PROGRAMME PREVISIONNEL

9h30 – 10h15 : Accueil autour d'un café

10h15 – 10h20 : Mots d'accueil

10h20 – 12h00 : 8 Présentations (10min + 2min questions)

12h00 – 13h00 : AG SPF

13h00 – 15h00 : Dégustation de produits à base d'algues par Scarlette le Corre (https://www.alguerie.com/) et Akira Peters (http://www.bezhinrosko.com/le-santec.html).

Déjeuner libre

15h00 – 16h00 : 5 Présentations (10min + 2min questions)

16h00 – 16h50 : Session Poster (6 Posters)

16h50 – 17h30 : 3 Présentations (10min + 2min questions)

10h20

Deciphering molecular cross-talks during kelp-endophyte interactions

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Kelps are important primary producers in temperate to cold northern hemisphere shores and key species in the formation of coastal marine habitats. As sessile organisms, these large brown algae are able to actively respond to external biotic stress by regulating transcription and metabolic pathways in order to cope with various associated micro- and macroflora. In kelps, small endophytic filamentous algae are known to invade stipes and fronds. For instance, the endophyte *Laminarionema elsbetiae* is highly prevalent in European populations of the sugar kelp, *Saccharina latissima*, but has also been found occasionally in *Laminaria digitata*. The presence of algal endophytes coincides with morphological changes in the hosts- such as dark spots, galls, twisted stipes and deformation of the blades. However, little is known about the molecular and chemical bases of this interaction, its physiological impacts on the hosts and its ecological role. To get further insight into host-endophyte interactions, we set-up an experiment to monitor the impact of the endophyte on growth of laboratory-grown kelp sporophytes. First results revealed that co-cultivation of *L. elsbetiae* does not induce strong oxidative responses of its main host *S. latissima*, neither affect its growth. On the contrary, growth of the occasional host *L. digitata* decreased significantly when co-cultured with the endophyte within less than a week. The molecular detection of endophytes associated with the kelp tissue by qPCR showed a lower prevalence in *L. digitata*, suggesting that defence reactions against *L. elsbetiae* were triggered in *L. digitata*, but not in *S. latissima*. Large-scale transcriptomic analysis of endophyte-induced early responses in both kelp species will help us to decipher the molecular and metabolic cross-talks during kelp-endophyte interactions.

10h33 Proposition d'inclusion des forêts de laminaires à la liste OSPAR, un exemple de soutien de l'expertise scientifique collective à la mise en œuvre des politiques publiques

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La Convention pour la protection du milieu marin de l'Atlantique du Nord-Est (dite "Convention OSPAR") a établi une liste des espèces et habitats menacés et/ou en déclin utilisée afin d'orienter les travaux pour améliorer la conservation et la protection de la biodiversité marine en Atlantique Nord-Est. En 2018, la France a repris une proposition initiale de l'ONG Oceana d'inclure les forêts de Laminaires comme nouvel habitat à la liste OSPAR qui sera présentée aux différents comités de la convention durant le cycle 2018/2019.

Les experts de cet habitat des différents Etats parties de la convention ont été mobilisés pour la rédaction d'un document synthétisant les connaissances disponibles pour permettre de répondre aux différents critères fixés par la Convention (importance globale, importance régionale, rareté, sensibilité, importance écologique, état de déclin, menaces, mesures de gestion existantes et à envisager). Ce document permettra de déterminer, pour chaque région de la zone OSPAR, si l'habitat doit être listé comme en déclin, et d'identifier les menaces qui pèsent dessus.

Une définition plus précise de l'habitat « Kelp forest » a ainsi pu être proposée, basée sur neuf espèces capables de former des canopées, et les connaissances disponibles pour chacune de ces espèces ont été synthétisées pour définir l'état de déclin pour chacune des 5 régions OSPAR.

Cette mobilisation collective est un parfait exemple de l'importance d'implication de la communauté scientifique pour l'appui à la décision et à la mise en œuvre des politiques publiques de conservation de l'environnement.

10h46 Species delimitation and phylogenetic analyses of the red algal genus Pterocladiella (Gelidiales, Rhodophyta) based on five-gene phylogeny

Ga Hun Boo, Line Le Gall

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Pterocladiella is an agar-producing red algal genus comprising 24 species from temperate and tropical seas. A molecular taxonomic study to investigate the species diversity of Pterocladiella at global scale was undertaken based on sequence data. We analyzed five markers (mitochondrial cox1, cob and plastid psaA, psbA, rbcL) from about 200 taxa, including type specimens of equivocal species. Species delimitation approaches were investigated with coalescent analyses based on mitochondrial cox1 sequences and revealed that Pterocladiella contains at least 37 species, confirming occurrence of 21 species among 24 species described in the genus. Thirteen species did not match up to the previously described species. Three previously described species, unavailable in the present study, are required to relate DNA sequences to type bearing names before to formally describe the remaining "molecular species" as new species. The concatenated five-gene phylogeny revealed the monophyly of Pterocladiella including six distinct groups. *Pterocladiella caespitosa* from South Africa, and *P. feldmannii* and *P. hamelii* from Madagascar were placed outside the large clade that includes 34 species, suggesting a southwest Indian Ocean origin of Pterocladiella. The remaining species were divided into five groups (I-V). Groups I and V were cosmopolitan; however, group II occurred from Pacific Ocean, group III in Australia, and group IV on the west coast of America.

10h59 Étude de la dépression de consanguinité chez l'algue brune *Saccharina latissima*

Christophe Destombe, Aurélien Baud, Bertrand Jacquemin, Julien Dhinaut, Jérome Coudret, Stéphane Mauger, Lucie Jaugeon, Myriam Valero

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La sélection artificielle est cruciale pour le développement de l'aquaculture d'algues et contrairement à de nombreuses espèces de plantes terrestres, le processus de sélection des algues marines est encore embryonnaire, en particulier en Europe. Le projet IDEALG a pour objectif de développer la recherche fondamentale pour le processus de sélection en aquaculture d'algues marines en y intégrant des aspects de génétique évolutive. En règle générale, les populations de macroalgues sont caractérisées par un niveau élevé de différenciation génétique, ce qui suggère que les populations sont adaptées à leur environnement local. Dans ce contexte, on peut supposer que les individus issus de croisement en autofécondation ou entre apparentés pourraient présenter des valeurs sélectives faibles en raison de la dépression de consanguinité. De façon similaire, les croisements entre parents trop différents génétiquement pourraient aussi conduire à des faibles valeurs électives en perturbant les complexes adaptatifs. Une distance de croisement optimale est donc attendue comme compromis entre ces deux effets (inbreeding et outbreeding depression). Pour vérifier ces hypothèses, nous avons mis en place des expériences de croisements chez la grande algue brune *Saccharina latissima*. Les effets des différents types de croisements (autofécondation, fécondation entre apparentés et entre individus de populations différentes) et de l'origine des individus (provenant de 5 populations plus ou moins génétiquement différenciées le long des côtes bretonnes) ont été testés en jardin commun (en mer).

11h12 Evolution de la voie de biosynthèse de l'acide abscissique chez les algues brunes

Delphine Negre[1,2], Arnaud Belcour[3], Meziane Aite[3], Loraine Brillet-Guéguen[2], Xi Liu[2], Philippe Bordron[2], Agneszka Lipinska[1], Catherine Leblanc[1], Anne Siegel[3], Simon Dittami[1], Erwan Corre[2], Gabriel Markov[1]

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L'acide abscissique est une phytohormone dérivée du métabolisme des caroténoïdes et impliquée dans de nombreux processus physiologiques chez les plantes terrestres. Chez certaines laminaires, sa présence a été démontrée par profilage métabolique (GC-MS pour trois espèces européennes, LC-MS/MS pour *Saccharina japonica*) mais la voie de biosynthèse correspondante n'a pas encore été annotée dans les génomes séquencés de macroalgues, et son rôle physiologique dans l'induction de la formation de sores fait débat. La reconstruction de réseaux métaboliques à l'échelle du génome chez la laminaire *Saccharina japonica* et chez une ectocarpale filamenteuse, *Cladosiphon okamuranus*, a permis de démontrer que l'essentiel des enzymes impliquées dans la voie de synthèse de l'acide abscissique chez les plantes terrestres sont conservées chez les algues brunes dont le génome est séquencé. En revanche, le locus codant pour l'enzyme catalysant l'étape finale de la voie a subi une duplication en tandem chez l'ancêtre des ectocarpales et des laminaires, suivi d'une perte des domaines centraux et d'une troisième duplication chez *Saccharina japonica*. Ces variations structurales soulèvent de nouvelles questions sur leurs origines et leurs possibles conséquences fonctionnelles sur la biologie des laminaires.

11h25 SeaProbes : développement d'anticorps monoclonaux pour l'étude des parois d'algues brunes

Sonia Kridi[1], Sue Marcus[2], Kevin Hardouin[1], Paul Knox[2], Cécile Hervé[1]

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Les algues brunes représentent une communauté dominante en milieu intertidal côtier. Leurs biomasses sont essentiellement constituées de polysaccharides, et notamment les polysaccharides de paroi peuvent représenter jusqu'à 50% du poids sec de l'algue. Ces parois cellulaires jouent des rôles physiologiques clés chez ces organismes, par exemple dans le développement, l'ajustement osmotique, les réponses de défense. En outre les distances phylogénétiques des algues brunes par rapport aux autres eucaryotes, explique la biochimie unique de ce compartiment. Les principaux polysaccharides constitutifs sont les alginates et les polysaccharides sulfatés contenant du fucose (FCSPs), incluant les fucanes. Les alginates sont largement utilisés comme agent texturant dans l'industrie et les FCSPs possèdent des propriétés biomédicales à exploiter. Malgré l'importance fondamentale et appliquée de ce compartiment, les connaissances sur la paroi des algues brunes sont fragmentées : elles reposent sur des études d'extractions chimiques réalisées à l'échelle de la plante entière, ou sur la purification de polymères d'intérêts. Ces techniques ne permettent pas de comprendre la dynamique des polysaccharides au cours du développement ou de réponses physiologiques, ni de cribler rapidement leur diversité sur des collections d'échantillons. Aujourd'hui nous avons développé dix nouveaux anticorps monoclonaux spécifiques de fractions d'alginates et de fucanes, que nous mettons à disposition de la communauté à travers SeaProbes (http://www.sb-roscoff.fr/en/seaprobes). Divers exemples d'utilisation de ces outils seront illustrés, notamment leur combinaison à des méthodes d'imagerie pour la localisation des polysaccharides de paroi dans des tissus complexes, où leur utilisation conjuguée à des méthodes moyen débit de type glycoarrays pour le criblage d'échantillons.

11h38 Detection of macroalgae by means of germling emergence

Akira F. Peters, Bezhin Rosko, Frithjof C. Küpper

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Germling emergence is a method for the isolation of macroalgae from the bank of microscopic stages present on the natural substratum. It not only allows the study of the hidden generation of seaweeds but also the detection of a size class of algae with thalli too small to be noticed by the common microscopic examination of field material. Germling emergence is also the method of choice for revealing the seaweed diversity of remote places and of sites with limited access time, such as the deeper euphotic zone. Examples will be given of classical and recent findings of such taxa.

11h51 Marie-France Simon 1931/2018

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Née le 2 septembre 1931 M.-F. Simon est décédée le 24 juillet 2018. Ses activités scientifiques et d'enseignement ainsi que des éléments de sa personnalité sont présentés à l'aide de 5 diapositives. Une notice complète est publiée dans le numéro 85 du Journal de Botanique (Décembre 2018).

12h03	AG **Bilan moral** **Bilan financier** **Bilan FEPS** **Lauréats Bourse étudiante SPF 2018**
13h00	Dégustation Produits à base d'Algue (13h-15h) par Scarlette le Corre(https://www.alguerie.com/) et Akira Peters (http://www.bezhinrosko.com/le-santec.html) Déjeuner libre
15h00	Chemical ecology of Benthic HABs: the impact of NW Mediterranean *Ostreopsis cf. ovata* on copepods **Anne-Sophie Pavaux, Julie Rostan, Laurence Guidi-Guilvard, Sophie Marro, Joanna Olivi, Stéphane Gasparini, Rodolphe Lemée** Sorbonne Université, CNRS, Laboratoire d'Océanographie de Villefanche, LOV, F-06230 Villefranche-sur-mer, France Blooms of benthic toxic dinoflagellates genus Ostreopsis have increased in frequency and intensity, notably in the Mediterranean Sea. Adverse effects on Human health of those microalgae have been recorded, with skin/eye irritations or breath difficulties among main symptoms. The toxicity of those dinoflagellates was attributed to the presence of palytoxin and analogs. Deleterious effects of Ostreopsis blooms on marine organisms have already been reported, without knowing if they were due to anoxic/hypoxic conditions or to the presence of toxic secondary metabolites. In this context, the aim of our work was to assess the effect of Ostreopsis cf. ovata on two copepods: *Sarsamphiascus cf. propinquus* (phytal meiobenthic species) and *Acartia clausi* (planktonic species). These two model were exposed, in vitro, to ecologically realistic concentrations of *O. cf. ovata* and *Licmophora paradoxa*, a non-toxic competitive benthic microalga. The potential toxic effect of *O. cf. ovata* was tested on survival rates, fecal pellets production (as a proxy of feeding) and reproduction (using fertility/fecundity ratio). Results indicated that *S. cf. propinquus* was the most tolerant organism to *O. cf. ovata* ever tested (LC50 (48h) > 20 000 cells.mL-1), even if hatching success and egg production were affected by the presence of the toxic dinoflagellate, suggesting a reprotoxicity. *Acartia clausi* was 4 000 times more sensitive (LC50 (48h) < 5 cells.mL-1) than the phytal meiobenthic copepods. These results highlight the necessity to study species-specific response to better understand the effects of *O. cf. ovata* on marine organisms.

15h13

Mediterranean long-term study shows correlation between *Ostreopsis cf. ovata* bloom timing and spring temperature

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Benthic toxic dinoflagellate *Ostreopsis cf. ovata* events are responsible for ecological, sanitary and economic issues in different temperate areas and need better understanding. Every summer, from 2007 to 2017, Ostreopsis cf. ovata blooms have been monitored in Larvotto beach (Monaco, NW Mediterranean Sea), allowing for the achievement of one of the longest time series of Ostreopsis blooms worldwide. Five sites were sampled each year during the bloom period, from mid-June to end August, with collection of benthic cells. Blooms phenology (timing, length and maximum cell abundance) was highly variable across the study period. Variations of net growth rate estimated during the phases of bloom development were analyzed as a function of Sea Surface Temperature (SST). Surprisingly, the highest growth rates were not associated with the maximal temperature records (27.5°C) but were estimated when temperature ranged between 21°C and 25°C. Many authors suggested that global warming might have influenced Ostreopsis expansion from tropical areas to temperate waters, such as the Mediterranean Sea. Current results do not directly support this hypothesis, but suggest a more complex role of temperature in bloom dynamics than a simple facilitation factor for algal growth, at least in temperate areas. In order to characterize in more details the role of SST on O. cf. ovata bloom phenology, SST anomalies were calculated as differences between temperature values and the mean SST value over the 11 years of survey. A positive correlation (Spearman test, $r_s = 0.766$, $p < 0.01$) was observed between positive anomalies of SST and the bloom precocity. This result indicates that blooms seem to occur earlier in the season when spring is warmer. The relation between SST and net growth rate as well as between SST anomalies and precocity of the blooms should be of great interest for modelers. Predicting the distribution and the phenology of Ostreopsis in European coastal waters is a crucial challenge, especially in a context of global warming.

15h26

Macroalgues et production d'actifs cosmétiques

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Les macroalgues synthétisent de nombreuses molécules comme polysaccharides, protéines et lipides, connues pour leurs potentielles applications cosmétiques. Ces composés algaux peuvent être utilisés comme ingrédients fonctionnels avec des propriétés texturantes mais également comme actifs avec des propriétés hydratante, antioxydante, anti-âge ou anti-inflammatoire (Deslandes & Bodeau, 2007; Guglielmo & Montanari, 2008; Majmudar, 2012). A l'heure actuelle peu d'études ont été reporté pour les macroalgues *Ulva sp.* (Chlorophyta, Ulvales) et *Solieria chordalis* (Rhodophyta, Gigartinales). Dans nos études, une technologie verte d'Extraction Assistée par Enzyme (EAE), a été appliquée pour la bioconversion de *Ulva sp.* et l'obtention de fractions riches en oligo/polysaccharide et protéines. Les traitements par endo-protéases ont conduit à un rendement d'extraction supérieur par rapport au contrôle (sans enzyme). Des extraits riches en polysaccharides et protéines préparés à partir de *Ulva sp.* ont montré une capacité à moduler l'activité cellulaire des fibroblastes humains, stimulation de leur prolifération et augmentation de leur activité métabolique. Ces résultats montrent le potentiel des macroalgues et de l'EAE pour produire des fractions actives en cosmétique en fonction de la biomasse algale, de la paroi cellulaire et de la sélectivité de l'enzyme utilisée. Le maintien de l'équilibre du microbiote cutané est essentiel pour la santé et la beauté de la peau. De futures applications cosmétiques des extraits en lien avec le microbiote cutané ainsi que d'autres activités sur le réseau matriciel et le renouvellement épidermique seront évaluées.

Références : Deslandes, E., & Bodeau, C. (2007). Composition cosmétique comprenant un extrait d'algue rouge comprenant une association de floridoside et d'acide iséthionique. EP 1 743 628 A1. Guglielmo, M., Montanari, D. (2008). Cosmetic composition with a lifting effect for sustaining relaxed tissues. Patent WO 2008146116 A2. Majmudar, G. (2012). Compositions of marine botanicals to provide nutrition to aging and environ¬mentally damaged skin. US 8318178 B2. Mots clés : macroalgues, *Ulva sp.*, *Solieria chordalis*, extraction assistée par enzymes, polysaccharides, protéines, applications cosmétiques, microbiote cutané

15h39

Involvement of allelopathically active metabolites on the surface of the cultivated *Halymenia floresii* in the biofouling phenomenon

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During the experimental culture of *Halymenia floresii* under IMTA (Integrated MultiTrophic Aquaculture) the surface of the *H. floresii* was observed to be remarkably free from settlement by fouling organisms. The presence of *Halymenia* appeared to limit the establishment of opportunist green algae and the colonisation of barnacles usually disturbing the cultures. This ecological phenomenon could reveal the release of allelopathic active compounds interfering with the settlement and growth of competitors. To study this phenomenon, *H. floresii* was cultivated under controlled environmental conditions and analyzed for its surface chemical defense metabolites. The surface-associated metabolites were extracted by the method of DIP (Nys *et al*., 1998), using different immersion solvents with increasing polarity and immersion periods. Using epifluorescence microscopy, the suitable immersion solvent and time were determined. N-hexane was the solvent that worked best at any of the immersion period tested. The chemical profiling of the different immersion solvents were performed by gas chromatography mass spectroscopy. The mean surface-area concentration of *H. floresii* (n=2) was observed to be 600 ng cm^{-2} which contrasted with the concentration of the whole-cell metabolites, 4.5 µg mg^{-1}. The surface of the healthy and bleached *H. floresii* (n=3) were observed under scanning electron microscopy (SEM) at different angles. The topography of the bleached *H. floresii* was completely disrupted by the deterioration of surface mat in comparison with that of the healthy surface. Further studies are in progress to better understand the chemical defense on the surface of *H. floresii.*

KEY WORDS: *Halymenia floresii*, IMTA, surface-area concentration, SEM, surface-associated metabolites, whole-cell metabolites.

15h52	## Contrôle de la réorientation métabolique chez la diatomée *Phaeodactylum tricornutum* par les facteurs de transcription **Thurotte A.[1], Scarsini M.[1], Moreau B.[1], Lagarde F.[2], Hu H.[3], Schoefs B.[1], Marchand J.[1]** **1** Metabolism, Bio-engineering of Microalgal Molecules and Applications (MIMMA) Mer Molécules Santé, IUML FR 3473 CNRS, Le Mans University, Le Mans (France) **2** Institut des Molécules et Matériaux du Mans (IMMM, UMR CNRS 6283), Université du Maine, Avenu Olivier Messiaen, Le Mans F-72085, France. 3Laboratory of Algal Biology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China Les lipides des diatomées constituent des molécules d'intérêt pour la production de biocarburants. Ils sont notamment synthétisés lorsque la micro-algue rencontre des conditions défavorables (stress), telles qu'une carence en azote. La croissance est alors ralentie voire arrêtée. Cette réorientation métabolique intervient à différents niveaux de régulation, et notamment sur la transcription des gènes codant pour les enzymes impliqués dans le métabolisme des lipides. Le travail présenté est motivé par la volonté d'identifier des FTs impliqués dans le contrôle de la synthèse des lipides, afin de créer de nouvelles souches dont le métabolisme sera orienté vers la production de lipides, sans pour autant sacrifier la croissance cellulaire. Pour identifier les FTs clefs chez la diatomée *Phaeodactylum tricornutum* Bohlin, une approche interdisciplinaire incluant bioinformatique, biologie moléculaire et physiologie a été adoptée. (1) Afin d'identifier des FTs candidats in silico, les séquences de FTs déjà décrits comme impliqués dans le métabolisme des lipides chez certaines plantes terrestres et microalgues ont été collectés puis comparés avec l'ensemble du génome de *P. tricornutum*. (2) Pour vérifier in vivo que des changements dans la quantité des FTs identifiés précédemment sont bien observés avant l'accumulation de lipides, l'abondance relative d'ARN messagers codant pour certains FTs d'intérêt a été étudiée en carence d'azote par PCR quantitative. (3) Des souches dont l'abondance d'un FT d'intérêt précédemment identifié sera modifiée (surexpresseur /silencing) sont en train d'être crée. Les souches modifiées seront criblées lors d'une carence en azote par spectroscopie infrarouge à transformée de Fourier.
16h05	## Session pour les Poster (45 min)

16h50

Dissipation of excess energy in the green microalga *Chlamydomonas reinhardtii*

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In the environment, light input fluctuates and frequently overwhelms photosynthetic CO2 fixation. Along the linear electron flow through photosystem II (PSII), cytochrome b6f, photosystem I (PSI) and RuBisCO, excess energy is dissipated by several photoprotective mechanisms. In cyanobacteria, photoprotection comprises the dissipation of excess electrons onto oxygen. This involves a unique pair of flavodiiron (Flv) proteins whose homologs in bacteria and archeae contribute to O2 or NO detoxification. Homologs to cyanobacterial Flv genes were also found in eukaryotic genomes. Interestingly, in microalgae, a photoreduction of O2 was evidenced 40 years ago but the catalyst remained unknown. To investigate Flv function in photosynthetic eukaryotes, we ordered mutant strains of Chlamydomonas reinhardtii (a model unicellular chlorophycea) in the CLiP library. In flv mutant strains, we observed at the onset of light an excess excitation pressure as compared to wild-type (WT) and a bottleneck located downstream the PSI. The flv mutants also exhibited decreased O2 photoreduction and higher sensitivity to short light fluctuations. Slower generation of transmembrane proton gradient was evidenced by slower fluorescence quenching and decreased electrochromic shift. Altogether, these data suggest that in *C. reinhardtii* Flv proteins catalyze a massive electron flow to O2 which is crucial to dissipate excess energy input and to rapidly generate proton gradient under fluctuating light. In addition to concomitant studies in bryophytes, this argues for conserved Flv function and alternative electron flow to O2. However, the loss of Flv in angiosperm remains very intriguing and Flv have received little attention in the secondary plastid lineages.

17h03

A bHLH-PAS protein regulates light-dependent diurnal rhythmic processes in the marine diatom *Phaeodactylum tricornutum*

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Periodic light-dark cycles govern the timing of basic biological processes in organisms inhabiting land as well as the sea, where life evolved. Although prominent marine phytoplanktonic organisms such as diatoms show robust diurnal rhythms in growth, cell cycle and gene expression, the molecular foundations controlling these processes are still obscure. By exploring the regulatory landscape of diatom diurnal rhythms, we unveil the function of a *Phaeodactylum tricornutum* bHLH-PAS protein, PtbHLH1a, in the regulation of light-dependent diurnal rhythms. Expression of PtbHLH1a mRNA phases towards the end of the light period, this peak being adjusted according to photoperiod changes. Ectopic over-expression of PtbHLH1a results in lines showing a phase shift in daily chorophyll synthesis compared to wild-type cells, and with altered rhythmic gene expression. These phenotypes were also observed when cells grew in absence of ligth-dark alternations, showing that the regulation of rhythmicity by PtbHLH1a is not directly dependent on light inputs. PtbHLH1a homologs are widespread in diatom genomes which may indicate a common function in many species. This study adds new elements to understand diatom biology and ecology and offers new perspectives to elucidate timekeeping mechanisms in marine organisms belonging to a major, but underinvestigated branch of the tree of life.

Poster

Synergistic effects of Triton X-100 and ultrasound on *Gracilaria gracilis* protein extraction

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Gracilaria gracilis, as others rhodophytes, present a high protein yield when compared to other macroalgae or vegetal species (i.e. XX% of dry weight). These proteins constitute a source of valuable compounds like the R-Phycoerythrin (R-PE). But there is a paucity of information regarding the diversity of the protein or peptide species that could be valorized from this red algae. Protein extraction from *Gracilaria gracilis*, like others macromolecules from seaweeds in general, is a long and expensive process. This hampers the study and valorization of this proteome. Moreover, a low temperature (i.e. close to 4°C) is mandatory to limit protein degradation, particularly by endoproteases, during the extraction process. We describe here an ice-cold process aiming to efficiently extract hydrosoluble proteins and peptides from *Gracilaria gracilis* by a combined action ultrasound (US) and the surfactant Triton X-100 (TX100). Results indicate that, separately, either US or TX100 significantly extracted proteins. Interestingly, the association of both US and TX100 induced a synergistic effect on protein extraction. The process has been optimized via an experience plan which eventually improved the protein extraction yield by a factor 3.84 when compared to a control maceration. This allows recovering an extract with 4.5g/L of hydrosoluble proteins.

Poster

Green life in the dark

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Although of fundamental importance for many phototrophs on Earth, the physiological mechanisms and molecular underpinnings that allow survival over long periods of dark remain a mystery. Diatoms, the dominant oceanic eukaryotic photosynthetic organisms, specifically *Fragilariopsis cylindrus* (polar pennate diatom with a sequenced genome) will serve as model to characterize the physiological, cellular, genomic, epigenomic, and metabolic state of cells during prolonged darkness and the return of light. Complementary expertise in our team (culturing of polar species, optics, photochemistry, genomics) will allow us to identify key adaptive mechanisms used by diatoms. Realistic light transitions from fall to early spring will be simulated to grow *F. cylindrus* under stable nutrient and cold temperature conditions. Cellular energy flow and allocation will be monitored before, during and after a simulated 6-month polar night: light absorption coefficients and pigment composition, absorption cross-sections, electron flows, concentrations and activity of RUBISCO, rates of C-fixation, respiration, and reduction level of storage compounds. *F. cylindrus* cells will also be examined for their transcriptional and translational activity, and will be subject to transcriptome and metabolome analyses. Mass spectrometry and immunoblotting performed on chromatin will identify major changes in DNA methylation and histone tail modifications. Chromatin and gene expression states arising in response to prolonged darkness and the return of light will be defined, informing metabolic maps and physiologies. In parallel, organellar structures will be assessed by electron microscopy. The results will have general significance for the many other organisms that survive long periods in the dark while remaining photosynthetically competent.

Poster

Allelopathic interactions of a toxic dinoflagellate on other phytoplankton species

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Despite the importance of phytoplankton in aquatic ecosystems, the level of understanding of the dynamics and structure of phytoplancton communities is limited. Different parameters could contribute to the structure of these communities, one of them is allelopathy. My PhD focuses on the latter: the species release secondary metabolites into the medium that have inhibitory effects on target species, these interactions are known as allelopathy. One of the main targets of these metabolites is the photosynthetic apparatus of competitors, making photosynthesis an ideal probe to study allelopathy. Little is known about allelopathy in marine microalgae, this is due to a number of methodological difficulties, including the lack of information on the relative physiologies of two microalgae in mixture. We chose a new approach to explore these interactions based on a physical phenomenon, the electro-chromic shift (ECS) of photosynthetic pigments, when subjected to the electric field generated across the thylakoid by photosynthesis. The ECS shows a different spectral signature in each photosynthetic microalgae clade, allowing the extraction of the photosynthetic responses of each species in an assembly. We can therefore measure the photosynthetic activity of a species A alone or in a mixture with species B, and highlight an allelopathic interaction targeting photosynthesis. In addition, all the complexes involved in photosynthesis contribute to the generation of a trans-thylakoïdal electric field (PSII, PSI, cytochrome b6f) or to its consumption (ATP-synthase). This allows to identify the target of the released metabolite on the photosynthetic apparatus. Thanks to the ECS we observed that the photosynthesis of some diatoms and prasinophytes was fully inhibited when mixed with the dinoflagellate *Amphidinium carterae*. The mechanism of inhibition seems to affect the electrochemical gradient of H+ that lead to the production of ATP by the ATP-synthase during photosynthesis.

Poster

A mutagenesis study of specific mRNA/OPR protein interactions in the chloroplast of *Chlamydomonas reinhardtii*

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During the endosymbiotic evolution of the chloroplast, many plastidial genes were lost or transferred to the nucleus. The chloroplast of Chlamydomonas reinhardtii retains roughly 100 genes. The various sub-units of photosynthetic complexes are encoded in part in the nucleus, in part in the plastidial genome. To achieve their correct assembly, the expression of the two genomes needs to be coordinated. Trans-acting factors (TAF), encoded in the nucleus, are proteins which can bind specifically to an organellar mRNA and act upon it. Among those TAF, the octotricopeptide repeat (OPR) family is abundant in green algae. The effects of deletion of specific OPR factors appear strictly specific to their targets in *C. reinhardtii*. The OPR repeat is a degenerate motif of 38 amino-acids, folding into a tandem of antiparallel α-helices which can bind to RNA. A repeat is predicted to interact with one specific nucleotide thanks to specificity-conferring residues at defined positions. This recognition mechanism is called the “OPR code”. A draft “OPR code” was established in our laboratory, using known OPR protein/mRNA couples. To confirm and build on the draft “OPR code”, I proceed in vivo, by mutating the plastidial targets of OPR factors to disrupt the binding and then try to restore the OPR protein/mRNA interaction by mutating the specific residues in the corresponding OPR. Trying to do so, I highlighted that OPR protein/mRNA interactions seem very robust, challenging our view of how the specificity of control of gene expression by the OPR factor is established.

Poster

Regulation of the xanthophyll cycle in diatoms

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Diatoms are the dominant primary producers in highly mixed waters, characterized by fluctuating light conditions. To avoid oxidative damage during exposure to high light, diatoms possess a very efficient fast response: the dissipation of excess light energy as heat, a mechanism visualized as the Non-Photochemical Quenching of chlorophyll a fluorescence (NPQ). In most diatom species, NPQ is proportional to the concentration of the xanthophyll pigment diatoxanthin, which is produced during the xanthophyll cycle. In saturating light conditions, the epoxidized xanthophyll diadinoxanthin is converted into its de-epoxidized form diatoxanthin, whereas the opposite reaction is observed in light-limiting conditions. Despite the central role of the xanthophyll cycle in light responses, the regulation of the rates of the de-epoxidase and epoxidase enzymes is not yet well understood. For example, most models still attribute a passive role to the epoxidation reaction despite the observations that its rate changes significantly with light conditions. We first measured the light regulation of the epoxidation reaction in the model diatom *Phaeodactylum tricornutum*, in conditions where the de-epoxidation step was inhibited with dithiothreitol. We could confirm that the epoxidation reaction has an optimum in light-limiting conditions, whereas its activity is strongly inhibited in saturating light conditions. We are now investigating the light regulation of the rates of the two enzymes by modeling the kinetics of NPQ in different light conditions.

Poster

Evidence supporting an antimicrobial origin of organelle transit peptides

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Transit peptides (TPs) are cleavable N-terminal extensions involved in the subcellular targeting of most nuclear encoded proteins localized in either the chloroplast or the mitochondria. Different TPs are very divergent in primary sequence, but share a propensity to form amphiphilic alpha-helix stretches when in contact with a membrane. In this respect they are very similar to a certain antimicrobial peptides (AMPs), which are used by most eukaryotes as well as some bacteria to kill microbial antagonists. In a neat analogy to post-import degradation of TPs, certain bacterial defense mechanisms against AMPs involve uptake and intracellular destruction of the attacking peptide. Might TPs be the result of an arms-race involving AMPs between the ancestors of mitochondria or chloroplasts and their hosts during the early phases of endosymbiosis (Wollmann 2016)? In support of this hypothesis, we show that (i) AMPs and TPs share key physic-chemical properties that set them apart from other classes of peptides, and that (ii) AMP peptide sequences target Venus fluorescent protein to the chloroplast or the mitochondria when expressed in *Chlamydomonas reinhardtii*. Wollman, F.-A. An antimicrobial origin of transit peptides accounts for early endosymbiotic events. Traffic 17, 1322-1328 (2016).

Poster

Evaluating the importance of cyclic electron flow around PSI in microalgae

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It is commonly assumed that in plants and green algae, cyclic electron flow (CEF) around photosystem I (PSI) plays a crucial role in optimizing photosynthesis. It allows the PSI and the cytochrome b6f to contribute to the electrochemical proton gradient, with no net product. It is therefore believed to have two main roles, (i) regulating the photosynthetic control and non-photochemical quenching in photosystem II (PSII), and (ii) providing the extra ATP required for carbon fixation. However, several decades of research did not provide a clear-cut answer about the mechanism, extent, regulation and conservation of CEF among different photosynthetic clades. This is mostly due to the absence of a consensus method to estimate this flow in physiological conditions. The most accepted approach compares the quantum yield of PSII and the one of PSI, accessible through absorption changes associated to the redox state of P700. But we could show that the latter method tends to underestimate the quantum yield of PSI, leading to aberrant conclusions regarding CEF. We propose an alternative method based on the electrochromic shift of photosynthetic pigments, which allowed us to estimate the extent and modes of regulation of CEF in various phytoplankton clades, in the laboratory and in the field.

Poster

Facilitation écologique entre deux espèces de microalgues *Chlorella sorokiniana* et *Scenedesmus acutus*

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Une facilitation écologique entre deux espèces de microalgues *Chlorella sorokiniana* et *Scenedesmus acutus*, classiquement observées dans les eaux fortement anthropisées, riches en ammonium (NH4+), est démontrée. En effet, des expérimentations en laboratoire ont montré que la croissance de *Scenedesmus acutus* est inhibée pour les très fortes valeurs d'ammonium (NH4+), à cause de la toxicité en ammoniac (NH3), ce qui n'est pas le cas pour *Chlorella sorokiniana*. Cette dernière espèce possède toutefois une affinité pour le NH4+ plus faible que *Scenedesmus acutus*. Une approche mathématique a été réalisée à partir de ces résultats expérimentaux et a permis de démontrer la succession écologique de ces espèces lorsqu'elles sont présentes ensemble. Ainsi lors d'apports très riches en NH4+, la croissance rapide de *C. sorokiniana* colonise en premier le milieu, réduisant la teneur en NH4+ (et donc en NH3) permettant à *S. acutus* de croître (facilitation) et même de gagner la compétition pour ce nutriment.