titre et acronyme du projet
LINKS-2 : Co-Limitation of light and nutrients on the mIxotrophy of nanophytoplanktonic communities in a coaStal ecosystem with strong environment gradient, the French Guiana coastal waters.

3. Type de projet : Projet collaboratif

4. Informations sur le projet
- Le projet est-il un nouveau projet ? **Non** Une re-soumission ? **Oui**, AO LEFE 2020
- Avez-vous soumis/comptez-vous soumettre le même projet ou un projet compagnon à un autre AO INSU ? **Non**
- Avez-vous un co-porteur à ce projet ? **Non**
- Le proposant a-t-il porté ou participé à un projet financé par le LEFE au cours des 3 dernières années ? **Oui**

Le proposant, Regaudie de Gioux Aurore, a porté et participé au projet LINKS financé par AO LEFE 2020. Dans ce projet initial (phase 1), le proposant demandait un financement de trois ans. La commission d'évaluation LEFE a accordé de financer la première année du projet en l'attente de voir l'acceptation de la flotte océanographique pour effectuer la campagne LINKS, la validation d'un post-doc SAD-Région BRetagne et la validation de certains points de la méthodologie. Le projet actuellement proposé LINKS-2 pour AO LEFE 2021 a été un peu modifié par rapport au projet initial LINKS afin de répondre aux questions des reviewers de la commission LEFE 2020. Le projet LINKS-2 est la deuxième phase du projet LINKS initial avec la mise en place de la campagne océanographique et des analyses.

5. Mots clés

Nanophytoplankton, mixotroph, limitation, coastal ecosystem

6. Intérêt scientifique, contexte et état de l'art

Planktonic photosynthesis, contributing approximately to the half of primary production of the biosphere (Field et al. 1998), is a fundamental process at global and at ecosystem scale influencing oxygen and carbon fluxes. In addition to the photosynthesis (called also phototrophy or autotrophy), the majority of phytoplanktonic organisms uses also organic substrates available in the ecosystem as a source of energy and nutrient (i.e. heterotrophy) (Stoecker, 1998). These organisms, called mixotrophs, are divided with respect to phototrophy between constitutive (e.g. phagotrophy that is the engulfment and digestion of another organism or other exogenous particles) *vs.* non-constitutive (e.g. kleptoplastidy) (Mitra et al. 2016). Following previous published arguments (Flynn et al., 2013, Mitra et al., 2014), osmotrophy (i.e. absorption of dissolved organic molecules through cell membrane) has not been included here as a characteristic for mixotrophic protists. The main reason is that osmotrophy appears to be ubiquitous from strictly autotrophs (i.e. diatoms) to strictly heterotrophs (microzooplankton) (Sanders, 1991; Glibert & Legrand, 2005; Burkholder et al., 2008). Mixotrophy, represented by 5 to 7 nutritional modes (Stoecker et al, 2017; Mitra et al., 2016), is so largely distributed in marine protists that it is hard to define a phytoplanktonic organism as strictly autotrophic (Selosse et al, 2017). However, most of carbon and nutrient fluxes modelling use the classic dichotomy (autotrophs *vs*. heterotrophs) to synthetize the functional trait of planktonic communities. By not integrating mixotrophy as a functional trait, the estimation of net primary production by modelling of planktonic community underestimates the efficiency of the biological pump. Indeed, the capability to modulate the different sources of carbon (C) would limit the competitive exclusion associated to inorganic sources (micro- and macro- nutrients) in ecosystems with strong environmental variations (Thingstad et al, 1996) and would allow higher trophic fluxes in stable ecosystems enhancing the competitivity of large size organisms (Ward and Follows, 2016). Mixotrophy constitutes thus an essential physiological trait in the understanding of structural and functional biodiversity of marine plankton. It is so fundamental to elucidate the importance of mixotrophic phytoplankton in planktonic communities, and particularly under limited conditions, to better understand marine ecosystems.

Several studies showed that mixotrophic organisms seem to play a major role into microbial loop (Zubkov *et al.* 2008, Mitra *et al.* 2010, Jeong *et al.* 2010, Hartmann *et al.* 2012, Flynn *et al.* 2013, Stoecker *et al.* 2017). Indeed, studies located in the Atlantic Ocean observed that phytoplanktonic protists represent between 40 to 90 % of all bacterivores in the euphotic layer (Zubkov *et al.* 2008, Hartmann *et al.* 2012) modifying our concept of microbial loop functioning. In oligotrophic ecosystems, as Mediterranean Sea, ciliates containing chloroplasts represent between 17 to 54 % of all ciliates and contribute more than 20 % to total chlorophyll concentration (Dolan & Marrase 1995, Pitta & Giannakouru 2000). In addition, eutrophic ecosystems characterized with nutrient over-enriched and light-poor conditions due to suspended sediment loading are promoting mixotrophy (Burkholder et al. 2008). Although several experiments on mixotrophic plankton organisms have allowed to highlight their metabolism (Sanders & Porter 1988, Sanders 2011), they did not clearly define the relative part of each nutritional mode as a source of energy and nutrients. It could explain that mixotrophy is still not considered in biogeochemical models (Zubkov & Tarran 2008, Mitra et al. 2010, Jeong et al 2010, Hartmann et al. 2012, Flynn et al. 2013, Stoecker et al. 2017). Furthermore, some studies observed the influence of environmental factors (e.g. light, temperature, nutrient concentration) on mixotrophs capacity to do phototrophy rather than phagotrophy and *vice versa* (Carvalho & Granéli 2010, Wilken et al. 2013, Millette et al. 2017, Jeong et al. 2018, Lim et al. 2019). Most studies observed that mixotrophic phytoplankton had their ingestion rates stimulating under low light conditions (Millette et al. 2017, Lim et al. 2019), nutrient deficiency (Carvalho and Granéli 2010) and with temperature increase (Wilken et al. 2013, Jeong et al. 2018, Lim et al. 2019). Furthermore, within the framework of the ANR-10-LABX-19 project PROMO (PI: **A. Regaudie de Gioux**, 2018–2019), additional experiments were done using a specific mixotrophic phytoplankton species (*Alexandrium minutum*) to test

the relative balance between different nutritional modes (osmotrophy, phototrophy and phagotrophy by bacterivory) and the influence of limiting light on this balance. We observed that the bacterial ingestion by *A. minutum* was higher under light limitation. All of these experiments were made with specific phytoplankton species and they were set up in laboratories using most of the time monospecific cultures. Very few works gave information on the importance of mixotrophy for phytoplanktonic communities and their capacities to privilege phototrophy and/or phagotrophy responding to limiting environmental factors in natural ecosystems. For example, Zubkov *et al*. (2005) and Hartman *et al*. (2012) showed the importance of bacterivory for small algae (nanophytoplankton) in the North Atlantic Ocean and Atlantic oligotrophic ecosystems. Seong *et al*. (2006) observed that natural nanoplanktonic communities of co-occurring red tide algae showed important bacterivory that can have a considerable grazing impact on bacterial populations. These works allowed highlighting that nutrients and light seem to be crucial factors for the importance of bacterivory in phytoplanktonic communities. Within the framework ANR-17-EURE-0015 and ANR-10-LABX-19 project REMEDIOS (PIs: **C. Jauzein** and **A. Regaudie de Gioux**, 2018), nutritional fluxes (i.e. osmotrophy, phagotrophy by bacterivory and phototrophy) of coastal nanophytoplanktonic communities were observed *in situ* using the pulse-chase method for bacterivory (Zubkov *et al.* 2008, Hartmann *et al.* 2012), the ^{14}C method for PP (Steeman-Nielsen 1952) and the ^{15}N technique for osmotrophy (Collos 1987). We observed that previous to a planktonic bloom (nutrient supply increasing), nanophytoplanktonic community showed lower bacterial ingestion rates in the surface waters (high light conditions) than in deeper waters and that during and after a planktonic bloom (nutrient supply decreasing), and showed higher bacterial ingestion rates above Deep Chlorophyll Maximum (DCM) than below. Light and nutrients limitation in a natural ecosystem seems to promote bacterivory (i.e. ingestion of bacteria by phagotrophy) by nanophytoplanktonic communities. Important efforts need to be done to get more information on the preference of natural mixotrophic nanophytoplankton communities to do phototrophy and/or phagotrophy when environmental factors are limiting *in situ*. This project proposes to improve our understanding of the factors influencing phototrophy and/or phagotrophy by nanophytoplankton focussing in particular into light and nutrients.

The previous published works as well as the projects leading by Dr Regaudie de Gioux (PROMO and REMEDIOS) indicate that marine ecosystems with strong light and nutrient limitations coupled with high temperature could promote mixotrophy over the phytoplanktonic community composition and stimulate the main metabolic pathways by mixotrophs. The marine coastal area of French Guiana is a complex and unique coastal system characterized by low seasonality and very strong environmental gradients from the shore towards the continental shelf: *i*) low productive beige waters near river mouths with high concentration of suspended matter (SM) limiting light penetration through the water column, *ii*) low productive greenish waters (< 20 m depth) close to the shore with low SM concentration and nutrient-limited, *iii*) productive brown waters (20 m < depth < 80 m) with high SM, silicate and chlorophyll concentrations (Chl) caused by the Amazon river retroflection, and *iv*) low productive clear blue waters (continental shelf) with nutrient limitation (e.g. Cadée 1975, Ternon et al. 2001, 2002). Despite the lack of seasonal variation, a gradient of functionally different microphytoplankton groups has been already observed from mainly autotrophs (diatoms in estuarian beige waters), mixotrophs (dinoflagellates community dominated by in greenish and brown waters) to mainly heterotrophs (mixotrophic and strictly heterotrophic dinoflagellates in blue waters) from the shore to the continental shelf of coastal French Guiana waters (Paulmier 1993, 2004). Although several studies described microphytoplanktonic species succession in these waters, it remains a lack of information on nanophytoplanktonic communities (< 20 µm) and how limiting environmental factors (here, light and/or nutrient) influence biodiversity (structural and functional) and nutritional modes of the nanophytoplanktonic communities. As for microphytoplanktonic communities, a succession of biodiversity and functioning (phototrophs, mixotrophs or heterotrophs) for nanophytoplanktonic communities might be observed across the four different coastal waters. Considering the expected proliferation of small phytoplankton cells in the ocean due to warming leading to ocean surface oligotrophication, studying the nutritional modes and the functional diversity of the nanophytoplanktonic communities present in this particular coastal ecosystem would allow to bring relevant information on the microbial loop functioning in coastal marine ecosystems co-limited by light and nutrient.

7. Objectif général et questions de recherche traitées

In this project, we propose to investigate the **effect of light and nutrient limitation on the mixotrophy of natural nanophytoplanktonic communities**. The main hypothesis of this project is that

different nanophytoplankton communities prevail in the different water types presenting different levels of mixotrophy relative to the present nutrient and light conditions. For this we propose here to describe *i)* the environmental and metabolic status of the different water masses and *ii)* the diversity and mixotrophic capacity of the nanophytoplankton using molecular methods and *in situ* experimental approaches. For that, this project will determine the relative part of different nutritional modes (phototrophy, phagotrophy (here bacterivory) and osmotrophy), the metabolism (autotrophy, heterotrophy or in balance), the photo-physiologic parameters and the biodiversity of the nanophytoplanktonic communities present under different limitation (light and nutrient: beige waters; nutrient only: greenish and clear blue waters; light only: brown waters). This project will allow better understanding of the productivity of coastal ecosystems under environmental limitations and improve our concept of microbial loop in such ecosystems. This project presents a strong originality considering the unique ecosystem that is the coastal waters of French Guiana with its strong turbidity gradient but also its interdisciplinarity combining genetic expression and nutritional fluxes.

The first year of the project (2021) will consist of a multidisciplinary oceanographic cruise where biogeochemistry, bio-optical and biology approaches will be applied, and finally the second year of the project (2022) will be devoted to the valorisation of the obtained results. The oceanographic cruise will be divided in two legs (Fig. 1) off the two largest rivers of French Guiana, the Maroni and the Oyapock where the four different coastal waters (see previously) are clearly distinguished.

Our specific objectives will be the following:

1- To describe the different coastal water masses
2- To assess the abundance and the diversity of natural nanophytoplanktonic communities depend on the environmental limitation present in this coastal ecosystem.
3- To assess the importance of mixotrophy in the nanophytoplanktonic communities of this natural ecosystem under variable limitations.

These questions are in line with the objective of LEFE-CYBER partly stating "*The general CYBER objective is to improve our understanding of biogeochemical cycles and marine ecosystems functioning that imply for a part to quantify stocks and biogenic element fluxes, to study process acting on organic matter production and its transfer to superior trophic level*". Particularly, our project would contribute to Theme 4 – Functional biodiversity, ecosystem functioning and biogeochemical cycles.

8. Plan de recherche, méthodologie et calendrier de réalisation

8.1. Research plan

Due to the exceptional Covid-19 pandemic and the national confinement, the preliminary tests proposed in the phase 1 of the LINKS project (AO LEFE 2020) were not yet done. However, after the analysis of the REMEDIOS results, we were able to adjust the bacterivory and osmotrophy methodologies and we are now able to manage them well. Furthermore, D. Marie (research engineer from SBR and partner of this project) showed that after a pre-filtration with 3 um nylon filter, turbid samples of phytoplankton communities from Mackenzie River where SPM was between 5 and 100 g m^{-3}, were well sorted. We proposed therefore to do this prefiltration to our samples dedicated for bacterivory measurements. LINKS-2 will be divided thus in two parts: the oceanographic cruise and the results analyses. The oceanographic cruise will be done in two legs, each of them cruising from off river mouth (Maroni and Oyapock rivers) for a description at the community scales of the main fluxes (PP, respiration, osmotrophy, phagotrophy and heterotrophy). By covering the area off these two rivers, we will be able to have a better coverage of this stable coastal ecosystem under environmental limitations. This project will provide the following deliverables:

Deliverable 1 – Water bio-optical properties
(Laboratoire d'Océanographie et Géosciences, LOG)

The analysis of the *in situ* particulate (phytoplankton, SPM) and dissolved (CDOM) matter optical properties (absorption and/or scattering) in addition with water radiometric measurements (luminance and reflectance) will allow a precise description of optical and biogeochemical properties and light conditions of the water masses. Furthermore, an analysis of the distribution of reflectance, chl-*a*, SPM and CDOM from ocean colour remote sensing observation (e.g. Sentinel 2, 3) will allow the characterisation of the optical water type for determining the location of the sampling stations.

Deliverable 2 -Physical and biogeochemical properties
(Ifremer-DYNECO-PELAGOS / Universidade de São Paulo, USP)

Hydrological parameters (temperature, salinity and conductivity) as well as nutrient concentration will be measured. These parameters will help to describe the physical and biogeochemical properties of the ecosystem. These data will allow also to observe the possible relationship between the biogeochemical parameters and the metabolic activity and the diversity of the mixotrophic phytoplankton in the planktonic communities present in this ecosystem

Deliverable 3 – Nutritional modes associated to the nanophytoplankton community
(DYNECO / USP / Sorbonne Université, SU)

Chl-*a* concentration, phototrophy (PP), phagotrophy through bacterivory, osmotrophy, metabolic balance of the planktonic communities (gross primary production, GPP, net primary production, NPP, and community respiration, CR) and photo-physiological parameters of the phytoplankton will be estimated in order to observe the variation of the phytoplanktonic metabolism, nutritional modes and physiological state between the different environmental limitations present in this natural and stable ecosystem. These data will allow estimating the relative part and the importance of the mixotrophy in the phytoplanktonic community under limiting environmental factors such as light.

Deliverable 4 – Phytoplanktonic biodiversity
(DYNECO / SU / LOG)

For quantitative estimates, microplankton abundance will be estimated using optical microscopy and flow cytometry, and nano- and pico-phytoplankton abundance will be analysed by flow cytometry. Through taxonomic and annotation matrices of morpho-trophic traits, we expect to describe the variability of mixotrophic protists in the phytoplanktonic community of a natural coastal ecosystem under environmental limitation. Taxonomic matrices will be obtained at least by optical microscopy for the microplankton size fraction. During the cruise and experiments, eDNA (metabarcoding) and cDNA (metatranscriptomics) samples will also be collected and preserved. eDNA and cDNA data will allow estimating qualitatively, and in the best case quantitatively, the genetic diversity of the phytoplanktonic species and functions in the different water masses and during the experiments. Molecular steps (extraction, purification/quantification and library preparation) will be done at DYNECO/PELAGOS and samples will be sequenced at Get-PlaGe France Genomics sequencing platform (Toulouse, France). The required funding for the molecular analyses here will consider only the sequencing. Bioinformatic analyses (cleaning, assembly, taxonomical and functional annotation) and ecological and biostatistics analyses (alpha and beta-diversity, community dynamics monitoring of species and functions based on co-occurrence networks) will be performed at the Sorbonne University.

7.2. Methodology

7.2.1. Oceanographic cruise – The oceanographic cruise (2021, validated by CNFC) will be divided in two legs (Fig. 1) during 15 days in order to describe coastal ecosystems limited by light and nutrient off the two largest rivers of French Guiana, the Maroni and the Oyapock. The National Vessel Commission informed us, once our cruise was validated, that the vessel required (ANTEA) will be in Antillas-Guyana waters in 2021 but not in 2022 and doubtedly in 2023. For that reason, the oceanographic cruise is proposed for 2021.

Cruise planning – During the first leg (Leg1) and the second leg (Leg2), we will sample seawater from coastal water off the Oyapock river's mouth to the continental shelf and off the Maroni river's mouth to the continental shelf, respectively. By covering the area off these two rivers, we will be able to have a better coverage of stable coastal ecosystems under environmental limitations. The final version of each transect will be decided on remote data (chl-*a*), coastal water reflectance and turbidity data. Thanks to these data, we will be able to find the four typical water masses of French Guiana coastal water (beige, greenish, brown and clear blue waters). Two kind of sampling stations will be sampled: complete stations (called "S") with hydrological, physical, optical, phytoplankton, biogeochemistry and biodiversity measurements (5 "S"

stations for each leg) and hydrological stations (called “c”) with only hydrological measurements (15 “c” stations for each leg).

During “S” stations, one or two hydrological CTD (conductivity, temperature, depth) profiles through the water column will be done to collect seawater temperature, salinity, oxygen concentration, fluorimetry, turbidity and PAR (photosynthetically active radiation) data. Additionally, seawater will be sampled between 2 and 5 different depths (depending on the water column depth) using a rosette sampler to analyse the salinity, the turbidity, the nutrient and oxygen concentrations, the alkalinity, the SM and CDOM concentrations, chl-*a*, PP, GPP, NPP, CR, the osmotrophy, the bacterivory, eDNA/cDNA, phytoplanktonic photo-physiology, bacterial production, grazing and abundance.

During “c” stations, only one or two hydrological CTD (conductivity, temperature, depth) profiles through the water column will be done to collect seawater temperature, salinity, oxygen concentration, fluorimetry, turbidity and PAR (photosynthetically active radiation) data.

Hydrological parameters – Temperature, conductivity, depth, oxygen concentration, fluorimetry and salinity will be determined at all stations using a CTD Sea-Bird SBE911 attached to the Rosette sampling system of the research vessel. The penetration of irradiance (PAR) at depth will be measured with a QCP-2350 submarine irradiance profiler that will be attached to the Rosette sampling system as well.

Physical and biogeochemical parameters – Turbidity will be measured at each “S” stations using a NKE water quality profiler. On each “S” station, seawater samples will be collected and filtrations will be performed immediately onboard at low vacuum pressure onto 25-mm glass-fiber filters (Whatman GF/F) for determination of the SM (g m^{-3}), particulate organic and inorganic carbon (POC and PIC; mg m^{-3}) and size-fractionated chl-*a* (mg m^{-3}) concentrations. Each filter will be stored in liquid nitrogen until laboratory analyses with a spectrophotometer. Additionally, chl-*a* concentrations will be also estimated through HPLC in order to have a better description of phytoplanktonic communities present there. Water samples will be also filtered under low vaccum onto 0.22 µm Millipore membranes for the determination of the absorption by CDOM ($a_{cdom}(\lambda)$, m^{-1}). The methodology is described in Loisel *et al*. (2009). Samples for nutrient (phosphate, silicate, nitrate+nitrite, ammonium) analyses will be collected at each station and kept frozen until analysis with a Bran Lebe AA3 autoanalyzer. Spectral reflectance, $R_{rs}(\lambda)$, measurements will be performed at each “S” stations using two Trios hyperspectral (every 3 nm) radiometers: one measuring the downwelling

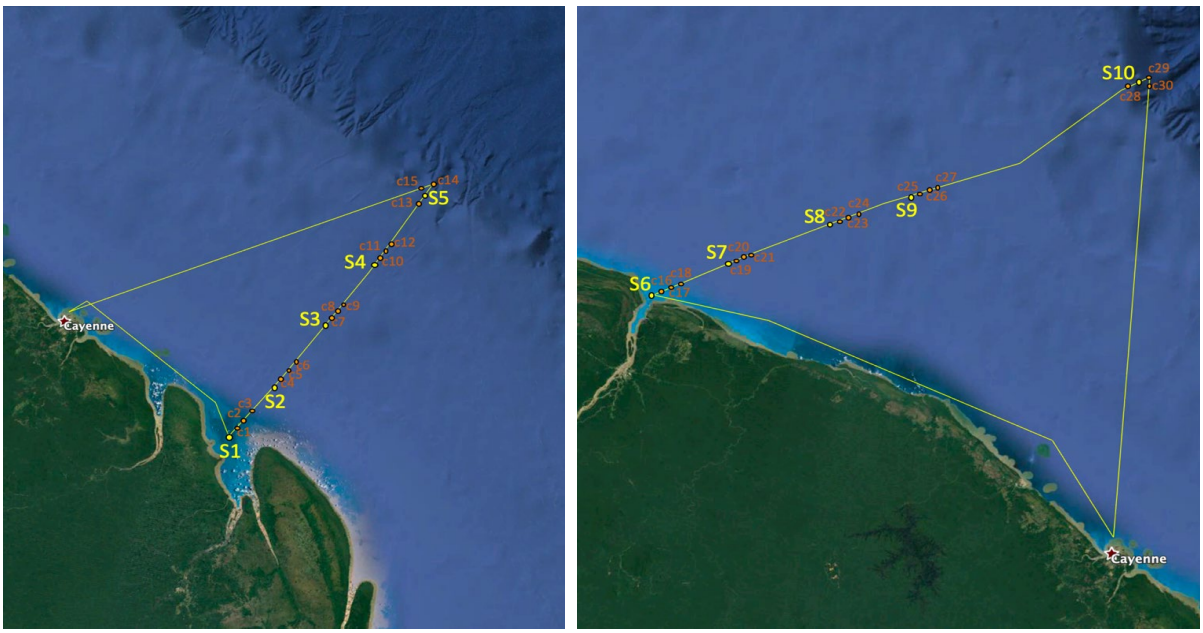

Figure 1 *— Leg 1 (right) and leg 2 (left). S: complete station; c: CTD only. The localisation of “S” and “c” stations is indicative and will be adapted in function of weather conditions, the time, the turbidity and the remote data.*

irradiance on the deck, $E_d(0^+, \lambda)$, and one measuring the in water upwelling radiance just below the surface, $L_u(0^-, \lambda)$. The immersion factors, as well as the impact of the self-shading are accounted for as in Lubac and Loisel (2007). These measurements will be performed from a small flat-bottomed boat, far from any perturbations of the main boat. $R_{rs}(\lambda)$ is calculated as follows: $R_{rs}(\lambda) = 0.543\ L_u(0^-, \lambda)/\ E_d(0^+, \lambda)$.

Phytoplankton metabolism (for each sampled depth at "S" stations) – Nanoplanktonic (3-20 µm by reverse filtration) community metabolism (GPP, CR and NCP; mmol O_2 m^{-3} d^{-1}) will be estimated from changes in dissolved oxygen concentration (O_2) over 24h in water samples. O_2 concentration will be measured by spectrophotometry (Labasque *et al.* 2004). Seven 100 ml opaque 'dark' and 7 transparent 'light' borosilicate glass bottles will be carefully filled with water from each sampled depth (previously size-fractionated by reverse filtration) and will be incubated for 24h under the same light and temperature than at the respective sampled depths using thermobath circulators. CR and NCP will be calculated from changes in O_2 concentration after incubation under 'dark' and 'light' conditions, respectively, and GPP will be calculated by solving the mass balance equation GPP = NCP+CR.

In situ nanophytoplanktonic PP will be measured by the ^{14}C technique (Steeman-Nielsen 1952). Water samples will be collected and delivered into transparent (light) and black masking tape-covered polycarbonate bottles (60 ml), and will be inoculated with 10 µCi activity of a ^{14}C working solution. These bottles will be then incubated during 2-3h under the same light and temperature than at the respective sampled depths using thermobath circulators. From each sample, the samples will be filtered through 20 µm and collected onto 3 µm 25 mm PC filters to determine particulate primary production. To remove inorganic ^{14}C, the liquid samples will be acidified with 100 µl of 10 % HCl and shaken for 12h, while the filters will be fumed with concentrated HCl (37%). Filters will be stored in liquid nitrogen until laboratory analyses with a scintillation counter.

For small phytoplankton (pico- and nano-phytoplankton), bacterivory will be estimated using dual-labelling pulse-chase method (Zubkov *et al.* 2008, Hartmann *et al.* 2012). 250 mL of seawater will be inoculated with $_L$-[^{35}S] methionine and $_L$-[4,5-^3H] leucine and incubated during 1h. Then, methionine and leucine non-radioactive will be inoculated to the sample to chase pulses of the radioactive amino acids in synthesized microbial proteins by sharply reducing the specific radioactivity of the tracer molecules and incubated during 1h. Subsample of 120 mL will be fixed with PFA 1% after 2h and 5h. Samples will be then stored at 4°C during at least 1 h and will be prefiltered with 3 µm pore-size nylon filter to remove the majority of small SMP. Then, 1.8 mL will be taken from these subsamples and placed into cryovials tube for bacterioplankton cells sorting and frozen (-20°C or -80°C). And 110 mL will be amended with pluronic solution and will be gently filtered through 0.8 µm pore-size polycarbonate filters, diameter 25mm (Whatmann), housed in Swinnex filter holder units (Millipore) with 60-ml plastic syringes. The concentrated cells were washed off filters and resuspended in 1.8 ml of the unfiltered, fixed sample and will be stored at -20°C or -80°C. To check for potential osmotrophic uptake of tracers by phytoplankton during the chase phase, additional so-called chase-pulse experiments will be carried out at two stations (one during Leg1 and one during Leg2). All subsamples will be sorted into three different populations (bacterioplankton, nano- and pico-phytoplanktonic communities) (N.B: radioactive samples can be sorted in the flow cytometry sorter that we are going to use) and then analysed with liquid scintillation counter.

Osmotrophy will be observed here as nitrogen uptake rate (Collos 1987). To estimate the assimilation of organic nitrogen ($^{15}N/^{13}C$ urea), triplicates of 100 ml of seawater will be inoculated with $^{15}N/^{13}C$ labelled urea and incubated during 2-3h. Here, we propose to adapt this method by adding a pr-sonication ?? addition of an anti-biotics?? Thus, once incubation time is over, samples will be xxx in order to remove bacteria on phytoplankton membranes, filtered through 20 µm mesh then 3 µm mesh to remove small SMP and finally, collected onto 1.3 µm A/E glass filters 25 mm diameter and filters will be dried. To estimate the assimilation of inorganic nitrogen (^{15}N nitrate), triplicates of 100 mL of seawater will be inoculated with ^{15}N labelled nitrate and incubated during 2-3h. Once incubation time is over, samples will be filtered through 20 µm mesh and collected onto 1.3 µm A/E glass filters 25 mm diameter and filters will be dried and stored. Later in the laboratory, filters will be analysed with mass spectrometry.

Photo-physiological data of the nanoplanktonic communities will be obtained with a Fluorescence Induction and Relaxation System (FIRe) bench-top fluorometer (Satlantic LLP). Fluorescence transients will be measured after saturating LED pulse and an iterative fitting routine will be applied to the

fluorescence induction curves as a function of the cumulative excitation light to derive the initial fluorescence (F_0), the maximal fluorescence intensity (F_m) and $\rho_{PSII(452)}$. From these parameters, we will be able to estimate the maximal photosystem II (PSII) photochemical efficiency (F_v/F_m) and the functional absorption cross-section for PSII (σ_{PSII}).

Micro- and nanophytoplankton abundance and diversity – Seawater samples will be collected, dispensed in 50 ml plastic bottles and preserved with Lugol solution (1ml l^{-1}) in the dark until abundance analysis in the laboratory with an optical microscopy. Additionally, duplicates of 1.5 ml of seawater sample will be dispensed in 2 ml cryovial tubes with 60 µl of glutaraldehyde 25 %. Samples will be then stored at -80°C until flow cytometry analysis in the laboratory to estimate the small phytoplankton and bacterial abundance.

Four stations (for each kind of coastal waters) on each leg (total 8) of *in situ* waters will be sampled at three different depths, prefiltered with 100 µm mesh (to remove larger organisms), filtrated (polycarbonate filters) onto 20 µm and 2 µm to determine the taxonomic composition of microplanktonic (100–20 µm) and pico- and nanoplanktonic eukaryotes (20–3 µm) and directly frozen at -80°C for molecular high-throughput sequencing (eDNA and global cDNA surveys). eDNA and cDNA will be extracted from the nanoplanktonic fraction (20–3 µm) (cf. molecular biology methods detailed in Alberti et al. 2017). Extracts will then be purified, quantified, and libraries for further Illumina sequencing will be prepared according to sequencing company recommendations. Based on the eDNA extracts, metabarcodes from the hypervariable V4 region of the 18S rRNA gene will be targeted using the primers TAReukFWD1 and TAReuk- REV3 (Stoeck et al. 2010). Metabarcodes will then be cleaned, filtered and sorted using the DADA2 pipeline (Callahan et al. 2016) and taxonomically assigned using the PR2 database (Guillou et al. 2013). From there, three distinct abundance matrices will be built and analysed using numerical ecology analyses (R packages). Metabarcodes will allow to unravel the composition and changes in the total planktonic community structure, and a specific focus will be done on the phytoplanktonic actors. Based on abundance matrices and (semi)quantitative parameters, we will try to detect the main lineages statistically linked to high bacterivory rates and to detect key actors of such subcommunities (Guidi et al. 2016).

Based on the cDNA extracts, a metatranscriptomic survey will be processed as described in Carradec et al. 2018. For taxonomic assignment of the transcripts, BLAST will be used against reference databases (e.g. MATOU catalogue (Carradec et al. 2018)). For functional assignment, coding domain prediction will be conducted with Transdecoder (Haas et al. 2013) to obtain peptide sequences of corresponding domains. From there, abundance matrices (one based on transcripts, one based on functions) will be built and processed through numerical ecology analyses and cooccurrence networks. The composition, structure, interactions and changes in the active functions, as well as the main function linked to bacterivory in the ecosystem will be explored. Based on previous *in silico* hypotheses (Burns et al. 2018), we will notably look for the expression of eukaryotic genes supposedly involved in phagotrophy.

Bacterial abundance, production and grazing – To estimate bacterial abundance (BA), samples will be collected and fixed with glutaraldehyde and stored in liquid nitrogen until laboratory analyses with a flow cytometer. Bacterial production will be measured from radioactive leucine incorporation (Kirchman et al. 1985). Samples will be inoculated with ^{3}H-leucine and incubated during 1h to 10h in the dark at *in situ* temperature. After incubation, samples will be killed using formalin and stored in liquid nitrogen until laboratory analyses with a scintillation counter. For grazing-dilution experiment, seawater will be filtered using GFF and 0.2 µm filters and mixed to different volumes (100%, 60% and 20%) of nanoplanktonic communities (< 20 µm). Individual samples will be sampled prior to (t_0) and at the end (t_{24h}) of the incubation period (*in situ* temperature and light to determine BA, phytoplanktonic abundance and chl-*a* concentration (see details above).

7.3. Planning

The **first year (2021)** of the project will be devoted to prepare the material, do the cruise (Sept. 2021) and do the analysis that have to be done in laboratory (salinity, turbidity, nutrient, POC, PIC, CDOM, chl-*a*, PP, phagotrophy, osmotrophy, microbial abundance, metabarcoding). During the **second year (2022)**, we will do the analysis that will remain to be done and present our results at national and international conferences. We expect to publish the obtained results in the next couple of years after the project.

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9. Résultats attendus

This project will allow improving our knowledge on the role of mixotrophic nanophytoplanktonic communities on microbial loop functioning in natural ecosystems and to better understand the productivity of coastal ecosystems under environmental limitations. The results of the LINKS-2 project will allow first to settle methodology for estimating phagotrophy on natural nanophytoplanktonic communities and secondly, to estimate the effect of environmental limitation such as light and nutrient on mixotrophic nanophytoplankton communities in a coastal stable ecosystem. More precisely, this project will allow:

- Assessing the influence of environmental limitation on the nutritional modes of nanophytoplanktonic communities.
- Observing the possible relationship between planktonic metabolism and photophysiology and the nutritional preference of nanophytoplanktonic communities present in a natural limited environment.
- Estimating the importance of the mixotrophic nanophytoplankton in the planktonic communities of this natural ecosystem under variable limitations.

A better understanding of these processes will be invaluable for a better modelling of the biogeochemical cycles. Our multidisciplinary approach will bring researchers in biogeochemistry, bio-optical, genetics, remote sensing and biochemistry from Dyneco/Pelagos, LOG, SU and USP, building new collaborations.

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11. Participation effective, prévue ou envisageable à d'autres programmes de recherche régionaux, nationaux et européens sur les mêmes problématiques

As already mentioned previously, research activities to resolve and better understand mixotrophic phytoplankton and their role in the microbial loop functioning are generally already funded by existing or future projects from ANR-LABX and regional programs. Here we list and briefly describe these programs:

- Post-doctoral fellowship co-funded by Ifremer and Britany region (2020-2022; supervisor: A. Regaudie de Gioux): During this post-doctoral fellowship, we will observe and estimate the importance of the phagotrophy for a mixotrophic phytoplankton species, *Alexandrium minutum,* under different environmental limitation *in vitro,* using monospecific cultures and *in situ,* during *A. minutum* blooms (Bay of Brest).

- ANR-LABX-19 PROMO (2018-2019, PI: A. Regaudie de Gioux): The main PROMO objective is to estimate the relative part of the nutritional mode (phototrophy, phagotrophy and osmotrophy) of a mixotrophic phytoplankton species, *Alexandrium minutum*, and how these nutritional modes vary under light limitation.

- ANR-LABX-18 REMEDIOS (2018, PIs: C. Jauzein and A. Regaudie de Gioux): During REMEDIOS, we estimated the nutritional fluxes (i.e. osmotrophy, phagotrophy by bacterivory and phototrophy) of coastal planktonic communities composing “Thin Layers of Phytoplankton” (TLP) in the Spanish Rías. We observed that previous to a planktonic bloom (nutrient supply increasing), nanophytoplankton community showed lower bacterial ingestion rates in the surface waters than in deeper waters and that during and after a planktonic bloom (nutrient supply decreasing), nanophytoplankon community showed higher bacterial ingestion rates above Deep Chlorophyll Maximum (DCM) than below.

12. Compléments

None.

Ressources nécessaires à la réalisation du projet

(1) Equipements disponibles pour la réalisation du projet

Dyneco-Pelagos: fluorometer, UV spectrophotometer, Bran Lebe AA3 autoanalyzer, scintillation counter, flow cytometer, epifluorescence and optical microscope, pre-PCR and PCR’s lab, bioinformatic cluster.

LOG: Trios hyperspectral radiometers, spectrophotometer, flow cytometer, epifluorescence and optical microscopes, SEM scanning electron microscope.

CEBIMar: Fluorescence Induction and Relaxation System (FIRe) bench-top fluorometer, WPI UltraPath liquid waveguide spectrophotometer.

SU: Bioinformatic cluster

(2) Co-financements attribués, en cours d'attribution, ou demandés dans le cadre d'autres programmes

A post-doctoral fellowship (2020-2022; supervisor: A. Regaudie de Gioux) has been approved by Ifremer and the Britany region. This post-doctoral fellow will observe and estimate the importance of the phagotrophy for a mixotrophic phytoplankton species, *A. minutum,* under different environmental limitation *in vitro,* using monospecific cultures and *in situ,* during *A. minutum* blooms in the Bay of Brest (France).

The *in-situ* experiments are common to the preliminary tests of the phase 1 of the LINKS project. Additionally, a Master student will be working on the bioinformatic, ecological and biostatistics analyses in the framework of master internships funded by the Institut Universitaire Français (project TROPHOMICS, 2020-2025, PI L. Bittner)

(3) Ressources demandées pour la réalisation du présent projet

❖ **Fonctionnement : *13570 euros***

2021: 13570 euros

- N liquid: 3*37 L (recharge before the cruise and after the cruise; total 2100 €)
- POC, PIC, CDOM and Chl-*a*: reactive (HCl, acetone, methanol: 200 €), material (25 mm GFF filters, 25 mm A/E filters, 3um 25mm PC filters, 2 ml cryovials: 500 €)
- Nutrients: material (Nalgene 60ml HDPE, Nalgene 15 ml HDPE, minisart 0.2um NML: 350 €)
- Planktonic metabolism: reactive (NaI, NaOH, $MnCl_2$, KiO_3, H_2SO_4; total 700 €), inverse filtration system (300 €)
- ^{14}C-PP: radioactive isotope ($NaH^{14}CO_3$; 700 €), reactive (scintillation liquid, PBS, HCl 37%; total 850 €), material (scintillation vials, filter 0.22 µm 25 mm; total 500 €)
- Bacterivory: radioactive isotopes (^{35}S-methionine, ^{3}H-leucine; total 3610 €), reactive (leucine, methionine, PFA 37%, pluronic solution, PBS, fluorescein, SYBR-Green; total 780 €), material (criovials, 0.8 µm PC filter, fluorescent beads; total 500 €)
- Osmotrophy: reactive (^{15}N/^{13}C urea, ^{15}NO3; total 600 €), material (filter A/E, 170 €), antibiotics (60 €)
- Biodiversity: reactive (lugol, glutaraldehyde 25%; total 150 €), material (50 ml bottles, 20 µm and 2 µm PC filter; total 500 €)
- Bacterial activity: radioactive isotope (^{3}H-leucine 700 €), reactive (glutaraldehyde, formaline: 100 €), material (criovials, filters GFF: 200 €)

❖ **Missions : *11000 euros***

2021 : 9500 euros

- Flights tickets (return-way): from France to French Guiana (9* 800 €; total: 7200 €), from Brazil to French Guiana (2300 €)

2022: 1500 euros

- Participation to an international conference

❖ **Analyses : *9165 euros***

- Nutrient analyses: 5 € / parameter / sample (5*5 parameters*10 samples *3 depths *3 triplicates, total 2250 €)
- HPLC analyses: 15 €/ sample (15*10*3, total 450 €)
- Sorter analyses: 10 € / sample (10*10 stations*3 depths*2 duplicates, total 600 €)
- Cytometry analyses: 7 € / sample (7*10 stations*3 depths*2 (phytoplankton and bacteria analyses)*2 duplicates, total 840 €)
- Mass spectrometry (EA-IRMS) analyses: 10.5 € / sample (10.5*10 stations*3 depths*3 triplicates, total 945 €)
- Metabarcoding sequencing production: 30 € / sample (30*8 stations*3 depths, total 720 €)
- Metatranscriptomic sequencing production: 140 € / sample (140*8 stations*3 depths, total 3360 €)

❖ **Campagne en mer**

The ship time ANTEA (INSU) has been accepted by the CNFC. After talking to the ANTEA's calendar responsible, the ANTEA will be present in Antillas-Guyana waters from mid-April 2021 to mid-September 2021. The ANTEA is not expected to go in these waters in 2022 and nothing is sure for 2023.

❖ **Demandes de label pour des ressources complémentaires**

TABLEAU RECAPITULATIF DU BUDGET EN € A REMPLIR OBLIGATOIREMENT EN COHERENCE AVEC LE TABLEAU DU FORMULAIRE EN LIGNE

Pour les projets multi-actions, les demandes budgétaires à chaque action (IMAGO, CYBER, CHAT, MANU, GMMC) devront être identifiées et différenciées dans le tableau

	Coût total	**Co-financements** (préciser la source, INSU ou pas)				**Demande LEFE**	
		Acquis année 1	Acquis année 2	Demandés Année 1	Demandés année 2	Demande LEFE année 1	Demande LEFE année 2
Missions	11000	0	0	5500 (FMAC)	4000 (ISBlue)	0	1500
Equipement *(< 50 k€)*	0	0	0	0	0	0	0
Fonctionnement	13570	0	0	0	0	13570	0
Moyens nationaux	0	0	0	0	0	0	0
Analyses *(à détailler dans tableau ci-dessous)*	9165	0	0	0	0	0	9165
Petit équipement *(<15k€)*	0	0	0	0	0	0	0

Récapitulatif (doit correspondre aux cases du tableau du formulaire informatisé)

Total financements déjà acquis/en cours d'acquisition (1) en € : 4000 € (we expect to submit a proposal with ISblue call for oceanographic cruise 2021 to helping financing four flight tickets to French Guiana for DYNECO/PELAGOS's team participating to the cruise); 5500 € (we expect to receive some funds from the FMAC (fonds mutualisé d'accompagnement financier des campagnes) to help financing the rest of the flight ticket)

Total financements demandés au LEFE (2) en € : 24235 €

Coût total du projet (1) + (2) en €: 33735 €

Annexe : Budget détaillé des analyses prévues (sur le terrain ou en laboratoire)
(donner dans le dossier scientifique la justification du coût unitaire des analyses)

POSTE	Nombre de mesures	Prix unitaire (€)	Montant total (€)
Nutrient analyses: LINKS cruise	90	25	2250
HPLC analyses: LINKS cruise	30	15	450
Cytometry analyses: LINKS cruise for phytoplankton + bacterial abundance	120	7	840
Sorting analyses: LINKS cruise for bacterivory (small phytoplankton)	60	10	600
Mass Spectrometry analyses: LINKS cruise for osmotrophy	90	10.5	945
Metabarcoding sequencing: LINKS cruise for biodiversity	24	30	720
Metatranscriptomic sequencing: LINKS cruise for biodiversity	24	140	3360
		TOTAL	9165