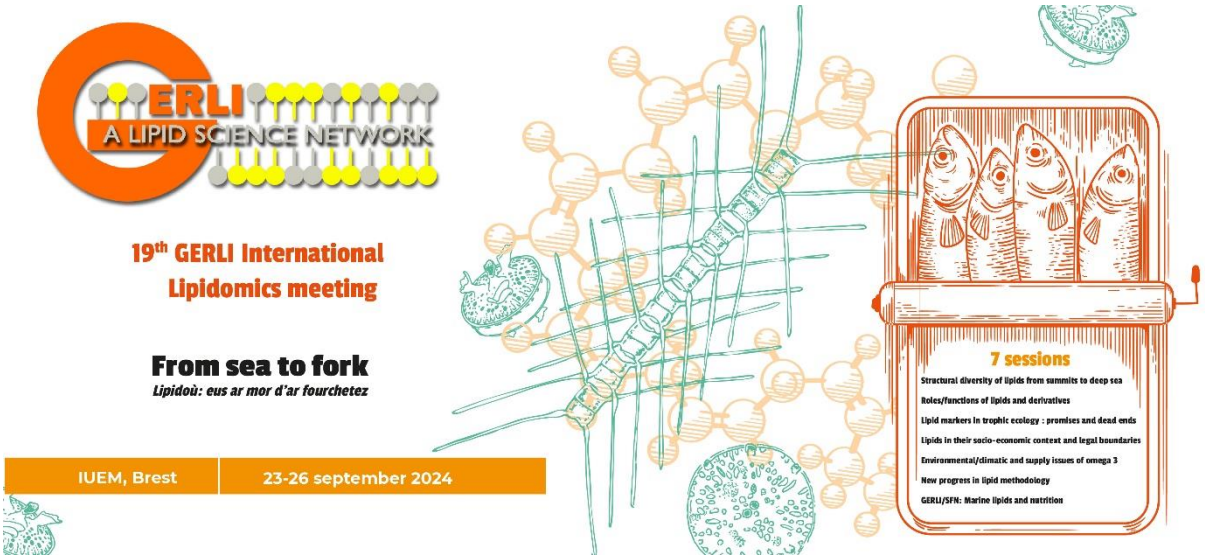

BOOK OF ABSTRACTS

THESIS PRIZES

Glycerol-3-phosphate and Phosphoethanolamine homeostatic switch triggers senescence by rewiring lipid metabolism.

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Cellular senescence affects many physiological and pathological processes and is characterized by durable cell cycle arrest, an inflammatory secretory phenotype and metabolic reprogramming. This latter is characterized by biosynthetic pathways reshuffling and lipid remodeling. Hence, finding common and specific metabolic signatures in senescence could provide targetable vulnerabilities to alleviate senescence burden in the organism. During my PhD project, by using dynamic transcriptome and metabolome profiling in human fibroblasts with different subtypes of senescence, we showed that a homoeostatic switch that results in glycerol-3-phosphate (G3P) and phosphoethanolamine (pEtN) accumulation links lipid metabolism to the senescence gene expression programme. Mechanistically, p53-dependent glycerol kinase activation and post-translational inactivation of phosphate cytidylyltransferase 2, ethanolamine regulate this metabolic switch, which promotes triglyceride accumulation in lipid droplets and induces the senescence gene expression programme. Conversely, G3P phosphatase and ethanolamine-phosphate phospho-lyase-based scavenging of G3P and pEtN acts in a senomorphic way by reducing G3P and pEtN accumulation. Collectively, this study ties G3P and pEtN accumulation to controlling lipid droplet biogenesis and phospholipid flux in senescent cells, providing a potential therapeutic avenue for targeting senescence and related pathophysiology.

High fat diet induces bioenergetic adaptations of small intestinal epithelial cells reducing cell differentiation and enhancing intestinal permeability in mice

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Obesity and overweight comorbidities are associated with low-grade inflammation linked to alterations of intestinal barrier. Intestinal homeostasis is supported by intestinal epithelial cells (IEC) mitochondrial function, that provides the energy needed for nutrient absorption, epithelial renewal, and maintaining of intestinal barrier. Yet, although numerous studies have described intestinal hyperpermeability in murine models of obesity, the mechanisms linking intestinal permeability, mitochondrial function and lipid metabolism in IEC are still not well described in obesity.

Male C57Bl/6J mice were fed a control diet (fat = 10% of energy) or a high fat diet (HFD, fat = 58% of energy). HFD mice displayed increased small intestinal permeability and triglyceride accumulation in small IEC. Bioenergetic analysis performed on isolated mouse IEC, and *in vitro* on IPEC-J2 enterocytes, revealed that lipid accumulation in enterocytes preceded a decrease in mitochondrial number, respiration and energy production. These bioenergetic adaptations of steatotic IEC were accompanied by increased antioxidant defenses and reduced β-oxidative activity and expression of enzymes involved in chylomicron export. Yet, reduced mitochondrial activity in IEC was associated with increased proliferation and reduced differentiation of IEC. Besides, enhancing mitochondrial function with the AMPK activator AICAR in jejunal organoids reduced proliferation and initiated IEC differentiation into mature enterocytes and conversely. Thus, reduced mitochondrial function of IEC recommitted cells towards proliferation. Furthermore, mitochondrial changes in enterocytes and intestinal hyperpermeability depend on the nature of the dietary fatty acids. Indeed, treating IPEC-J2 with C16:0 and C18:0 induced hyperpermeability *in vitro* due to the decrease in mitochondrial ATP production together with enhanced mitochondrial fragmentation and ROS production, contrary to C12:0 or C14:0.

Excess lipid intake diminishes mitochondrial number and energy production in IEC, reducing IEC differentiation that contribute to the increased epithelial permeability involved in low-grade inflammation and obesity-associated comorbidities.

SESSION 1
STRUCTURAL DIVERSITY OF LIPIDS FROM SUMMITS TO DEEP SEA

Influence of glycolipids on the physical properties of lipid membranes: insights through x-ray and neutron scattering

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Biological membranes often contain considerable fractions of glycolipids. These can strongly influence the membrane characteristics in terms of their interactions with adjacent membranes, with ions, as well as their in-plane organization, among others. We use various scattering techniques with x-rays and neutrons and complementary computer simulations to elucidate these phenomena on the molecular level [1-5].

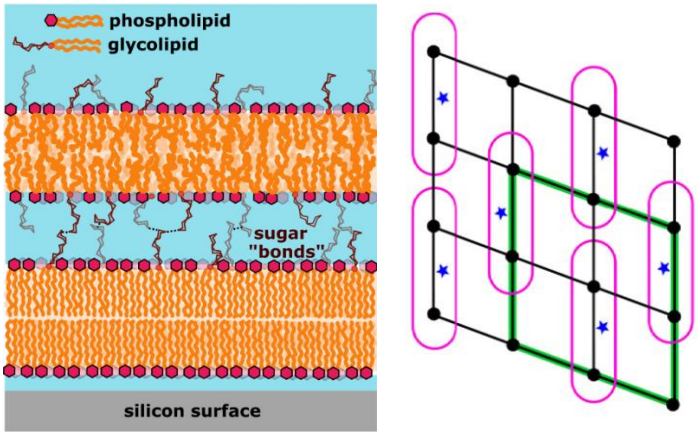

Figure 1: (left) Cartoon of a solid-supported double lipid bilayer containing glycolipids [5]. (right) Schematic representation of the molecular superlattice induced by a tail-saturated glycolipid with a monosaccharide headgroup [4].

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Biodiversity, bioactivity and lipid diversity of microphytobenthic biofilms from coastal intertidal sediments

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Microphytobenthic biofilms are complex communities of microeukaryotes, cyanobacteria, other bacteria and archaea that occur on intertidal mudflats and sandflats. These biofilms are a significant contributor to the total primary production of coastal ecosystems and persist and function despite extreme variation in environmental conditions over short timescales and high biological competition between taxa. Given the extent of biological competition and co-evolution, highly variable environmental conditions, and their accessibility, intertidal microphytobenthic biofilms are also suitable targets for marine natural product discovery. Despite their important natural role and potential for human application, comprehensive studies of community composition, function and chemical diversity such as metabolites are lacking. In this study, microphytobenthic biofilms were sampled from intertidal settings in temperature waters across Ireland. A multi-omic workflow to characterise biological diversity using culture-independent sequencing technologies and lipid diversity using mass spectrometry-based approaches is being conducted. The qualitative and quantitative distribution of lipids were analysed by targeted and untargeted GC-MS and LC-MS with data dereplication and compounds identification being assisted using the Global Natural Product Social molecular networking platform. Preliminary data highlight significant differences in community composition and abundance between sites. Community composition, biogeography and lipid diversity differences are matched by significant variation in anticancer, antimicrobial and antioxidant potential based on bioactivity screening of lipid extracts.

Characterization of digalactosyldiacylglycerol synthases in the model diatom *Phaeodactylum tricornutum*

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Oceans are the "lungs of the Earth." They are characterized by a vast biodiversity of photosynthetic organisms. Diatoms, a large group of unicellular photosynthetic microalgae, are the primary producers in the ocean. Alone, they are responsible annually for approximately 40% of photosynthetically fixed CO_2 in the ocean and 20% of the oxygen produced on Earth. Diatoms originate from secondary endosymbiosis, and their plastid is bounded by four membranes instead of two. In recent decades, they have attracted increasing attention because of their ability to produce high-value biomolecules, mainly lipids. However, little is known about lipid synthesis in diatoms, and it has yet to be studied in detail. In photosynthetic organelles, galactolipids are highly abundant and crucial for maintaining optimal photosynthesis efficiency. They are composed of a glycerol backbone, two fatty acids (FAs), and one or two galactosyl moieties (MGDG or DGDG, respectively). In *Phaeodactylum tricornutum*, a model diatom, MGDG synthases (*PtMGDα, PtMGDβ, and PtMGDγ*) have been recently characterized (Gueguen et al., submitted). Yet, DGDG synthases need to be studied. In *Phaeodactylum tricornutum*, four isoforms were identified (*PtDGDα, PtDGDβ, PtDGDγ and PtDGDδ*). In this study, the four *DGD* genes were functionally characterized. After each gene had been subcellularly localized by eGFP fusions, they were knocked out using the CRISPR-Cas9 technology. The selected mutants were morphologically, physiologically, and lipidomically phenotyped under different culture conditions. A striking phenotype was observed for *PtDGDα* knock-out, with a consistent reduction of 20:5-containing DGDG species, showing potential substrate specificity. This study also highlights the first subcellular localization of a glycerolipid in diatoms through immunolabeling of DGDG and the evolutionary history of DGDG synthases in diatoms.

Microbial membrane lipids as a tool to track microbial metabolisms in the environment: case study in continental wetlands

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Wetlands encompass very diverse ecosystems, from ponds to peatlands, and are key ecosystems in the regulation of the carbon cycle on Earth. They store vast amounts of carbon, via organic matter burial, while being the largest non-anthropogenic source of methane emissions worldwide. However, we are currently unable to determine the future fate of carbon in these ecosystems. This is, in large part, due to our lack of understanding of the microbial metabolisms processing organic matter in the wetland (sub)soil. This talk will highlight how the quantification and determination of the carbon isotopic composition of microbial membrane lipids can inform on the *in situ* metabolisms of environmental microorganisms. It will notably show recent results we obtained on a novel archaeal phylum, *Candidatus* Bathyarchaeota. This phylum was recently observed to predominate in wetland subsoils worldwide and its carbon metabolisms are so far unknown.

Sterol Biodiversity in Holothuroids

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A striking feature of the phylum Echinodermata is the dichotomy between the Crinoidea (feather stars) and Echinoidea (sea urchins) which contain Δ^5-sterols (such as cholesterol) like most metazoans, while the Asteroidea (starfish) and Holothuroidea (sea cucumbers) contain complex mixtures of sterols in which molecules with a Δ^7 double bond are predominant. Interestingly, the same dichotomy also appears in the production of saponins, as only the classes of holothuroids and of asteroids synthesize saponins. However, the biosynthetic adaptations that have resulted in this unique sterol composition in Holothuroids remains unknown. A detailed study of the diversity of sterols (free and conjugated) found in two contrasting species of holothuroids, the temperate suspension feeder *Cucumaria frondosa* and the tropical deposit feeder *Holothuria scabra*, along with their dietary intake, was conducted. Despite the differences in taxonomy, habitat, feeding behavior and potential dietary sterols, both sea cucumbers (*H. scabra* and *C. frondosa*) contained similar sterol diversity. Both sea cucumbers had a majority of lathosterol (Δ^7) and other Δ^7-sterols and surprisingly showed a high abundance of cholesterol and other Δ^5-unsaturated derivatives, but almost exclusively found as conjugated sterol sulfate fractions. Furthermore, parkeol (a $\Delta^{9(11)}$isomer of lanosterol) and other $\Delta^{9(11)}$-sterols were also detected in the free sterol fraction of both holothuroids. These finding suggest holothuroids have evolved a novel pathway for triterpenoid biosynthesis derived from parkeol and not lanosterol as is the case for the rest of the animal kingdom. The expression of sterol biosynthetic enzymes from *H.scabra* in yeast and plants is implemented to characterize such a novel pathway.

Total structural analysis of the diversified lipid in *Micrococcus luteus* cells by multiple stage mass spectrometry with high resolution mass spectrometry.

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Micrococcus luteus (*M. luteus*), is a Gram-positive, spherical bacterium known to its distinctive yellow or golden color. An obligate aerobe, *M. luteus* is ubiquitous, and can be found in a wide range of environments throughout terrestrial, aquatic, and particularly, marine environments. It can live in soil, dust, water and air, survive as part of the normal microbiota of human skin, colonize in the human mouth, mucosae, and upper respiratory tract. Notably, it was recently isolated from 120,000,000-year-old amber deposits.

Micrococcus are generally considered nonpathogenic in healthy individuals, but can be opportunistic pathogens for immunosuppressed patients. Due to their ability to produce enzymes and pigments, some strains of *Micrococcus luteus* have been investigated into their potential in the biotechnological applications. it is also used as a model organism in laboratory studies due to its relatively small genome and ease of cultivation. The potential in applying *Micrococcus spp* as a promising source for new drug discovery has been suggested.

M. luteus cells are known to contain a variety of lipids, including cardiolipin, phosphatidylglycerol and dimannosyldiacylglycerol (DMDG), which constitute the major lipids in the bacterial membrane, along with traces (0 3%) of phosphatidylinositol. DMDG serves as a lipid anchor precursor in the assembly of the membrane, and studies has been focused on the distribution of these lipids in the membrane of the bacterium cells. However, there is a paucity of research into the structural detail of lipids.

Here, we apply LIT MSn with high resolution mass spectrometric approach combined with GC/MS analysis for a complete structural characterization of the lipid structure, including the details of the fatty acid chains. We revealed the lipid repertoire, including the PG, CL, DMDG, and PI that were reported previously. Importantly, we revealed novel phosphatidylpropanediol lipid and the prenyl phosphate lipid families that have not been reported previously.

Function and biotechnological application of the glycine-glucolipid in the marine bacterium *Alcanivorax borkumensis*

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The hydrocarbon-degrading marine bacterium *Alcanivorax borkumensis*, initially isolated from sediments in the North Sea, has been described to be globally distributed, playing a crucial role in degrading hydrocarbons derived from cyanobacterial production or petroleum spills. Notably, the cells produce a unique glycine-glucolipid which consists of four esterified 3-hydroxy decanoic acid in amide linkage to glycine, and in glycosidic linkage with glucose. The function and distribution of this glycine-glucolipid was enigmatic. The glycine-glucolipid was isolated from the bacterial cell-broth, cell-pellet, or cell-free supernatant. It was mostly found in the cell-broth and cell-pellet, rather than in the supernatant, and in all cases, it had the same structure containing a glycine residue. In contrast to previous reports, which suggested that a glycine-free form of the glucolipid exists which is secreted into the supernatant. Therefore, the glycine-glucolipid of *A. borkumensis* is resident to bacteria, presumably bound to the cell wall. We identified the genes involved in the synthesis of the glycine-glucolipid, and generated *A. borkumensis* mutants lacking the glycine-glucolipid. These mutants not only enabled us to verify the essential role of these genes in the biosynthesis but also facilitated the investigation of the *in vivo* function of this glycolipid. Deficiency in the glycine-glucolipid affects the capacity of the cells to attach to the oil/water interface, and causes a decrease in the growth of mutant cells with hexadecane, demonstrating the glycine-glucolipid is crucial for the uptake and metabolization of alkanes. The glycine-glucolipid is a potential biosurfactant for applications in agriculture, e.g. as an additive of foliar fertilizers. When we added the glycine-glucolipid to a foliar fertilizer with phosphate, the glycine-glucolipid facilitated phosphorus uptake by *Nicotiana bentamiana* leaves to the same extent as the ecotoxic surfactant Triton X-100. This suggests the glycine-glucolipid is a promising, environmentally friendly additive for enhancing nutrient absorption by leaves in agriculture.

SESSION 2
ROLES/FUNCTIONS OF LIPIDS AND DERIVATES

Exploring the status of polyunsaturated fatty acids and isoprostanoids in marine species exposed to environmental stress

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Polyunsaturated fatty acids (PUFA) are abundant in marine species, sourced from algae or through the ingestion of smaller organisms in the food web. Marine species rely on algae for sustenance, contributing to ecosystem balance and biodiversity. However, a change in the environment disrupts the metabolic stability, leading to reduced food resources due to altered seasonal patterns. To survive, marine species seek alternative food sources. Elevated temperatures cause extreme strain, leading to oxidative stress in marine species. Here, we studied the PUFA and isoprostanoids, which are non-enzymatic oxidized PUFA products or oxylipins of marine species (corals, oysters, mangrove crabs, sea urchins, and sea snails for example) under various thermal or environmental conditions. A cleaner environment appears to give a healthy fatty acid profile of these marine species namely, corals and mangrove crabs. Changes in the environmental temperature and contamination had an impact on isoprostanoid levels such as F_{2t}-isoprosanes, Phytoprostanes and F_{4t}-Neuroprostanes, in oysters, corals, mangrove crabs and sea urchins. In summary, understanding these complex interactions sheds light on adaptation mechanisms and potential implications for marine ecosystems. Isoprostanoids may be a potential valuable biomarker to evaluate the health of these marine species while status of in vivo PUFA provide clues of the eating behaviour when the environment is modified.

Oxygenated PUFA metabolites as biomarkers in marine environment

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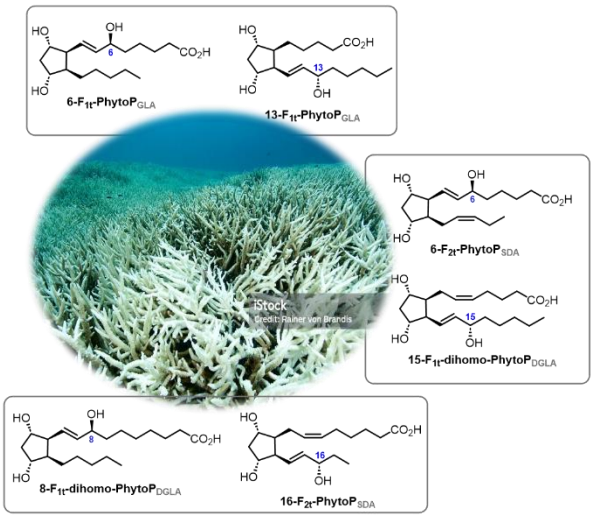
A vast array of oxygenated metabolites derived from polyunsaturated fatty acids (PUFAs) has been uncovered and named oxylipins. Radical cascades play a pivotal role in the biosynthesis of these metabolites, with radical initiation occurring either within the active site of an enzyme[1][2] in the extracellular environment or within membrane[3][4] on phospholipids/glycolipids. The advancement of convergent and adaptable chemical methodologies by organic chemists, coupled with the refinement of sophisticated mass spectrometry techniques, has significantly expanded our understanding of PUFA metabolites. Therefore, these oxygenated fatty acid metabolites have emerged as crucial indicators of oxidative stress in marine environments. This presentation will provide a brief overview of the biosynthetic pathways leading to oxylipins, chemical strategies for obtaining pure compounds, and examples of LC/MS-MS quantification of oxylipins in marine matrices such as algae and corals.



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The Seipin protein of *Phaeodactylum tricornutum*: structure and function specificities

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Microalgae are a diverse group of photosynthetic unicellular organisms found in a wide variety of habitats. In response to abiotic and biotic stresses, microalgae produce lipid droplets (LD), dynamic organelles involved in the storage of carbon- and energy-rich molecules and remodeling of membrane lipids.

The biogenesis of LD is a complex mechanism in which the Seipin protein is a key player. Seipin has been studied in a variety of organisms. It is a transmembrane protein forming an oligomer embedded in the ER membrane, and is functionally involved in various aspects of LD biogenesis: orientation of LD budding, control of LD size and control of triacylglycerols (TAG) flux into the LD.

In the oleaginous diatom *Phaeodactylum tricornutum*, a Seipin isoform (PtSeipin) has been identified, and has been shown to be involved in LD biogenesis. The KO of PtSeipin revealed a strong phenotype with oversized lipid droplets remarkably coupled to accumulation of TAG, in particular in response to high light stress. Such a strong effect on TAG accumulation has not been observed in other organisms, suggesting that it has specific molecular functions. In order to understand these specificities and better understand LD biogenesis and functions in diatoms, we are exploring the structure of PtSeipin and its interactions with other proteins.

Transcription factors for reprogramming lipid metabolism in response to temperature in the simple green picoalga *ostreococcus tauri*

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Lipid composition of marine microalgae varies as a function of temperature. In particular, production of Very-Long Chain Polyunsaturated Fatty Acids by phytoplankton is inversely correlated with temperature in the ocean, and its predicted decline because of global warming poses a serious ecological and economic threat. However, we still have very little detailed knowledge of the regulation of lipid metabolism by temperature and of how lipids contribute to the overall physiological adaptation of marine microalgae. Whether or how the transcriptional regulators of temperature responses and of lipid metabolism are connected is unknown in marine microalgae. Gene networks are useful tool for providing molecular blueprints of physiological associations. Here, we use the simplest green marine picoalga *Ostreococcus tauri* to gain insight in the reprogramming of lipid metabolism by temperature. In particular, we aim at identifying key transcription factors involved in membrane lipid remodelling as well as in linking membrane adaption to complementary biological processes required for temperature acclimation. We characterized the transcriptomic and lipidomic response of *O. tauri* to chilling and warming. Through the establishment of co-expression network we identified transcription factors putatively activating lipid metabolism genes. We further considered lipid metabolism genes relevant for the observed lipid changes to assess the binding of transcription factor to promoters using Yeast One-Hybrid assay. Our results suggest that the plant CBF, bZIP and SPA1 homologues are important transcriptional activators of genes involved in lipid remodelling during chilling, in particular of desaturases.

Lipid-mediated signaling mechanisms in plant innate immunity

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Recognition of conserved patterns of potential aggressors by plasma membrane localized receptors activate immune responses in plants, a process termed pattern triggered immunity (PTI). We recently discovered that phosphatidylcholines (PCs) from eggs of the large white butterfly *Pieris brassicae* are perceived by the model plant *Arabidopsis thaliana* to detect early signs of herbivore attack. PCs are released from insect eggs and induce defence gene expression, the accumulation of salicylic acid and cell death in Arabidopsis, all of which constitute a hallmark of PTI. Induction of plant immune signaling in response to PCs is dependent on functional Brassica-specific L-type lectin receptor kinases LecRK-I.8 and I.1, which we previously reported to be early components of insect egg perception in Arabidopsis. Recent *in-vitro* analytical approaches investigate interactions of LecRK-I.8 and I.1 with PCs and PC-derivatives. The ubiquitous presence of PCs and other lipids in biological membranes and as storage lipids of organisms which interact with plants led to the question whether lipidic compounds from different sources might be detected by plants as conserved features from potential enemies. This research hypothesis is supported by preliminary experiments that revealed an immunogenic activity of lipid extracts of different microbial plant pathogens, indicating that microbe-derived lipids act as general elicitors of plant immune signaling. Our recently established research group at the European Institute of Chemistry and Biology (IECB) at the University of Bordeaux focusses on the identification of novel microbial derived lipidic elicitors of plant immune signaling and their perception mechanism in Arabidopsis. In order to achieve this, we combine analytical chemistry methods (chromatography, lipidomics, MS, NMR) and classical molecular biology and biochemistry methods.

Characterizing molecular actors from brown algal oxylipin pathways in defense signaling through metabolomics and functional genomics

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The oxylipin pathways are known to be involved in defense signaling in plants and animals. In brown algae, an evolutionary independent eukaryotic lineage, oxylipins deriving from both C18- and C20- Polyunsaturated Free Fatty Acids are produced during stress defense responses. Their biosynthetic pathways and roles as signals molecules during biotic interactions are still unknown. Genomic approaches have identified several CYP5164 genes, homologous to the plant CYP74 gene family, which may play an important part in defense signaling between brown algal host and endophyte. To decipher the biological functions of these genes in the model brown algal, *Ectocarpus sp.7,* targeted and un-targeted metabolomic analyses were performed to compare the global metabolome of control and stressed algal cultures. High-Resolution Liquid Chromatography Mass Spectrometry (LC-MS) analysis was used to mine for differences in the overall metabolic profiles and to investigate the occurrence or absence of specific oxylipins in CRISPR knock-out mutants for the CYP5164B1 gene locus compared to wild-type strains. In addition, recombinant CYP5164B1 proteins were produced to characterize *in vitro* biochemical activities by Gas Chromatography Mass Spectrometry (GC-MS) and to identify brown algal-specific substrates. These approaches will indicate whether the profiles of mutant oxylipins are consistent with the previously determined catalytic activity of the recombinant enzyme. The combination of *in vivo* metabolomic approaches and targeted biochemical characterization will enable CYP5164 activity to be integrated into the global metabolic brown algal model. Moreover, these results will contribute to a better understanding of CYP-based defense and chemical signaling in brown algae during biotic interactions.

Non-enzymatic oxylipin production in a mudflat microphytobenthic biofilm: evidence of diatom response to light

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The microphytobenthos (MPB) is a diatom dominated microbial community of primary producers inhabiting the mudflat sediments. On one hand, the benthic diatoms display photo-protective strategies to face extreme light variations susceptible to generate cellular oxidative stress. On the other hand, oxidative stress induce the production of reactive oxygen species (ROS) that generate oxylipins, oxygenated metabolites of polyunsaturated fatty acids (PUFAs), which may represent the best described signaling molecules in diatoms. However, non-enzymatically generated oxylipins known as isoprostanoids or isofuranoids are poorly studied in diatoms. To better understand the roles of the latter in migrational MPB light response, we investigated the effect of different light irradiances corresponding to dark (D), low light (LL, 50 and 100 µmol. photons. m^{-2}. s^{-1}PAR), medium light (ML, 250 µmol. photons. m^{-2}. s^{-1} PAR), high light (HL, 500, 750 and 1000 µmol. photons. m^{-2}. s^{-1}PAR), on the isprotanoids production by the biofilm's organisms. The PUFAs precursors of the varying oxylipins evidenced a diatoms response to light irradiance. Under 1000 PAR condition, the total amount of isoprotanoids increased, indicating an oxidative stress response. Isoprostanes (IsoP) and prostaglandins (PGF) characterized the HL conditions and evidenced lipid peroxidation probably linked to the higher generation of ROS by the photosynthesis. On the contrary, the phytoprostane (PhytoP) characterized the LL and ML where the de-epoxidation state was low and ROS scavengers probably not overwhelmed. The involvement of various PUFA precursors in light responses suggests that different cellular sites may be implicated depending on the levels of irradiance. This first investigation of non-enzymatic oxylipins production by a microphytobenthic biofilm under different light irradiances highlighted the interest to explore their potential signaling roles related to MPB light responses.

Phytosterol metabolism and homeostasis

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Sterols are mandatory cellular components, as building blocks of membranes and as precursors of phytohormones, both functions having specific molecular structural requirements. Sterol homeostasis is an essential process in eukaryotic development. Strikingly, genetic mechanisms implied in sterol homeostasis in mammals, fungi and plants are different. However, a common aspect between plants and other organisms is a key role of upstream enzymes implied in the mevalonate/isoprenoid pathway. To identify major components of the regulatory circuits at play in isoprenoid and phytosterol homeostasis, we have characterized high sterol esters producers in *Arabidopsis thaliana*, *Nicotiana benthamiana* and *Nicotiana tabacum.* In the latter species, a high phytosterol producer previously isolated in the laboratory carries a semi-dominant mutation responsible for sterol esters accumulation in lipid droplets. Genetic reprogramming in this tobacco mutant and the phenotypes of its hypersterolemic leaves and lipid droplets are investigated to determine processes and proteins associated with sterol ester formation and mobilization at the cellular level.

Role of peroxisome and peroxisomal β-oxidation in lipid remodeling and plant adaptation to phosphate starvation in higher plants

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Phosphate (Pi) starvation is a frequent nutrient stress significantly impacting plant growth and crop yield. To adapt to Pi starvation, plants activate different mechanisms to ensure their survival, including increasing Pi uptake from soil and remobilizing intracellular phosphorus (P) reserve. Notably, during Pi starvation, phospholipids (PL) located at extra-plastidial membranes are partially degraded to release Pi, while plastidial digalactosyldiacylglycerol (DGDG), which does not contain Pi group, is transferred to extra-plastidial organelles to substitute PL in order to maintain their integrities and functions. Previous studies have revealed that the DAG backbone of newly formed DGDG originated from extra-plastidial PC, indicating that the partially degraded PC is recycled to synthesize DGDG via DAG. However, we uncovered that the total amount of lipids per cell weight drastically decreased under Pi deprivation. Given that, we hypothesized a potential association between lipid remodeling and β-oxidation, a lipid degradation pathway occurring in peroxisomes. It prompted us to further investigate if the β-oxidation pathway would be important in recycling free fatty acids derived from the partially degraded PL under Pi deprivation and, more generally, if peroxisomes would be involved in lipid remodeling during Pi starvation.

Thus, I optimized a peroxisome purification protocol in order to analyze the lipid composition of isolated peroxisomes in response to Pi starvation. In addition, in order to elucidate the role of β-oxidation process for lipid remodeling and the resilience of plants to this stress, I employed multiple approaches, such as gene expression profiling, growth phenotype assessment, lipidomic analysis and subcellular imaging onto wild type and β-oxidation deficient mutants during Pi starvation.

SESSION 3
LIPID MARKERS IN TROPHIC ECOLOGY: PROMISES AND DEAD ENDS

Lipids and stable isotopes in trophic ecology: what are we measuring?

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The use of fatty acids in food web sciences is based on the assumption that their presence within the tissues of consumers has a trophic origin, and their specificity towards food sources provides informative properties about the nature of dietary relationships. Metabolic processes, ranging from the transfer of nutrients across the intestinal wall to the remobilization of storage lipids (during periods of low food availability or gametogenesis, for example), are likely to modify the fatty acid composition of consumers. This can lead to non-dietary related differences in the fatty acid composition of food sources and their consumers, differences that are currently poorly understood compared to the better-known processes in stable isotope ecology. In this presentation, I will try to identify, based on a comparison between the two research fields, future research directions to improve our understanding of how and when fatty acids (and other lipid biomarkers) can provide reliable information about trophic interactions occurring within natural ecosystems.

Trophic ecology of three deep-sea coral species, interannual comparison using trophic biomarkers

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The seabed covers nearly 71% of the Earth's surface, with an area of 362 million km² of which 90% is represented by the deep ocean. In these light-deprived areas, distant from productive surface waters, reefs formed by a community assemblage of heterotrophic scleractinian corals thrive. However, food resources availability is one of the factors limiting the distribution of deep-sea coral reefs. In this *in-situ* study, we focus on the species *Desmophyllum pertusum*, *Madrepora oculata* and *Solenosmilia variabilis* which cohabit and share trophic ressources in the Lampaul Canyon off the coast of Western Brittany. Our work aims at describing the use of food resources among these three species over a period of three years. Our results have shown differences in dietary habits among the three species. Although all feed on varied prey, *Desmophyllum pertusum* and *Solenosmilia variabilis* preferentially feed on zooplankton while *Madrepora oculata* predominantly consume phytoplankton. These results highlight the efficient partition of trophic resources within the cold-water coral's reefs of the Lampaul Canyon.

Sterol biosynthesis and phytosterol bioconversion in *Crassostrea gigas* larvae

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Dietary sterols are important for bivalve larval growth and survival. Sterol incorporation in Pacific oyster *Crassostrea gigas* larvae has been studied here by means of a flow-through larval rearing technique allowing accurate quantification of microalgal ingestion. Two sets of experiments were done using two cultured microalgae: *Tisochrysis lutea* (T) and *Chaetoceros neogracile* (Cg) as mono- and bi-specific diets. Accumulation of tissue sterols in oyster larvae, in addition to those present in the diet, indicate that *C. gigas* larvae appear to have the ability to synthetize sterols *de novo* under low dietary sterol supply, e.g., when fed the T diet. Sterol synthesis was dependent upon sterol dietary supply; larvae fed T exhibited greater sterol incorporation at the pediveliger stage than larvae fed TCg (2.7 *vs* 1.3-fold sterol incorporation compared to sterol ingestion, respectively). Larval sterol compositions under the different dietary regimes indicate likely bioconversion pathways modifying dietary sterols. Larvae fed T bioconverted dietary brassicasterol mainly to cholesterol *via* a 22-dehydrocholesterol intermediate. Brassicasterol was also actively synthetized in larvae fed T and TCg, even though it predominated in larvae fed T, suggesting a possible metabolic role of this sterol in *C. gigas* larvae. Apparent desmosterol synthesis under all experimental conditions suggests a role as a membrane component or as an intermediate in cholesterol synthesis. Our data also indicate that *C. gigas* larvae require approximately 13 ng cholesterol $larvae^{-1}$ to achieve competence for metamorphosis.

Impact of dietary lipid compounds on the metabolic rate of an aquatic consumer

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Lipid markers constitute a powerful approach in trophic ecology since they can provide varied information relating to the presence of organisms, energy flows and the physiological state of consumers. This conference will mainly focus on the essential compounds that determine nutritional quality for aquatic consumers and will present the results of a study in which we quantified the impact of dietary lipid compounds on the energy expenditure of *Daphnia magna*. Here, we measured the resting metabolic rate (RMR) of *Daphnia magna* along ontogeny when undergoing various (non-energetic) nutritional constraints. All types of dietary (co)limitations (Fatty acids, Sterols, Phosphorus) induced an increase in mass-specific RMR up to 128% between highest and lowest quality diets. We argue that quantifying the energetic cost imposed by food quality on individual RMR may constitute a common currency enabling the integration of nutritional and metabolic ecology.

Algae to alkanes: new insights into the potential of alkanes as detrital biomarkers from kelp degradation experiments

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In many coastal areas, kelp forests support complex food webs with many marine consumers relying on kelp as their basal organic matter source. It has become evident that kelp detritus may be a significant resource and enhance secondary production within and around kelp forests. Understanding the contribution of detritus to marine food webs is particularly important in the current changing ocean scenario, where climate change related stressors are driving large scale changes in kelp abundance and distribution, with consequences for productivity. However, we are still lacking ways to detect which macroalgae contribute to the food web as detritus. Here we are focusing on n-alkanes, present in the lipid fraction of algal biomass, where they contribute to the structural integrity of cell membranes and other cellular compartments. We collected fresh and degraded samples of dominant macroalgae species (*Laminaria hyperborea*, *L. digitata*, *Saccharina latissima*, *Sacchorhiza polyschides*, *Himanthalia elongata*, *Ascophyllum nodosum*, *Fucus serratus*, *Palmaria palmata*, *Mastocarpus stellatus*, *Ulva* sp.) from Brittany beaches to understand differences in n-alkane composition and proportion among species and their detritus. Additionally, over the course of eight weeks, we observed the experimental degradation of four different species of macroalgae (*L. digitata*, *S. latissima*, *F. serratus*, *and P. palmata*). Regularly, subsamples were taken for the extraction and analysis of n-alkanes. Preliminary results show significant differences in the proportion of n-alkanes among different species and between fresh and degraded algae. The n-alkane C17 was particularly prominent in the two red algae species, whereas fresh and detrital algae seemed to be differentiated by the proportion of the n-alkanes C25, C27, and C29. Marine animals do not produce n-alkanes themselves, but can bioaccumulate them through their diet, indicating their potential as biomarkers for different algae species and/or degraded algae.

Temperature-driven changes in fatty acids from diatoms to copepods modulate direct effects of temperature on copepod fitness

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Copepods are aquatic invertebrates with a key role at the basis of marine food webs due to their high biomass and nutritional quality. They are rich in EPA and DHA, which they predominantly obtain from their food. The rise in the sea surface temperature leads to changes in copepods mortality, metabolism and phenology. In addition, rising temperatures reduce the availability of fatty acids (FA) in primary producers such as diatoms, decreasing its food quality. How this reduction transfers to the copepods and its potential interactive effects on the direct effects of temperature on their fitness remains largely unknown. In this study we aim to disentangle direct from indirect effects of temperature on copepod's FA profile, nauplii production, body size and elemental composition. In a fully crossed factorial design we exposed the benthic harpacticoid copepod *Tachidius discipes* (Giesbrecht, 1881) to a low (15 °C) and a high (24 °C) temperature treatment from hatching until maturity. Additionally, copepods were fed with a good and poor food quality treatment created by culturing diatoms at the two same temperatures. We found that temperature produced a shift in the FA profile of the diatoms resulting in a decrease in DHA(%), EPA (%), PUFA/SFA and the ω3/ω6 ratio that was transferred to the copepods (except for DHA). We also found that food quality modulated the direct effects of the temperature treatment: poor food quality resulted in smaller maximum sizes, less carbon content and less offspring, although the effects are not uniform but depended on the temperature considered. We also found a strong positive correlation of the ω3/ω6 ratio with nauplii production, max body length and carbon content. Together, indirect and direct effects of temperature on copepods will reduce its size, abundance and nutritional quality compromising the quantity and quality of food available for higher trophic levels.

SESSION 4
LIPIDS IN THEIR SOCIO-ECONOMIC CONTEXT AND LEGAL BOUNDARIES

An Industry Perspective on Microalgae Lipids: Economics, Regulations, and Societal Impact

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Algae-derived compounds, including lipids, are emerging as novel resources with significant socio-economic implications. Algae can be cultivated on non-arable land, contributing to rural development, and can produce more biomass per square meter than any traditional agricultural crop. Their applications span diverse industries such as foods and beverages, animal feeds, nutraceuticals, pharmaceuticals, and cosmetics. However, due to the high production costs associated with this young and highly technological field, most algae-derived products are currently confined to niche markets. Despite these challenges, new trends are fostering market growth, driven by increasing consumer demand for sustainable and eco-friendly products. For example, algae-based products are becoming viable alternatives to unsustainable products like fish oil. However, legal and regulatory barriers pose significant challenges to market expansion. Compliance with regulations, especially those governing novel foods, can be time-consuming and costly. Intellectual property issues and lack of Freedom to Operate (FTO) further hinder market growth. Additionally, maintaining product quality and traceability is crucial for ensuring product integrity and consumer trust. Therefore, navigating the complex legal landscape set by the European Union is essential for achieving compliance and market success. The continued growth and development of the algae sector will depend on overcoming existing challenges and leveraging new opportunities. Technological advancements, increased investment, and adapted government policies will be key to unlocking the full potential of algae-derived products.

Eating Omega 3 rich products: knowledge and perceptions according to sources

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Claims about Omega 3 content have dramatically increased on food packaging these two last decades in a context of higher awareness of the vital role of unsaturated fatty acid for human health. Numerous research showed that Omega 3 has become an attribute in food choices among health-oriented consumers while most of them struggle to make sense with this nutritional lexicon and subtle differences in the type of omega 3 and potential plant-based vs fish-based sources. In the context of climate change and of the rise of alternative diets that lead consumers to decrease their animal-based food, sometimes evicting any fish, there is a need to better understanding the building process of representations associated with Omega 3 and any sources. Based on a national, quantitative survey (n=1000) with French consumers, our presentation will explore the different meanings and representations associated with omega 3 in food and their role in health, depending on the type of food and on respondents' food profiles (omnivorous, flexitarians, vegetarians or pescovegetarians). Moreover, a focus will be carried on fish food, omega 3 rich food but whose representations bring together fragmented elements of knowledge, beliefs and symbolical thoughts that connect the type of fishes and their perceived properties. Finally, some theoretical considerations will elaborate on the experts/nonexperts divide about Omega 3 and on the different forms of rationalities that lay eaters rely on to grasp the nutritional content of their food and/or to combine health with pleasure and taste. Conclusive remarks will suggest some paths to better informing eaters on the issue of Omega 3 while taking into account their different expectations when it comes to food and to plant-based/animal-based food arbitrage.

Lipid traceability by molecular and isotopic fingerprinting

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Lipids used in the dietary supplements, infantile food or cosmetic markets can originate from different sources such as marine organisms, terrestrial plants or synthetic products. Certain of these sources become more depleted or are protected, while others not, and it leads to large differences in their market price. Such price disparity could induce fraudulent behaviours, aiming to make profits by selling mislabelled lipid products at the cost of expensive lipid sources, while containing only cheap ones. For ethic, sanitary and commercial reasons, it is of primary importance to be able to pinpoint these malevolent activities and identify the origin of the lipids in supplier and commercial products. The present study aims to present the recent advances in lipid traceability for different markets.

We combined the analysis of fatty acids (FA), squalene and sterols by conventional GC-FID/MS with the compound specific isotope analysis (CSIA) on carbon of FA and unsaponifiable compounds. Marine and terrestrial oils from different sources were analysed and a database of molecular and isotopic fingerprints was created.

The molecular fingerprints of unrefined products can distinguish different sources of lipids. The identification of the source in refined products is more sensitive and only CSIA can achieve it. Depending on the sources considered, a single analysis or a combination of and carbon isotopes is needed to achieve a satisfactory distinction. For instance, 13C-CSIA analysis on DHA, EPA and sterols allowed identifying microalgal oils cut with fish oils.

This study highlights the potential of CSIA on different lipids to reveal the quality and the origin of a product. Squalene and sterol CSIA are still rarely performed but were revealed a promising tool for lipid traceability, as well as hydrogen CSIA. Such approach involves nonetheless building an exhaustive database with reliable suppliers to ultimately target lipid sourcing certification.

Sardines or how to meet the challenges of sustainability and food security in the context of climate change

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The food transition required in the current climate change context is forcing us to rethink our consumption patterns. This means changing our diets, which are all too often based on red meat and/or processed products. In this context, fishery and aquaculture products, also known as 'blue foods', are set to play an important and growing role (see EAT-Lancet recommendation). However, many obstacles remain to be overcome, such as the imbalance of certain stocks, the impact of certain fishing and aquaculture techniques on habitats and environment, and the scale of international trade in these products.

However, this general observation needs to be refined and many fish species, such as small pelagic, are of great interest in this transition. Firstly, these species are rich in omega-3 fatty acids and contain nutrients that are important for human health. Secondly, small pelagic are wild species that are easy to catch using fishing gear that has a relatively low impact on habitats and a relatively low ecological footprint (greenhouse gas emissions). Finally, they are relatively affordable products in seafood markets that are often considered too expensive.

In France, small pelagic, and sardine in particular, have a very important historical and cultural heritage. Sardine fishery has historically been present along the entire coast of mainland France, and canneries are rooted in many local social histories. However, while the consumption of sardines in France has increased over the last 20 years, the stock has recently faced conservation problems and a decline in its nutritional qualities.

Using the results of 2 research projects (DEFIPEL and OMEGA), we will present the organization and dynamics of the sardine industry in France. We will then look at the nutritional expectations of sardine consumers and the strategies adopted by canners to meet these expectations. Finally, we will consider how this economic research can be useful for managing this fishery and understanding the economic and social disruptions caused by the current climate change context.

SESSION 5
ENVIRONMENTAL/CLIMATIC AND SUPPLY ISSUES OF OMEGA 3

Production of omega-3 LC-PUFAs in transgenic *Camelina sativa*: A vision for extending the story from nutraceutical to therapeutic applications

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The importance of an alternative and sustainable supply of omega-3 long chain polyunsaturated fatty acids (omega-3 LC-PUFAs) has long been established. As these biologically active fatty acids are essential constituents of human nutrition and have key roles in growth and development, there is an ever-increasing demand for oils containing eicosapentaenoic (EPA) and docosahexaenoic acid (DHA). In fact, there is growing evidence from clinical studies that the presence of omega-3 LC-PUFAs in the human diet has therapeutic effect in conditions such as cardiovascular diseases, obesity, metabolic syndrome. Recent studies even suggests that Omega-3 intake in midlife, and high levels of omega-3 in the blood, may be associated with a lower risk of dementia and Alzheimer's disease [1]. These fatty acids, primarily produced by marine microorganisms, enter our diet through the consumption of fish, but the depletion of wild fish stocks and pollution of the marine environment indicate an urgent need for an alternative and sustainable source of omega-3 LC-PUFAs. Given the growing importance of the aquaculture industry in delivering healthy food, and its requirement for large amounts of omega-3 oil, the question of how to resource the supply of LC omega-3 then arises; traditional sources of EPA and DHA (fish oil) are challenged, whilst vegetable oils do not contain EPA or DHA. Therefore, research efforts have focused on the successful reconstitution of LC omega-3 biosynthesis in oilseeds and micro-organisms. The engineering of oil crops to synthesize high levels of DHA and/or EPA is the most cost-effective solution for large-scale production of LC-PUFAs. The expression of optimised combinations of the genes required to produce these fatty acids in the seed of *Camelina sativa* has been achieved and the utility of this approach demonstrated [2, 3, 4]. This represents a significant breakthrough in the provision of an effective alternative to the use of omega-3 fish oil by the global aquaculture industry. The recent approval of our GM camelina varieties by the United States Department of Agriculture (USDA) has now opened the road to commercialisation of omega-3 camelina oils for both aquafeed and human nutrition.

In vertebrates, some specialised cell types contain essential, ultra-long chain (UL; >C28+) PUFAs derived from LC-PUFA precursors. The retina has a highly specialised membrane organisation in which these ultra-long PUFAs are actively incorporated into phospholipids. In humans, mutations preventing UL-PUFA formation have been linked to various diseases such as the Stargardt-like macular dystrophy (STGD3) causing juvenile-onset macular degeneration [5], but also to poor sperm quality leading to male infertility [6]. Recent animal studies by our US-based collaborators (Dean A. McGee Eye Institute, Oklahoma) have demonstrated reversal of retinal disease states by the exogenous delivery of these ultra-long PUFAs [7, 8] but chemical synthesis is technically extremely challenging and consequently, prohibitively expensive. Using our transgenic camelina platform we are generating a new source of ultra-

long PUFAs, suitable for evaluation as a therapeutic treatment for retinopathies. Specifically, we are extending the current EPA and DHA synthesis capability via additional specific elongating activities in conjunction with appropriate acylating enzymes to direct these novel FAs into PLs or TAG. Using a small panel of different background lines synthesising either EPA, DPA or DHA, it will be possible to generate in planta a lipid library of C28-C36:5/6 ultra-long PUFAs which can be tested for efficacy in appropriate cell and animal models. Just as for omega-3 LC-PUFAS before, this approach has great potential for providing a sustainable source of UL-PUFAs initially for the treatment of STGD3. However, as more knowledge is gained into other conditions linked to deficiency in UL-PUFAs, it could have medical applications in a wider range of conditions including male infertility and age-related macular degeneration (AMD).

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The heart's energy secrets: lipids to grease mitochondrial performance in sardines, *Sardina pilchardus*

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Global change may decrease the production of omega-3 long-chain polyunsaturated fatty acids in primary producers. This decline in key nutrients such as docosahexaenoic acid (DHA) at the base of the food chain can reduce DHA availability in higher trophic levels, affecting the DHA content in their cell membranes, including mitochondrial membranes, thus impacting mitochondrial ATP production and individual metabolism. Over the past two decades, multiple sardine populations have shown declining body condition and size-at-age, primarily due to trophic alterations induced by climate change. Our hypothesis is that reduced dietary DHA would affect sardines' cardiac mitochondrial performance, contributing to physiological disturbance. Our experimental approach involves feeding wild adult sardines a control diet or a DHA-deficient diet for 6 months. Our findings indicate that DHA-deficient sardines had lower membrane DHA content in the heart compared to controls and less efficient mitochondria in ATP production (ATP/O ratio), with increased oxygen consumption for proton leakage (LEAK) and decreased oxygen consumption for ATP production (OXPHOS). Further analyses are ongoing to clarify the relationship between fatty acid membrane composition and variation in mitochondrial performance among individuals. Overall, our results demonstrate that dietary DHA limitation reduces mitochondrial ATP production capacity while increasing oxygen consumption in proton leakage. Impaired mitochondrial performance can lead to cardiac dysfunction and reduced individual performance, potentially impacting observed life history trait alterations in sardines, such as growth or condition, with implications for highly productive trophic networks if occurring in the wild.

Spatial and ontogenetic modulation of fatty acid composition in juvenile European sea bass (*Dicentrarchus labrax*) from two French estuaries

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This study aimed to fill critical knowledge gaps regarding the adaptive capacity of wild fish to changing environmental conditions, focusing on the role of fatty acids (FA) in this process. We compared the FA composition in liver, muscle, and brain tissues of wild European sea bass juveniles from two French estuaries (Loire and Seine), focusing on the variability among ontogenetic stages (first, second and third year, i.e. G1, G2, G3, respectively). In all tissues and groups, the main saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids (PUFA) were identified as palmitic acid (16:0), oleic acid (18:1n-9) and docosahexaenoic acid (DHA; 22:6n-3), respectively. We highlighted tissue-specific patterns, with the brain showing a distinct FA composition from the two other tissues. Ontogenetic stage and estuary influenced the overall FA profile of fish, and, in particular, the proportions of essential FAs (EFA), such as DHA, eicosapentaenoic acid (EPA), and arachidonic acid (ARA) proportions in all tissues. These results might reflect changes in diet, metabolic demands, or adaptations to environmental conditions. We showed that the Seine G1 had a lower hepatic DHA proportions, which were associated with activation of PUFA biosynthetic pathways at the molecular level, as shown by the *fads2* (fatty acid desaturase) gene expression. This may be related to (i) a lower PUFA content in their diet, combined with (ii) a higher DHA requirement due to a higher cellular turnover at this ontogenetic stage. This would suggest that wild European sea bass is able to compensate to some extent for a PUFA dietary limitation in their natural environment. This study provides valuable insights about FA dynamics in euryhaline fishes during juvenile stages, improves our understanding of their metabolic and trophic interactions, and highlights the need to further investigate the potential effects of FA depletion in a changing trophic environment.

Omega-3 fatty acids and their influence on feeding behavior in rainbow trout: unraveling the sensory mechanisms

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In the current global expansion of aquaculture, where it has become crucial to alleviate pressure on wild fish stocks, which partially supply fish farms (providing fishmeal and fish oil for feed), the need arises to explore alternatives and new raw materials like vegetable ingredients. However, their total replacement with plant-based ingredients leads to a significant alteration in the survival and growth performance of rainbow trout, starting from the first meal and throughout their life cycle. Increasingly, studies are identifying altered feeding behavior as a hypothesis, although few mechanistic studies have confirmed this so far. While the removal of fish meal and fish oil results in the removal of omega-3 intake (DHA/EPA), few data exist regarding the impact of these nutrients on the feeding behavior of farmed fish. Yet, nutrient detection plays a crucial role in food selection, assessment, and ingestion in animals. Therefore, understanding the mechanisms that regulate the feeding behavior of rainbow trout, such as the involvement of omega-3 fatty acids, is a prerequisite for acquiring crucial knowledge to develop new nutritional strategies.

Our research reveals that dietary omega-3 levels profoundly influence rainbow trout feeding preferences and behaviors. Fatty acid profiles and metabolites within the central nervous system correlate with these behaviors. Moreover, our research demonstrates that the nature of the diet (diet with fish meal and fish oil vs. plant-based) influences the sensory detection of nutrient, especially omega-3 sensing receptor at the gustatory system level in trout. Even after a single plant-based meal or 30 days of feeding, serotonin regulation—critical for taste information transmission and behavior—is altered.

These findings highlight both the disruption of integrated feeding responses by plant-based diets and the trout's remarkable sensitivity to new food sources. Addressing aquaculture challenges, our study aims to enhance fish feed quality and identify indicators for dietary resilience against ecological fluctuations

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Processing seafood: a major driver in omega-3 and trace element composition?

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Seafood represents a major part in human nutrition. This part can be particularly important for populations living on the coast, especially in West and Central Africa WCA. Seafood consumption is determined by cultural habits, which comprise both favored species, but also the way they are processed prior consumption. In a nutrition context, transformation is thus an essential step to consider to evaluate the nutritive value of a food. In WCA, smoking and drying are the most common transformations of seafood, allowing its conservation and availability all year. However, processing can alter seafood composition. Nutritional benefits and risks associated with seafood consumption depend on its composition in nutrients omega-3 fatty acids, essential trace elements, proteins and in hazardous elements, such as heavy metals or trace elements exceeding a certain threshold. The goal of this study is to understand how different processing methods alter seafood composition, and how the benefits-risks balance associated with their consumption are modified. To answer this question, six popular African species (five pelagic fishes and one bivalve) from contrasted locations in WCA and characterized by different transformation processes fresh, smoked, dried, canned had been analyzed. Several sources of variation have been observed. For instance, for *Ethmalosa fimbriata* (a small coastal oily fish), we observed no differences in EPA+DHA and trace elements between fresh and smoked fish. However, EPA+DHA contents varied between fishing seasons suggesting that environmental conditions and/or fish biology play a stronger part in fish nutritional composition than the way it is processed.

Replacement of fish oil by a high-DHA microbial oil in salmon diets: effect on lipid molecular species and gene expression

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The omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are essential nutrients for farmed fish and for their human consumers. Fish oil is one of the main sources of these long-chain polyunsaturated fatty acids; however, due to an increasing demand for EPA and DHA and with a limited supply from wild fisheries, there is a need for alternative sources that are sustainable and cost effective for aquaculture. Here we investigated replacement of fish oil with a high-DHA, low-EPA oil extracted from single-celled thraustochytrids. This provided a unique opportunity to determine how these dietary fatty acids independently influence lipid metabolism and physiological pathways in salmonids. A 16-week feeding trial was conducted with Atlantic salmon fed diets with a complete or partial replacement of fish oil with microbial oil. There was no significant difference in growth performance among the dietary treatments but we observed differences in lipid composition and gene expression. Using shotgun-based mass spectrometry analysis we investigated lipid molecular species, focusing on triacylglycerols (TAG) and diacyl-phospholipids. DHA in the microbial oil diet was efficiently incorporated into phospholipid structures on feeding, followed by accumulation in salmon muscle. The microbial oil diet elevated the level of certain EPA-containing TAGs, such as TAG C52:5 (16:0_16:0_20:5) and TAG C54:6 (16:0_18:1_20:5), indicating that the microbial oil diet may be an excellent source for enhancement of the abundance of ω3 lipids. Further, prostaglandins (PGs) PGE2 and PGF3α were identified and quantified for the first time in salmonid tissue. These results correlated with muscle, and especially hepatic lipid metabolism biomarkers: *hmgcr*, *fasb*, *pparaa*, *pparab*, *12lox* and *acly*; however, some transcript levels were the same with high dietary DHA (high microbial oil) and high EPA (fish oil) indicating successful replacement of fish oil with microbial oil.

Autotrophic and heterotrophic production of omega-3 long chain polyunsaturated fatty acids (n-3 LC-PUFA) in the Iroise Sea

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In the marine environment, both autotrophs and heterotrophs are able to biosynthesize *de novo* n-3 long chain polyunsaturated fatty acids (n-3 LC-PUFA) through different pathways. These molecules, including 20:5n-3 (eicosapentaenoic acid, EPA) and 22:6n-3 (docosahexaenoic acid, DHA), are crucial for the growth, development and survival of all organisms. Hence, because of their ability to produce n-3 LC-PUFA rich oils, some heterotrophs, such as thraustochytrids, are now being increasingly studied at industrial scale and have prompted attention to their contribution to primary production in the natural environment. The leading objective of this study is to assess the heterotrophic contribution to the marine production of n-3 LC-PUFA, over the autotrophic one, on a fine spatial scale, during spring blooms. To this, samples of suspended particulate organic matter (SPOM) were collected in the mixed layer and filtered for lipid analysis along two transects in the Iroise Sea during two different cruises (June 2023 and May 2024). The study of the lipid classes helped to identify glycolipid-rich and –poor samples. Glycolipids being specific to autotrophs, this suggests differences in the heterotrophic and autotrophic lipid production. Spatial variability in SPOM fatty acid composition was also evidenced along the two studied transects. Samples were mainly composed of EPA or DHA depending the station, thus involving a variation in communities of primary producers. This suggests a variable contribution of heterotrophic to autotrophic organisms to n-3 LC-PUFA production related to different biogeochemical parameters. However, the actual contribution of heterotroph is still difficult to assess as there are yet no known fatty acid biomarkers specific to heterotrophs. In order to quantify the proportion of n-3 LC-PUFA resulting from heterotrophic production, LC-MS-MS analyses could be carried out to study molecular species and the combination of fatty acids in each lipid class and try to identify new biomarkers specific to heterotrophs.

SESSION 6
GERLI/SFN: MARINE LIPIDS AND NUTRITION

Marine lecithin: From rich LC-PUFA sources to health benefits

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The marine environment represents a vast reservoir of biomolecules of interest in the fields of nutrition and health. However, the current exploitation of essential maritime resources, with a population of 8 billion individuals in 2024, must involve the rational management of species stocks and optimized valorization of these resources.

These marine resources are rich in nutrients and bioactive substances, such as long-chain n-3 polyunsaturated fatty acids (LC-PUFAs) that global health authorities recommend consuming for their health benefits in the prevention of numerous diseases, neural development, and the prevention of stroke and Alzheimer's disease. From microalgae to krill, small pelagics such as sardines, herring, anchovies, mackerel, or salmon and large pelagics like tuna, represent a pool of lipids of interest due to their richness in essential fatty acids such as docosahexaenoic acid (DHA C22:6 n-3) and eicosapentaenoic acid (EPA C20:5 n-3). These fatty acids, esterified in varying proportions on different lipid classes such as triacylglycerols and polar lipids, are the subject of numerous studies due to their implications for health and their different vectorization. The physicochemical and structural properties of these LC-PUFAs in emulsion or liposomal form will be presented with a focus on the interest of these amphipathic molecules (DSC, SAXS, AFM, TEM, etc.), which allow for the vectorization of both hydrophilic and hydrophobic molecules. Questions regarding their stability against initiated oxidation reactions or their fate under the action of lipolytic enzymes during digestion will be addressed.

Examples of vectorization of biomolecules (antioxidants, vitamins, calcein, curcumin, enzymes, peptides, etc.) will illustrate the interest of these polar lipids, as well as in vitro studies on their actions in transfers at the intestinal and blood-brain barrier membranes.

Chub mackerel and quinoa improve brain DHA levels and n-3/n-6 ratio in Alzheimer's disease

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With the life expectancy increase, the incidence of Alzheimer's disease (AD) became a major concern due to its socioeconomic impact. In the brain, docosahexaenoic acid (DHA, 22:6 n-3) is crucial to maintain neuron function and have been correlated with several neurological disorders like AD. As brain is almost completely dependent upon diet uptake of DHA, it is plausible to infer that the ingestion of this fatty acid (FA) may restore its imbalanced levels. To check this, 32 5xFAD transgenic mice (animal model for AD) were distributed into four groups, and fed with following diets: Control (C) (standard AIN-93M diet); Chub Mackerel (CM) (AIN-93M supplemented with 10% chub mackerel); Quinoa (Q) (AIN-93M with 5% quinoa); and Chub mackerel+Quinoa (CMQ) (AIN-93M with both 10% chub mackerel and 5% quinoa). Mice brain lipid fraction was analysed for its FA profile. When compared to Q and C groups whose DHA levels ranged from 11.4±2.1% to 12.3±1.1% of total FA, mice from CM group stood out with the highest contents (15.4±1.6%). This also led to a significant increment of n-3 PUFA registered in this later group (16.4±1.7%) in opposition to Q and C groups (11.8±2.0% and 13±1.1%, respectively). The reduction of arachidonic acid (ARA, 20:4 n-6) levels, a FA associated with inflammation, in groups fed with diets formulated with chub mackerel (6.1±0.6% and 5.5±0.2% determined in CM and CM+Q), should also be highlighted (C group: 7.8±0.6%), along with an improvement n-3/n-6 ratio (from 1.1±0.1 in C to 1.8±0.2 and 1.5±0.1 in CM and CM+Q). These results suggest that chub mackerel contributed to counteract the reduced DHA brain levels associated with AD condition. When combined with quinoa, chub

mackerel resulted in decreased levels of ARA and n-6 PUFA. Ultimately, this combination may mitigate inflammation, which is a key hallmark of AD.

Metabolic and brain effect of marine lipids as phospholipids vs triacylglycerides on aged mice: a lipidomics plus proteomics research

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Dietary choices and patterns are modifiable lifestyle factors which can modulate the process of aging. Current research suggests that diets rich in fish and fats containing antioxidants and omega-3 fatty acids, decrease cardiovascular diseases and obesity risk, protect the brain from aging, and promote an overall healthier life. Among the common features in aging, oxidative stress and low-grade chronic-inflammation are mechanistically implicated in the loss of homeostasis in both, metabolic and age-related diseases.

Consumption of n-3 PUFA including EPA and DHA occurs mainly through intake of fish, seafood or fish oil where PUFA are usually esterified to triacylglycerides (TGs). But they can also be found esterified to polar lipids as phospholipids (PLs). PLs are reported to concentrate higher n-3 PUFA content, more stable than TGs, suggesting that PLs baring n-3 PUFAs are a more beneficial form of PUFAs. A better bioavailability was also attributed to PLs.

This work proposes the intake of marine oils rich in TGs versus PLs to counteract the putative negative effect of chronic inflammation and oxidative stress occurring during aging. For that, we addressed the effect over the metabolic-brain-axis in an aged animal model fed marine lipids as TGs and PLs. Proteomics and Lipidomics on liver and brain cortex illustrated the pathway-oriented profiling of lipid metabolism and protein oxidation associated to oil intake. Behavioural research of the aged animals completed the study.

Results demonstrated that marine oils retarded brain lipid and protein alterations and working memory decrement in aging. Lipidomics and redox proteomics demonstrated that aging leads a pro-inflammatory phenotype, in both, liver and brain, through the activation of COX and sEH pathways. Moreover, PLs-marine oil showed the higher effects reducing oxidative stress and driving anti-inflammatory phenotypes.

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Production, molecular modeling and investigation of lysophospholipids-DHA crossing using an in vitro human model of blood brain barrier

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The aim of the present study was to evaluate toxicities, and passage of docosahexaenoic acid (DHA) in comparison to Lysophosphatidylcholine (LysoPC-DHA) and other lysophospholipids (LysoPL-DHA) exhibiting vital functions in the brain including lysophosphatidylethanolamine (LysoPE-DHA) and lysophosphatidylserine (LysoPS-DHA) using an in vitro model of human blood-brain barrier (BBB). The human brain-like endothelial cells (hBLECs) monolayer tightness, evaluated by the parallel assessment of the permeability of fluorescent marker Lucifer yellow (LY), revealed the absence of toxicity of non-esterified DHA and all LysoPL-DHA towards hBLECs. At 30, 60 and 120 min post-incubation, all tested LysoPL-DHA displayed a higher recovery in the abluminal medium in comparison to non-esterified DHA. Among all, LysoPS-DHA revealed the highest apparent coefficient permeability (Papp) 85.87 ± 4.24 x 10-6 cm.sec-1 and was significantly different than DHA, LysoPC-DHA and LysoPE-DHA exhibiting respectively 26.04 ± 0.11 x 10-6 cm.sec-1, 63.71 ± 5.7 x 10-6 cm.sec-1 and 75.04 ± 1.99 x 10-6 cm.sec-1. More interestingly, when studying the time course of Papp of DHA, LysoPC-DHA and LysoPE-DHA at 10 µM at 30, 60, 120 and 180 min, this permeability decreases with time especially for LysoPC-DHA and LysoPE-DHA, not for DHA. Furthermore, LysoPS-DHA exhibited the highest intracellular accumulation (10.39 ± 0.49%) in hBLECs in comparison to all other tested lipids. Finally, differences in 3D structures and molecular electrostatic potential maps calculation of produced LysoPL-DHA could explain the dissimilar cerebral uptake of LysoPL-DHA. Altogether, our findings suggest that LysoPS-DHA crosses preferentially the BBB and these findings could be of great significance to pharmaceutical applications.

Direct impact of dietary plant-based glycolipids on human gut microbiota and inflammation: first evidence from in vitro models

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Metabolic diseases are associated with an altered intestinal health, including imbalanced microbial communities and an impairment of the intestinal epithelium integrity, causing an increased permeability and associated low-grade intestinal inflammation. In the framing of plant-based diet promotion, it is necessary to better understand the roles and mechanisms of action of plant nutrients on health. Plant glycolipids found in many green vegetables and fruits remain poorly studied, and their effects on gut health have not yet been investigated. We thus explored here the impact of plant glycolipids on fecal human microbiota (healthy donor vs donor with intestinal inflammation) cultured in vitro using an innovative high-throughput microbiota modelling system, the MiniBioReactor Array (MBRA). Bacterial load, microbiota composition and pro-inflammatory potential (load of bioactive LPS and flagellin) were measured longitudinally. Lipidomic and metabolomic analyses were also performed on culture media. On the host side, intestinal HT29-MTX cells were exposed to glycolipids after a 24h DSS pro-inflammatory challenge to evaluate their effects on epithelial cell integrity, mucus synthesis and inflammation.

Using the MBRA system, we observed that plant glycolipids can directly stimulate bacterial load and significantly affect microbiota composition in dysbiotic donor. Regarding microbiota-associated metabolites, SCFA content was modified by plant glycolipid only in healthy microbiota, whereas some groups of metabolites (purines, aromatic amino acids, conjugated bile acids) were impacted by plant glycolipids in both microbiota. Moreover, plant glycolipids harboured anti-inflammatory potential by reducing levels of bacterial LPS and flagellin in

microbiota from both eubiotic and dysbiotic donors. Beneficial effects were also observed in the DSS challenged epithelial cells, with reductions in both expression and secretion of pro-inflammatory cytokine IL-8 after glycolipid treatment. These results show that plant glycolipids can directly impact gut microbiota composition and function in a manner expected to reduce intestinal inflammation, suggesting plant glycolipids as beneficial nutrients for gut health.

Impact of structuring DHA in marine lipids on its intestinal absorption and metabolic fate

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Docosahexaenoic acid (DHA) is an essential fatty acid (FA) with proven health benefits. Given the latest consumption surveys, improving its bioavailability become a public health challenge. Among strategies, structuring FA represents a new approach for increasing DHA bioavailability. In this context, we studied the impact of several DHA-rich formulas from microalgal oil, differing in their molecular structures: ethyl ester (DHA-EE), monoglyceride (DHA-MG) or triglyceride added to vegetable phospholipids (DHA-TG+ PL) or not (DHA-TG), on the intestinal absorption of DHA. We therefore monitored the lymphatic bioavailability of DHA in rats submitted to the different formulas. More, the lipid characteristics of the resulting lymphatic chylomicrons (LMC) were determined by the DHA composition of lymphatic TG and PL.

Results showed that DHA-EE was the least conducive to DHA bioavailability, whereas the MG-DHA structure presented a significantly higher Cmax of DHA (+50%) and AUC (+89%) values compared to the other structures. This improvement was explained by a by-pass of the lipolysis step with a faster Tmax (1-2h less) in MG group. In addition, DHA-MG structure allowed to DHA-enrich the lymphatic TG (+40%) and PL (+50%) more favourably compared to EE or TG structures.

By modifying the composition and structuration of LMC, the vectorisation and the metabolic fate of DHA could be different in the organism to specifically target some tissues. Thus, the molecular form of vectorisation of FA may therefore be a means for improving the digestion and the intestinal absorption of essential FA such as DHA, up to modulating the characteristics of LMC and thus directing its tissue and metabolic fate. Based on previous data, we hypothesise that structuring DHA as TG or as EE, DHA could be more prone to target tissues and liver metabolism, whereas structuring DHA as MG could target nervous tissues, such as the retina or brain.

Marine omega 3 fatty acids and insulin-resistance

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Marine n-3 fatty acids improve most of the biochemical alterations associated with insulin resistance (IR) especially inflammation of adipose tissue considered as a main trigger of IR associated to obesity. Experimental models of dietary-induced IR in rodents have shown their ability (often at a very high dose) to prevent IR, but with sometimes a tissue specific effect. In healthy subjects and insulin- resistant non-diabetic patients, most trials and meta-analyses conclude to an insulin-sensitizing effect. Concerning the risk of T2D, the most recent meta-analysis allows to conclude to a very probable protective effect, even if further studies using more homogeneous doses, and sources of n-3 would be useful.

SESSION 7
NEW PROGRESS IN LIPID METHODOLOGY

Marine lipidomics: a valuable tool to design innovative solutions for the valorisation of marine organisms

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Marine ecosystems harbour a vast diversity of life forms, which represent a rich source of inspiration for the development of biotechnological solutions. The unique adaptations and biochemical pathways developed by marine organisms to survive in extreme and varied conditions produce novel bioactive compounds and biomolecules. In recent years, the study of marine lipids origins has gained momentum, driven by the search for new ingredients and advances in analytical techniques. Among these lipids, fatty acids are the most studied; however, these compounds are usually found in very low amounts in their free form. Instead, they are mainly esterified into more complex lipids, such as polar lipids and triglycerides. Characterization of these complex lipids provides crucial insights to address various challenges in marine environments.

High-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) and tandem mass spectrometry (MS/MS) have been instrumental in characterizing the structures of polar lipids and elucidating their functionalities. Lipidomics studies across various groups of marine organisms have found significant applications in marine compound bioprospecting, seafood traceability, and ecological research. The analysis of lipidomes from marine oils has facilitated the bioprospecting of algal and fungal compounds with beneficial health effects. Furthermore, the adaptability of marine lipidomes to biotic and abiotic changes has been effectively utilized to trace the geographic origin of seafood. In ecological studies, lipidome analysis provided insights into the role of lipids in the adaptation and survival strategies of marine organisms.

The diverse applications of lipidomics studies in marine organisms highlight the increasing importance of obtaining a comprehensive view of marine lipids. From the discovery of new health-beneficial compounds to improving seafood traceability and advancing ecological research, lipidomics serves as a valuable tool to protect and sustainably use of marine ecosystem resources.

Bioproduction of ^{13}C-labeled docosahexaenoic acid (DHA) by the protist *Aurantiochytrium mangrovei* and the dinoflagellate *Crypthecodinium cohnii*

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Stable isotope labeling is a potent technique with promising applications, allowing the direct analysis of the metabolism, through the conversion of substrates into metabolites, and the fate of resulting metabolites. ^{13}C-labeled long-chain polyunsaturated fatty acids (LC-PUFA) serve as metabolic tracers to investigate their uptake and metabolism in the human body. However, the availability of ^{13}C-labeled LC-PUFA is limited and costly due to their expensive chemical synthesis. An alternative method involves the biosynthesis of ^{13}C-labeled LC-PUFA using heterotrophic microorganisms cultivated on ^{13}C-substrates. Despite this, achieving uniformly ^{13}C-labeled docosahexaenoic acid (DHA, 22:6n-3, $C_{22}H_{32}O_2$), an essential LC-PUFA, has been challenging. This study focuses on ^{13}C-DHA enrichment using cultures of the protist *Aurantiochytrium mangrovei* and the dinoflagellate *Crypthecodinium cohnii*, grown on a medium containing uniformly ^{13}C-labeled glucose as the sole carbon source. Other nutrients were supplied in inorganic forms to avoid ^{12}C dilution effects of organic compounds. Gas chromatography coupled with mass spectrometry (GC-MS) allowed the quantification of stable isotope labeling within fatty acids, particularly the ratio of ^{13}C to total carbons, while employing Selected Ion Monitoring (SIM) mode increased sensitivity and facilitated the quantification of various DHA isotopologues. GC-MS analysis of ^{13}C-enriched DHA obtained from *A. mangrovei* and *C. cohnii* culture revealed that the dominant isotopologues of the molecular ion corresponded to $^{13}C_{22}H_{32}O_2$, $^{13}C_{21}{}^{12}CH_{32}O_2$ and $^{13}C_{20}{}^{12}C_2H_{32}O_2$. This analysis estimated the isotopic enrichment of ^{13}C-DHA over 90% for *A. mangrovei* and over 80% for *C. cohnii*, after a single cycle of ^{13}C enrichment culture. This work underlines the potential of using these microorganisms as an innovative "factory" for the biosynthetic production of ^{13}C-labeled DHA.

In-situ monitoring of lipid hydrolysis at the molecular and supramolecular level by Nuclear Magnetic Resonance

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Lipid hydrolysis is often monitored at the molecular level upon extraction from reaction mixtures in organic solvent. This extraction step is considered as a prerequisite for the collection of high-resolution data such as the molecular mass (mass spectrometry), proton or carbon nuclei frequency and couplings (Nuclear Magnetic Resonance), molecular vibration frequencies (infra-red spectroscopy). Besides, the substrates and products of hydrolysis reactions can also be characterised by chromatography thanks to molecular standards.

The advantage of this strategy is that it enables controlling specific reaction conditions (temperature, mixing). In particular the supramolecular organisation of lipids is critical: the presence of surfactants enables the presentation of substrate of hydrolysis under different supramolecular organisations (micelles, mixed micelles, nanodiscs, liposomes…) that can favour (or disfavour) the enzymatic activity of the lipolytic enzymes. Over the course of hydrolysis, this supramolecular organisation is likely to be remodelled according to the critical micelle concentration of the products. Unfortunately, the above-mentioned methods do not enable the monitoring of such supramolecular transitions.

Here, we show that Diffusion Ordered NMR spectroscopy (DOSY) can be used to probe for the supramolecular organisation(s) of substrates and products of hydrolysis in situ. Because liquid NMR is a non-destructive method, the NMR tube can be considered as a mini-reactor in which the conditions of temperature are controlled. The hydrodynamic properties of the supramolecular assemblies composed of different compounds is probed using Pulsed-Field Gradient. DOSY diffusion coefficients measurement is an orthogonal from the classic NMR chemical shift measurement, and both data are measured in a two-dimensional data matrix. The molecular compositions of the supramolecular assemblies are thus also determined in contrary to Dynamic Light Scattering measurement. In summary, in-situ DOSY measurement can be used to determine the molecular and micellar transitions that occur during lipid hydrolysis.

Comparative omics provide insights into the synthesis of unusual fatty acids in alternative crops

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To address global issues related to food and energy security, my research group is studying alternative crops, historically considered as weeds until recently being identified as promising sources of renewable specialty fuels and other industrially-relevant chemicals. Indeed, *Physaria fendleri* (aka. lesquerella) and *Thlaspi arvense* (aka. field pennycress), winter annuals closely related to *Arabidopsis thaliana*, produce and store in their seeds unusual fatty acids (FAs) that can replace several petrochemicals currently used in industry. Our long-term goal is to advance these plants—that can be grown off-season—as dedicated bioenergy and industrial crops. However, for these oilseed crops to become economically viable sources of unusual FAs, oil synthesis needs to be improved. A lack of knowledge of the metabolic pathways underlying FA synthesis in *Physaria* and pennycress seeds presents a major constraint. This study aims to find potential biochemical step(s) that limit(s) oil synthesis, which will serve as targets for future crop improvement.

To advance towards this goal, we analyzed the endosperm composition by LC-MS/MS to develop and validate culture conditions that mimic the development of the embryos *in planta*. Using developing *Physaria* and pennycress embryos in culture, we were able i) to determine the efficiency with which embryos convert substrates into biomass components, and ii) to replace the substrates by ^{13}C-labeled ones and monitor the flow of ^{13}C-carbon in central metabolic pathways leading to oil synthesis. Our studies demonstrated that pennycress embryos metabolize carbon into biomass with an efficiency significantly higher than other photosynthetic embryos. Interestingly, *Physaria* and pennycress use non-conventional pathways to channel carbon into oil. In parallel, comparative metabolomics and transcriptomics identified potential metabolic engineering targets to improve oil content and composition. This study describes the combination of innovative tools that will pave the way for controlling seed composition in promising alternative crops.

Determination of lipid oxidation status in different matrices and determination of fat parameters for edible oils by NMR

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For the analysis of edible fats and oils, various methods have already been established. A particular focus in oil analysis is on determining the most important classical fat parameters as indicators of lipid oxidation status (peroxide value, anisidine value, iodine value, acid value). The classical methods for determining fat parameters involve various titrations. These are known to be nonspecific, and their results are influenced, for example, by accompanying fat components.

Nuclear magnetic resonance spectroscopy (NMR spectroscopy) offers, as a modern measurement method, the possibility to determine these fat parameters using a single quantitative ^{1}H-NMR experiment. In this process, the analytes underlying the fat parameters are specifically determined. By converting these into the fat parameters, comparability of the results with those of the classical methods is established. Sample preparation takes less than 5 minutes, and a measurement takes up to 15 minutes.

We would like to present our results from the application of this method in the analysis of various sample matrices of different oxidation stages, as well as demonstrate how the oxidation process of vegetable oil or lecithin samples can be tracked using ^{1}H-NMR. In 2024, a study on the determination of oxidation parameters in algae oil products using NMR is planned, and we would like to additionally present its initial results.

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The NMRlipids Databank: Enhancing Lipidomics Research through Programmatic Access to Molecular Simulation Trajectories

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We present the NMRlipids Databank—a community-driven, open-for-all database of molecular dynamics (MD) simulation data of lipid membranes, quality-evaluated against high-resolution experimental data.

Using the overlay databank format, it collects experimental and MD simulation data scattered in various locations and formats, and makes them accessible both through a GUI (Graphical User Interface) and an API (Application Programming Interface).

We envision that programmatic access through the API allows flexible implementation of data-driven and machine learning applications, thus unlocking possibilities beyond current MD simulation and experimental studies to decipher the detailed effects of lipid composition on the functionality of cellular membranes. As a proof-of-principle, we demonstrate one such application: The analysis of water diffusion through membranes, which as a rare event is beyond the sampling capabilities of regular simulation volumes, but highly relevant for applications such as diffusion tensor magnetic resonance imaging and drug penetration through membranes.

Kiirikki et al. **Overlay databank unlocks data-driven analyses of biomolecules for all** *Nature Communications* 15 1136 (2024)

12-Hydroxyeicosatetraenoic acid is the only enzymatically produced HETE increased under brain ischemia

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Brain ischemia is characterized by an arrest of cerebral blood flow, causes irreversible brain damage, and is the leading cause of death and adult disability. Brain ischemia results from ischemic stroke, cardiorespiratory arrest, traumatic brain injury, and aging, among other pathological conditions, but might also accompany surgeries and anesthesia. Hydroxyeicosatetraenoic acids (HETE) are dramatically increased under brain ischemia and significantly affect post-ischemic recovery. However, the exact mechanism of HETE increase and their origin under ischemia are poorly understood. HETE might be produced *de novo* through lipoxygenase (LOX) -dependent synthesis with possible esterification into a lipid storage pool, or non-enzymatically through free radical oxidation of esterified arachidonic acid (20:4n6). Because HETE synthesized through LOX exhibit stereospecificity, chiral analysis allows separation of enzymatic from non-enzymatic pools. In the present study, we analyzed free HETE stereoisomers at 30 sec, 2 min, and 10 min of ischemia. Consistent with previous reports, we demonstrated a significant, gradual increase in all analyzed HETE over 10 min of brain ischemia, likely attributed to release of the esterified pool. The R/S ratio for 5-HETE, 8-HETE, and 15-HETE was not different from a racemic standard mix, indicating their non-enzymatic origin, which was in opposition to the inflamed tissue used as a positive control in our study. However, 12(S)-HETE was the predominant isoform under ischemia, indicating that ~90% of 12-HETE are produced enzymatically. These data demonstrate, for the first time, that 12-LOX is the major LOX isoform responsible for the enzymatic formation of the inducible HETE pool under ischemia. We also confirmed the requirement for enzyme inactivation with high-energy focused microwave irradiation (MW) for accurate HETE quantification and validated its application for chiral HETE analysis. Together, our data suggest that 12-LOX and HETE-releasing enzymes are promising targets for HETE level modulation upon brain ischemia.

POSTER SESSION 1
STRUCTURAL DIVERSITY OF LIPIDS FROM SUMMITS TO DEEP SEA

Atmospheric deposition (wet and dry) is a significant source of fatty acids for ecosystems?

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Fatty acids are fundamental in functional ecology and are widely studied in terrestrial and aquatic environments. Despite their functional importance, no study has focused yet on fatty acids in the atmosphere. The fatty acids within atmospheric biological matter, such as micro-organisms and debris, are transported by wind dynamics and deposited on ecosystems through wet and dry deposition. Moreover, one recent study using Fourier-Transform ion cyclotron resonance mass spectrometry (FT-ICR MS) have highlighted that lipid-like compounds represent up to 50% of the molecular formula of organics dissolved in cloud water (Pallier et al., 2024).

In this study, we first attempt to identify the fatty acids in different phases of the atmosphere, i.e. the particulate phase (aerosols) and the aqueous phase (cloud and rain), and then to estimate the deposition fluxes of fatty acids from the atmosphere to surface environments. A one-year environmental observation is ongoing, with the aerosols and clouds sampled at the summit of puy de Dôme (1465m a.s.l.), and rain collected at the periurban site Opme (655m a.s.l.). Here, we present the preliminary results obtained from four aerosol samples collected in 2015. Saturated fatty acids with even chains were the most varied fatty acids from 10:0 to 28:0. The samples showed few monounsaturated and polyunsaturated fatty acids, 18:1ω9, 18:1ω7, and 18:2ω6. For all four seasons, the major fatty acids were 16:0, 18:0, and 18:1ω9. In addition, the samples showed low levels of the fatty acids commonly found in bacteria, 15:0, 17:0, and iso15:0. Lastly, we also observed other compounds, phthalates, and dicarboxylic acids. This could be due to atmospheric ageing.

The results obtained are promising and represent the first identification of fatty acids from the particulate phase of atmosphere. The further analysis of cloud and rain samples will provide insights on their concentration in the aqueous phase and deposition.

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Taxonomic identification by DNA barcoding of several Mauritanian macroalgae and comparison of their lipid profile

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Banc d'Arguin National Park (BANP) is a Mauritanian marine protected area, created in 1976 with an area of 12,000 km² (maritime and continental). The BANP presents a very rich and diversified marine biodiversity with the presence of many animal and plant species. A particularity of this ecosystem is that it is subjected to drastic and fast changes such as sandstormsor slower ones such as the anthropization of the environment, which have a significant impact on the populations of local flora and fauna. The study of this ecosystem which adapts to these particular environmental conditions becomes relevant in the context of global climate change. The macroalgae of this protected site are very little known and are not much studied, despite their importance in the food chain for the fauna of the BANP, their interest for the study of biodiversity and their potential for development. We recently initiated the characterization of the galactolipid macroalgea membranes because they represent up to 80% of the fatty acid stocks, including a large proportion of polyunsaturated fatty acids such as α-linolenic acid (ALA). In this study, several species from 3 different classes of macroalgae (green, red and brown algae) were identified by DNA *barcoding* technique. Total lipids were extracted using Folch's method and isolated by preparative TLC. Isolated products were characterized by TLC, NMR and MALDI-tof mass spectrometry. Total fatty acid content was assessed by GC.

Multi-step total synthesis of phenolic phytoprostanes

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Lipophenols, polyphenolic compounds acylated by a fatty acid, have recently been identified in food matrices naturally rich in both polyphenols and fatty acids, making them natural derivatives present in human diet. The identification of natural lipophenols is particularly relevant to understand their pharmacological actions, metabolism or to use them as analytical standards.

As an example, hydroxytyrosol (HT) linked to polyunsaturated fatty acids (PUFA) is naturally present in extra virgin olive oil (EVOO)[1] and should participate to its antioxidant properties. As a preliminary work, the chemical synthesis of HT lipophenols allowed UHPLC-MS/MS quantitative study in EVOO during a 12 months period, mimicking both commercial and inappropriate conditions of storage. The results highlighted HT-OA as a relevant marker for the monitoring of oil storage conditions and quality.[2] Based on this study, an emphasis was put on HT-ALA, exhibiting a different analysis pattern than its analogues. This result might be due to oxidation of this compound to form **phenolic phytoprostanes**. Phytoprostanes (PhytoPs) are non-enzymatic lipid peroxidation products coming from ALA, biomarkers of oxidative stress in plants.

This hypothesis was strengthened by the literature, showing that phytoprostanes coming from ALA were present in some vegetal oils,[3] as well as preliminary oxidation studies on HT-ALA in flask.

The first stereoselective total synthesis of phenolic PhytoPs as analytical standards was therefore performed in 20 steps with a 3% global yield (84% average yield by step) from commercially available 1,3-cylooctadiene. The lactol key intermediate was synthesized in 11 steps with controlled stereochemistry, which then allowed the introduction of the two side chains using Wittig and HWE reactions.

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Exploring the variability of cuticle compounds in different grapevine cultivars

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Plant cuticle is the first shield which protects aerial epidermis against environment constraints, notably by limiting water loss, UV irradiation damage, pest infestation and pathogen infection. This lipophilic barrier is subdivided in two parts: (i) an external wax layer constituted of very-long-chain fatty acids, primary alcohols, alkanes, aldehydes or triterpenoids, and (ii) a deeper cutin polymer made of hydroxy-/epoxy- fatty acid monomers, which is to a certain extent integrated to the cell wall. Cuticular compounds identification and quantification are well established in different species such as grapevine, but rarely over a wide range of cultivars. By targeting a single organ maturity, we provide the first leaf and berry cuticle composition comparison study which integrates about fifty grape varieties from the same vineyard. GC-MS coupled with GC-FID analyses indicate that leaf waxes are mostly composed of primary alcohols 1-hexacosanol or 1-octacosanol while berry waxes are dominated by triterpenoids oleanic and ursolic acids. Classically, cutin of both organs contains predominantly hydroxylated palmitic acid and hydroxylated or epoxylated stearic and oleic acids. Despite the similar composition for each organ cuticle, wax and cutin compounds were differentially accumulated according to cultivars. This study supplies an overview of cuticle diversity within the same species taking grapevine as model. Cuticle relationship with agronomic traits observed during the sampling year will be highlighted as well as variety tolerance against abiotic and biotic stress.

Uncovering the functional lipidome of *Dunaliella salina*: bioactive lipids with potential nutraceutical applications

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Non-communicable diseases (NCDs) are a burden in Western society, caused by unhealthy diets and poor eating habits. The high intake of saturated fats and trans fatty acids (FA), and the deficit in omega-3 polyunsaturated fatty acids (PUFA), among others, contribute to oxidative stress and inflammation, which can aggravate age-related disorders and NCDs. Microalgae are gaining attention as a potential solution to achieve sustainable and healthy diets, namely as source of omega-3 PUFA, polar lipids, and pigments.

Dunaliella salina is a green microalga, known for its richness in high-value biomolecules, namely carotenoids and lipids. This edible microalga has been extensively explored for ®-carotene production. Besides, *D. salina* is commonly rich in essential FA, such as ⟨-linolenic (ALA), as well as in oleic acid (OA). However, FAs are mainly esterified to complex lipids, such as triglycerides (TG) as well as to membrane phospholipids (PL) and chloroplast glycolipids (GL). Nevertheless, its lipidome has not yet been characterized so its valorization as a source of functional lipids has been neglected.

In this work, a detailed lipid profiling of *D. salina* was determined using both liquid chromatography and gas chromatography coupled to mass spectrometry, and its bioactive potential was studied using *in chemico* assays.

A total of 306 lipid molecular species were identified, including phospholipids, glycolipids, betaine lipids, sphingolipids, and neutral lipids, rich both in ALA and OA. Additionally, some of the identified lipid species have already been reported with bioactive properties. In fact, lipids

from *D. salina* showed anti-inflammatory activity by inhibiting cyclooxyenase-2 activity, antioxidant scavenging activity, and antidiabetic activity by inhibiting ⟨-glucosidase activity.

Altogether, these results highlighted the potential of this microalga to be used as a functional food ingredient or in nutraceutical industries.

Upgrading the lipidome of *Gelidium corneum* biomass and by-products as sources of added-value lipids and bioactive ingredients

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Seaweeds are a relevant source of high-value compounds, including lipids, pigments, and other compounds, which have applications in various biotechnological sectors. *Gelidium corneum* is a red macroalga that is commonly known for producing agar. However, the industrial extraction of agar from its biomass creates a subsequent residue erroneously treated as waste. This poorly valorized by-product can be a potential source of high-value compounds. This study is dedicated to characterizing and bioprospecting the lipid profile of *G. corneum* biomass and its industrial residue post-agar extraction (from Iberagar). Results revealed a total of 9 fatty acids (FA) identified for the biomass and residue after transmethylation and gas chromatography-mass spectrometry analysis. Arachidonic (omega-6 C20:4) and eicosapentaenoic (omega-3 C20:5) acids were found in greater abundance in the biomass, while linoleic acid (omega-6 C18:2) was detected with higher abundance in the residue. The lipidome profiling obtained using reverse-phase liquid chromatography-mass spectrometry showed that both biomass and residue contain several classes of phospholipids, glycolipids, and sphingolipids, although a marked decrease of glycolipids was observed in *G. corneum* residue compared with initial biomass. In addition, both lipid extracts showed antioxidant activity by scavenging the 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical cation ($ABTS^{\cdot+}$) and the 2,2-diphenyl-β-picrylhydrazyl radical ($DPPH^{\cdot}$), with a slightly higher effect promoted by the lipids from *G. corneum* biomass. Additionally, a mild antidiabetic activity was detected through the evaluation of α-glucosidase inhibition, showing a slightly higher effect in *G. corneum* residue, which is probably promoted by the lipids. Overall, these results contribute to the valorization of the biomass and the industrially processed waste of *G. corneum* as sources of added-value ingredients like bioactive lipids for application in a variety of industries, such as cosmetics, cosmeceuticals, and nutricosmetics.

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Exploring the lipidome of *Ulva rigida*: unlocking its potential as a source of bioactive lipids

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Edible macroalgae *Ulva* spp. are widely distributed across the globe, offering significant promise as a valuable resource for blue bioeconomy. However, its potential remains largely untapped, particularly concerning bioactive lipids. Algal lipids, renowned for their healthy benefits, represent a compelling alternative to traditional fish oil sources. However, *Ulva*'s lipidome remains relatively underexplored.

This study aimed to unravel the lipidome of sustainably cultivated *Ulva rigida* using liquid chromatography coupled to high-resolution mass spectrometry (LC-MS) as an approach to screen and bioprospecting of bioactive lipids with antioxidant and anti-inflammatory properties.

The lipidome of *U. rigida* revealed the presence of beneficial *n*-3 fatty acids for human health, namely alpha-linoleic acid (ALA, 18:3 n-3) and docosapentaenoic acid (DPA, 22:5 n-3). Additionally, a diverse array of lipid species, namely glycolipids, betaine lipids, and phospholipids, were identified, including some species bearing polyunsaturated fatty acids which bioactive properties have been already reported.

Furthermore, *U. rigida* lipid extract demonstrated antioxidant and anti-inflammatory activities through ABTS and COX-2 inhibition assays, respectively. This research sheds light on the untapped potential of *U. rigida* as a source of bioactive lipids and underscores the importance of mass spectrometry as a sensitive tool for lipidome analysis. Moreover, it paves the way for further exploration into the multiple applications of *Ulva*-derived lipids across industries, spanning from pharmaceuticals to functional foods.

Lipid metabolism during dormancy of sweet cherry floral buds: preponderance of 3-hydroxylated FAs in MGDG

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Since winter and spring temperatures greatly influence bud dormancy progression, with acute impacts on flowering and fruit production, understanding the mechanisms that take place in buds during dormancy onset, maintenance and release, appears crucial in the current context of global warming. Recently, abundant lipid droplets have been observed in dormant buds suggesting that neutral lipids accumulate during dormancy. The presence of neutral lipids in dormant buds is enigmatic, and their role in dormancy release stays unexplored.

In order to better understand the lipid metabolism occurring during flower bud dormancy, we have determined the kinetics of lipid accumulation and followed the transcriptional events associated with lipid metabolism and lipid droplet biogenesis during bud formation and dormancy breakage in sweet cherry (*Prunus avium* L.) flower buds. Our results show an accumulation of neutral lipids during dormancy, mainly TAGs and steryl esters. Surprisingly, important amount of peculiar 3-hydroxylated fatty acids were also detected, that represent around 50% of the MGDG fatty acids in flower buds. The unusual accumulation of these 3-hydroxylated fatty acids in cherry buds at the entry of dormancy, and their total disappearance at blooming, suggest that they may serve as a marker of dormancy. Their functional significance in dormant buds and the enzymes responsible for their accumulation are still under investigation.

First characterization of the lipid composition of a giant virus membranes

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Moumouvirus australiensis, a giant virus belonging to the family Mimiviridae [1], infects species of Acanthamoeba. It enters its host through phagocytosis, releasing a nucleoid compartment into the host cytoplasm after its internal membrane fuses with the phagosome. Despite 20 years of research on Mimiviridae, little is known about the origin and composition of their membranes, which are crucial for productive infection. This study presents the preliminary characterization of the moumouvirus membrane lipid composition using two-dimensional silica gel high-performance thin-layer chromatography (HPTLC) and LC-MS/MS, on lipids extracted from purified capsids. The lipidomic analyses identified various lipid classes, including Triacylglycerol (TAG), Free fatty acid (FFA), Diacylglycerol (DAG), Monoacylglycerol (MAG), Diacylglycerol trimethylhomoserine (DGTS), Monoacylglyceryl-trimethylhomoserine (MGTS), and Diacylglycerylcarboxy-N-hydroxymethyl-choline (DGCC). Interestingly, no phospholipids were identified. Given that Acanthamoeba cells can synthesize these lipids and that Mimiviridae viruses recycle ER-derived vesicles to construct their membranes [2], our findings question whether the ER membranes share the same composition as moumouvirus membranes or if the virus modifies the membrane composition to create its own. Ongoing studies are tracking host-derived fatty acids using Acanthamoeba cells grown in minimal media with 13C-glucose to help distinguish host-derived lipids from virus neo-synthesized lipids.

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Functional characterization of a plastidial cytochrome b5-fused Δ4 desaturase from *Ostreococcus tauri* in higher plants

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Marine microalgae are the primary producers of important lipids in oceanic ecosystems. In particular, marine microalgae fuel the food web with omega-3 very-long chain polyunsaturated fatty acids (ω3-VLC-PUFAs), which provide a protective role against a range of human metabolic disorders and are therefore considered highly health beneficial. *Ostreococcus tauri* is a pico-eukaryote that contains 22:6 (DHA) in microsomal lipids as well as many ω3-PUFAs, including 18:5 and 16:4, which are highly abundant in plastidial galactolipids but also found in betaine and neutral lipids (Degraeve-Guilbault et al., 2017).

The genome of *O. tauri* contains 13 genes putatively encoding fatty acid desaturases. In the present study, we characterized the enzyme encoded by OT_ostta13g01550 (CEG00114.1) as a plastidial cytochrome b5-fused delta-4 desaturase involved in 16:4 biosynthesis. Transient heterologous expression in *Nicotiana benthamiana* resulted in the production of $16{:}4^{\Delta4,7,10,13}$ and $16{:}3^{\Delta4,7,10}$, but failed to produce $18{:}5^{\Delta3,6,9,12,15}$ when coexpressed with plastidial Δ6-desaturases. Stable transgenic Arabidopsis lines were also generated, and their characterization will be presented.

Reference:

Degraeve-Guilbault C, Bréhélin C, Haslam R, Sayanova O, Marie-Luce G, Jouhet J, Corellou F (2017) Glycerolipid characterization and nutrient deprivation-associated changes in the green picoalga *Ostreococcus tauri*. Plant Physiol 173: 2060–2080

Temperature and salinity induce modulation of the lipidome of the microalga *Tetraselmis striata* CTP4: a lipidomics investigation

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Tetraselmis striata CTP4 is a euryhaline, eurythermal microalga, rich in lipids, including omega-3 polyunsaturated fatty acids (PUFAs). However, little is known about how salinity and temperature affect its fatty acid profile and distribution of the membrane polar lipids and reserve neutral lipids across this microalga lipidome. Thus, this work sought to evaluate the plasticity of the fatty acid profile and the lipidome of *T. striata* CTP4 grown under different combinations of salinity (5, 20 and 35 ppt) and temperature (10, 20, 30 and 40°C) by gas-chromatography (GC-MS) and liquid-chromatography coupled to mass spectrometry (LC-MS). *Tetraselmis striata* biomass produced under all combinations of salinity and temperature showed a high abundance of PUFAs, especially omega-3 PUFAs. Temperature was the variable that mostly affected the FA composition of *T. striata* CTP4, while little to no changes were observed under different salinities, for the same temperature. The cultures grown under the condition T30-S20 achieved the highest accumulation of PUFA (biomass of 108.0±19.4 mg FA.g-1), and omega-3 PUFA (biomass of 66.3±12.2 mg FA.g-1). The content in omega-3 PUFA decreased in cultures at 20°C when compared to 10°C, and accompanied by an increase in the omega-6/omega-3 ratio. Fatty acids from *T. striata* were distributed across different polar and neutral lipids which were altered by salinity and temperature. Lipid species up-regulated at 30°C and at all salinities appear to correspond to less unsaturated species, while at 10°C and 20°C the up-regulated lipid species appeared to be more unsaturated. *Tetraselmis striata* CTP4 grown at 40°C had higher monounsaturated FA and triacylglycerol productivity, although imposing severe limitations on its survival. Overall, the different combinations of salinity and temperature allowed the production of biomass with dissimilar lipid composition and nutritional value with promising biotechnological applications.

POSTER SESSION 2
ROLES/FUNCTIONS OF LIPIDS AND DERIVATES

Investigating herbicide targets in the very long-chain fatty acid biosynthetic pathway

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Very-long-chain fatty acids (VLCFA), with 20 or more carbons, are essential molecules with significant physiological and structural functions in plants because they are components of key lipids such as membrane phospholipids and sphingolipids, seed triacylglycerols, cuticular waxes and root suberin. The elongation of VLCFA is carried out by the Fatty Acid Elongase complex which performs a four-step reaction cycle that adds two carbons to the growing fatty acyl chain. KCS enzymes, which are responsible for the first condensation reaction, are known for their high genetic diversity in plants, and functional characterization of several Arabidopsis KCS genes has revealed different chain-length specificities.

Herbicides that inhibit VLCFA synthesis (HRAC group 15) play a crucial role in weed management strategies. The mode of action of these compounds was initially identified based on the characteristic phenotype observed in treated plants, suggesting that they target the KCS subunit of the FAE complexes. Most commercially available herbicides exhibit stronger activity against grass plants (monocotyledonous) than broadleaf plants (dicotyledonous), though the reasons for this selectivity are not well understood.

The objective of this project was to further explore the inhibition of VLCFA synthesis by HRAC 15 group herbicides. To do so, a phylogenetic analysis and a comparative functional analysis of KCS from various plant species were performed using a yeast heterologous system reconstituting complete plant FAE complexes. In addition, in vivo and in vitro studies using yeast expressing active KCSs treated with several herbicides, were conducted to investigate their mode of action and selectivity.

Lipid dynamics in the cold-water coral *Dentomuricea aff. meteor*: effects of ocean warming and reproductive condition

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Dentomuricea aff. meteor is an important bioengineering cold-water coral gorgonian, in the Azores seamounts. It forms extensive coral gardens mostly at depths of 200 to 400 meters, that provide essential habitat, used as shelter, breeding and feeding grounds for other organisms, contributing significantly to the biodiversity in the Azores region. However, these coral gardens face increasing threats, such as ocean warming.
In corals, lipids constitute up to 40% of dry organic matter and are vital for their health, and physiological processes such as metabolism and reproduction. These essential nutrients are categorized into neutral lipids, serving as energy reserves, and polar lipids, contributing to cell membrane structure. Lipid class composition respond to environmental factors such availability of food and changes in environmental parameters (temperature and pH), making them valuable trophic biomarkers and indicators of physiological performance and stress.
Considering the threat presented by ocean warming and the importance of lipids in coral physiology, the primary focus of this study was to investigate the potential dynamic changes in the lipid class composition of *D. meteor* colonies under different temperatures: 14°C, 16°C, corresponding respectively to the species' minimum and maximum natural temperature range, and 19°C corresponding to the IPCC RCP8.5 prediction scenario (+3°C from the species' maximum natural temperature range). The study was conducted over a period of 6 weeks to evaluate potential changes over time. Additionally, we explored these variations during the reproductive season. Lipids were extracted using a modified Folch method then separated by High Performance Thin Layer Chromatography. For the results, we anticipated variabilities in the distribution of neutral lipids *vs* polar lipids, and even within the polar lipid classes due to adaptation to the different temperatures.
Furthermore, we expected higher lipid concentrations in females compared to males and before spawning due to their higher reproductive investment. Then, a post-spawning decrease in lipid content is expected for both sexes, particularly pronounced in females at higher temperatures, considering the energetic costs associated with spawning and ocean warming.

Oxidative stress mitigation in horticultural crops using foliar applications of *Ilex paraguariensis* extract: a dose-dependent study

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Abiotic stress has been shown to induce the formation of reactive oxygen species (ROS) in plant cells. When the level of ROS surpasses the capacity of the endogenous defence mechanism, oxidative stress status is reached, leading to plant damage and a drop-in crop productivity. Under oxidative stress conditions, ROS can react with polyunsaturated fatty acids to form oxidized derivatives called phytoprostanes (PhytoPs) and phytofurans (PhytoFs), which are recognized as biomarkers of oxidative damage advance. Modern agriculture proposes the use of biostimulants as a sustainable strategy to alleviate the negative effects of oxidative stress on plants. This work evaluates the dose effect of natural antioxidant extract to mitigate the oxidative-stress deleterious effects in melon and sweet pepper exposed to thermal stress. The plants were sprayed with Ilex paraguariensis (IP) aqueous extract in three different concentrations before exposure to abiotic stress. PhytoP and PhytoF levels were determined in the leaves of melon and pepper plants. IP1 and IP2 were effective against oxidative stress in both plants, with IP1 being the most protective one. IP1 decreased the levels of PhytoPs and PhytoFs by roughly 44% in both melon plants and pepper plants. The yield, with IP1, increased by 57 and 39% in stressed melon and pepper plants, respectively. IP3 foliar application in melon plants induced a pro-oxidant effect rather than the expected mitigating action. However, in sweet pepper plants, IP3 decreased the oxidative stress progress and increased the fruit yield.

Changes in lipid and fatty acid contents of gonad during the reproductive cycle of the Mediterranean swordfish *Xiphias gladius*

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Swordfish (*Xiphias gladius*) is a large pelagic fish distributed worldwide and is heavily exploited for human consumption. Despite its popularity, there is limited knowledge of its reproductive biology, particularly concerning lipid dynamics in gonads. In teleost fish, reproductive success and offspring survival are associated to lipid availability for gamete synthesis. This study aimed to analyse the lipid composition, including lipid classes and fatty acids (FA) in both cell membrane and reserve lipids (i.e., polar and neutral lipids, respectively; PL and NL), throughout the development of male and female gonads from the swordfish population from Corsican waters in the Mediterranean Sea. Overall, swordfish gonads contained less than 2% wet weight of total lipids, with testes and ovaries showing similar fat content. Lipid classes and FA concentration remained unchanged during testes maturation, while the concentration of phosphatidyl choline (PL), triaglycerol (NL) and certain FAs like 16:0, 18:1n-9, and 22:6n-3 followed an "inverted U-shaped" relationship with the ovarian maturation. In both PL and NL, 22:6n-3 was the predominant polyunsaturated FA (>20% of total FA), while 20:5n-3 and 20:4n-6 were minor (3-6%) and showed little variation during maturation. 22:6n-3 and 18:1n-9 were selectively allocated to the ovarian maturation until spawning. Finally, swordfish ovaries could serve as a valuable food source for humans, considering150 g serving can meet the daily omega-3 requirements. However, further research on pollutants is essential to assess their impact on swordfish reproduction and the safety of gonads for human consumption.

POSTER SESSION 3
LIPID MARKERS IN TROPHIC ECOLOGY: PROMISES AND DEAD ENDS

Cracking the Code: advancing fatty acid analyses in Antarctic benthic species by Gas Chromatography-Mass Spectrometry

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Fatty acid (FA) composition is a useful information for investigating trophic ecology of marine organisms and how they interact within food webs. In Antarctica, the diversity and availability of food sources depends on the ice cycle and disturbances. Benthic organisms receive variable quantities of sinking sea ice algae or phytoplankton. Benthic primary producers (e.g. macroalgae, microphytobenthos) may also fuel the food web. The TANGO expedition, held in austral summer 2023, aimed at characterizing the FA profiles of benthic consumers along the West Antarctic Peninsula. Here, the focus was on a frequently encountered benthic mollusk, the limpet *Nacella concinna*. After transmethylation of lipids and formation of FA methyl ester (FAME), gas chromatography coupled to ionized flame detector (GC-FID) revealed a high diversity of FA. Biomarkers of bacteria (18:1n-7) and macroalgae (20:4n-6) were identified in different proportions depending on the stations. GC coupled to mass spectrometry (GC-MS) highlighted specific aliphatic chains, such as dimethyl acetal (DMA) and non-methylene-interrupted (NMI) FAME, known for their specific association to membrane lipids and their role in stress resistance. Nevertheless, some FAME identification, especially locating double bonds, was not feasible with certainty. Given this, 4,4-dimethyloxazodine (DMOX) derivatives were investigated to clarify molecular structures of these FA. Different protocols were compared in terms of feasibility and efficiency in converting FAME directly into DMOX derivatives. Analysis of various known matrices, such as commercial and lab-made standards with specific FA composition, has enabled protocol development and optimization. Then, analysis of *N. concinna* DMOX derivatives allowed to assess its FA diversity. Pushing the molecular precision of FA content ultimately results in the creation of libraries covering the variety of compounds present in an organism. Subsequently, a reference base can be established for the identification of future contents and use as trophic markers to delineate complex trophic ecology.

The yellow mullet fish oil from the Banc d'Arguin Imrâguens in Mauritania: An example of polyunsaturated fatty acids transfer from diatoms to the fish within the alimentary chain

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The Banc d'Arguin National Park (PNBA) in Mauritania is listed by the UNESCO World Heritage. It is characterized by an exceptionnal marine biodiversity with numerous endemic species and it provides a major site of reproduction for western Africa fish. The Imrâguens form fisherman communities established at Banc d'Arguin, who live upon fishing the yellow mullet (*Mugil cephalus*) during its migration and derived products.

The analysis of the fish oil produced by Imrâguens from mullet heads is rich in omega 3 polyunsaturated fatty acids (37.7 % of total fatty acids). The main fatty acid is eicosapentaenoic fatty acid (EPA ; 20.18 ± 0.01 %). This fatty acid is particularly abundant in diatoms, that contribute to 20- 30% of mullet feeding. The identification of 16:4n-1 also provide a good trophic marker for yellow mullet feeding on diatoms. The lipases potentially involved in the mobilization of these fatty acids in the course of digestion of diatoms were identified from the analysis of *Mugil cephalus* genome. Genes encoding a lipase homologous to gastric lipase and four lipases homologous to pancreatic carboxylester hydrolase or bile-salt stimulated lipases were identified. These later could be involved in the lipolysis of galactolipids, the main lipids present in diatom photosynthetic membranes which are rich in EPA.

These data provide an added value to the traditional fishing practice of Imrâgens and highlight the nutritional value of the fish oil they produce.

POSTER SESSION 4
LIPIDS IN THEIR SOCIO-ECONOMIC CONTEXT AND LEGAL BOUNDARIES

Episomal CRISPR-Cas9 and polyploidization methods for microalgae, production of non-GMO mutants according to UE standards

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In 2023 the European Commission published a proposal about regulation of plants obtained by certain new genomic techniques (NGT) stating that some of these techniques are safe for consumers and the environment and bring benefits to society as a whole. Moreover in some cases, products containing or consisting of plants with genetic modifications introduced by NGTs cannot be differentiated from products containing or consisting of plants bred with conventional breeding methods. Leading to the conclusion that, by meeting certain conditions, mutants produced with such methods would not fall under Genetically Modified Organism (GMO) restrictions.

These regulations are focused on crop plants nevertheless marine phytoplankton being responsible for about half of the primary biomass production and producing a wide variety of valuable products some being lipids such as omega-3 very long chain poly-unsaturated fatty acid that are essential to human nutrition. Some of these species are already used to produce supplementation for such fatty acids, if we are to optimize some strains for diverse industrial applications we need to develop tools that allow us to fit in the criteria. To produce CRISPR-Cas9 mutants that would not be considered as GMO we explore episomal CRISPR-Cas9 and aim to the development of non-GMO selection methods that would also act as « suicide cassette » using auxotrophy and reproduce « traditional methods » effects.

Polyploidization has long been used for crop plants and falls in the « traditional methods » category. The usual method is chemical treatment with colchicin, we explore a new method using episomal over-expression of a centromeric histone and episome curation to achieve similar results. The development of these research tools will allow gene characterization and to describe a phenotypic signature of polyploidization among different microalga groups.

POSTER SESSION 5
ENVIRONMENTAL/CLIMATIC AND SUPPLY ISSUES OF OMEGA 3

Evaluation of n-3 LC PUFA synthesis in the liver of European sardines deficient in DHA for two months

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Climate change is expected to alter the production of n-3 long-chain polyunsaturated fatty acids (n-3 LC PUFA) by phytoplankton at the base of the trophic web. This may have implications for the transfer of these molecules toward marine consumers. Many of them are suspected to be weakly able to synthesise sufficient quantities of n-3 LC PUFA including DHA (docosahexaenoic acid) to meet their physiological needs. Our main objective was to evaluate the synthesis capacity of n-3 LC PUFA in the liver of sardine (*Sardina pilchardus*), a species of high ecological and economic importance. For two months, fish were either fed a DHA-deficient diet or a control diet. We hypothesised that (i) a DHA deficiency would be reflected in the hepatic lipid reserve and structure and (ii) deficient individuals would counteract the deficiency by stimulating the expression of genes involved in the n-3 LC PUFA synthesis pathway to synthesise DHA from precursor fatty acids. We found that the percentage of DHA in reserve and structural lipids was significantly lower in deficient sardines compared to fish fed the standard food. This was associated with a similar expression of genes encoding elongases (elovl2, elovl5) and desaturase (fads2) involved in the n-3 LC PUFA synthesis pathway in the two groups. However, the expression of the elovl5 gene was correlated with that of fads2 and elovl2 only in the deficient individuals, suggesting the activation of compensation mechanisms at molecular level. DHA-deficient individuals also exhibited a higher expression of the phospholipase A2 gene (pla2) and a higher percentage of ARA (arachidonic acid), which is a precursor of pro-inflammatory molecules. At this stage, we cannot conclude whether there are compensation mechanisms in the liver in response to a dietary DHA deficiency, so it would be interesting to investigate the gene expression after prolonged conditioning.

Seasonal plasticity of algae lipidome in response to environmental variations: evidence from *Ulva rigida* cultivated in a sustainable integrated multi-trophic aquaculture

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The green macroalgae belonging to the genus Ulva are currently traded worldwide for human consumption and as source of premium ingredients for nutraceutical and cosmetic applications. The bioactive compounds of Ulva include polar lipids, especially glycolipids bearing omega-3 polyunsaturated fatty acids (PUFA) that have been recognized for their health benefits. The possibility of predicting the plasticity of Ulva lipidome in response to environmental variations is essential to fully exploit the nutritional value of Ulva lipids and their biotechnological applications. In this study, we evaluated the lipid composition of *Ulva rigida* cultivated in Portugal on a land-based integrated multitrophic aquaculture (IMTA) and the seasonal variations of its total lipid content, esterified fatty acids (FA) and polar lipids (the major FA carriers). Along winter, spring, summer and autumn samplings, the lipid content and abundance of unsaturated FA, namely omega-3 PUFA, were highest in winter and lowest in summer. Independently of the seasonal variations, *U. rigida* presented a low omega-6/omega-3 ratio, which is associated to positive health effects. Among the polar lipids, those that varied the most were betaines, glycolipids, and phospholipids, including a high number of lysolipid species that increased during autumn and spring. Multivariate analysis using principal component analysis revealed a better discrimination of the four seasons when the dataset on molecular lipid species was used. An in-depth knowledge of the seasonal variability of lipid composition in biomass of *Ulva rigida* can be used to better explore this green macroalga as a sustainable source of bioactive polar lipids with high market value.

POSTER SESSION 6
GERLI/SFN: MARINE LIPIDS AND NUTRITION

Study of the impact of a diet enriched in chub mackerel and quinoa on the hepatic lipid fraction and its DHA status in Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegenerative condition marked by a decrease in cognitive function. Scientific evidences highlighted a correlation between AD and diminished levels of docosahexaenoic acid (DHA, 22:6 n-3) in neurons, reason why it is believed that enhancing brain health through dietary measures could hold cognitive impairment. Recognizing liver's pivotal role in lipid processing, this study endeavors to assess how a DHA-rich diet influences both the lipid profile and the accumulation of this fatty acid within the liver of mice with AD. Hence, 32 5xFAD transgenic mice (animal model for AD) were allocated to four groups, and fed with different diets: Control (C) (standard AIN-93M diet); Chub Mackerel (CM) (AIN-93M supplemented with 10% chub mackerel); Quinoa (Q) (AIN-93M with 5% quinoa); and Chub mackerel+Quinoa (CM+Q) (AIN-93M with both 10% chub mackerel and 5% quinoa). The hepatic lipid fraction of these mice was studied for its fatty acid (FA) distribution both in the total lipids and the most relevant lipid classes (phospholipids, free fatty acids [FFA] and triacylglycerols [TAG]). Diets containing chub mackerel led to a significant increment in hepatic n-3 polyunsaturated fatty acids (n-3 PUFA). Such increment was mainly leveraged by DHA that accounted 12.2±2.8% of total FA in CM+Q group, and 7.9±3.3% in CM in opposition to those levels determined in C and Q groups (4.4±3.2% and 3.8±1.2%, respectively). As for the FA profile of main lipid classes, significant differences were only observed for FFA and TAG fractions, with higher levels of DHA in groups CM (2.9±1.2% and 7.5±3.6% of total FA, respectively) and CM+Q (3.0±1.0% and 8.2±2.1%, respectively). These findings suggest that foods rich in quinoa and chub mackerel could potentially boost hepatic DHA levels.

Additionally, supplementation facilitated increased availability of free DHA, which could be transported or integrated into various lipid classes.

Diets enriched in chub mackerel increase erythrocytes DHA levels and improve omega-3 index in Alzheimer's disease

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Docosahexaenoic acid (DHA, 22:6 n-3), is a fundamental fatty acid (FA) to maintain a healthy neuronal function. Numerous studies have related a deficiency on DHA with several neurological conditions, including Alzheimer's Disease (AD). Before being available for absorption by brain cells, the DHA found in foods must be digested and transported through the blood stream. Hence, efforts have been made to use the omega-3 index (sum of eicosapentaenoic acid [EPA, 20:5 n-3] and DHA) to predict the cognitive decline associated with ageing. However, as lipid metabolism is altered in AD patients' additional studies are needed to clarify this correlation. To understand the effect of a diet formulated with chub mackerel (naturally rich n-3 polyunsaturated fatty acids [n-3 PUFA] and particularly DHA) in circulating DHA in AD, 16 5xFAD transgenic mice (animal model for AD) were divided in two groups and fed with following diets: Control (C) (standard AIN-93M diet) and Chub Mackerel (CM) (AIN-93M supplemented with 10% chub mackerel). Mice erythrocytes were collected and analysed for its FA profile. Significant differences were observed in the FA of mice erythrocytes from CM group when compared to C group counterparts. Results show former doubled their DHA content regarding C group (from 4.4±0.9% to 8.7±0.5% of total FA), while reduced to nearly half their arachidonic acid (ARA, 20:4 n-6) levels (from 10.0±1.7% to 5.4±0.3%). As a result, n-3 PUFA contents were incremented and the n-6 PUFA decreased in CM mice. Such trend positively modulated both the n-3/n-6 ratio (0.3±0.0 and 1.0±0.0 determined in C and CM groups, respectively) and the omega-3 index (4.6±0.9 and 11.0±0.7 estimated in C and CM groups). By doing so, these results suggest that a diet enriched in chub

mackerel might be beneficial to modulate lipids uptake while improving DHA and omega-3 erythrocytes *status* in patients suffering with AD.

Chondroitin sulfate nano vectorized by LC-PUFAs nanocarriers, both extracted from salmon (*Salmo salar*) by green process.

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Among the different families of glycosaminoglycans, chondroitin sulfate (CS) exhibits desirable properties for health due to its numerous physiological functions. CS plays a role in controlling peri-cellular ions, protecting connective tissues, promoting bone growth, preventing age-related skin damage, and inhibiting bacterial infections. CS can also participate in anticoagulant, anti-inflammatory, and analgesic processes, but its most widespread clinical application currently is the treatment of osteoarthritis.

This study presents the results of the valorization of lipids and chondroitin sulfate, both extracted from salmon co-products (salmon head from *Salmo salar*) through controlled enzymatic processes. The polar lipids, naturally rich in long-chain polyunsaturated fatty acids (DHA C22:6 n-3 and EPA C20:5 n-3), and the chondroitin sulfate, primarily located in the nasal cartilage, were separated and concentrated before being characterized using various techniques (FTIR, DSC, HPLC, CPG-FID, Iatroscan®). These compounds were then used to formulate liposomes encapsulating chondroitin sulfate, whose physicochemical characteristics were studied (size, electrophoretic mobility). The liposomal solution was tested on chondrocytes from elderly individuals suffering from knee joint inflammation: LDH, MTT, proliferation tests, expression of various inflammation markers (Cox-2, mPGES-1, PGE2), nitrate levels, and the amount of collagenase produced. The results showed that chondroitin sulfate, in synergy with the liposomes, played a positive role in combating chondrocyte inflammation. This molecular assembly project, derived from the valorization of fish co-products and providing health benefits, fully aligns with an environmental approach.

Polar lipids from marine microalgae show strong anti-inflammatory effects against LPS-induced macrophages

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Chronic inflammation represents an imbalanced physiological response with consequences to cells, tissues and organs that can ultimately activate the onset of chronic diseases, such as cancer, diabetes, cardiovascular disease, among others. Natural anti-inflammatory compounds have been proposed to tackle chronic inflammation without the risk of severe side effects from the conventional anti-inflammatory drugs Microalgae represent a natural and sustainable source of anti-inflammatory compounds, and recently microalgal lipids, particularly, glycolipids such as monogalactosyldiacylglycerol (MGDG), have been highlighted for their potent action against pro-inflammatory stimuli. This work aimed to assess the anti-inflammatory effect of three polar lipid fractions enriched in different polar lipid classes namely fraction 1 with digalactosyldiacylglycerol (DGDG) and sulfoquinovosyldiacylglycerol (SQDG), fraction 2 with MGDG and fraction 3 with diacylglyceryl-trimethylhomoserine (DGTS) and phospholipids (PL), isolated from *Nannochloropsis oceanica* and *Chlorococcum amblystomatis*, through measurement of nitric oxide (NO) production and transcription of pro-inflammatory genes *Nos2, Ptgs2, Tnfa* and *Il1b* in lipopolysaccharide (LPS)-activated macrophages. Fractions 1 and 3 of both algae promoted a reduction in LPS-induced NO production from macrophages. Although at different extents, *N. oceanica* and *C. amblystomatis* lipids significantly reduced the transcription of *Nos2, Ptgs2, Tnfa* and *Il1b* induced by LPS. Fraction 3 represented the most active *N. oceanica* lipid, highlighting the composition of this alga in DGTS and PL, while Fraction 1 was the most active *C. amblystomatis* fraction, highlighting this alga's composition in DGDG and SQDG. These results emphasize the strong anti-inflammatory capacity of microalgae lipids and may be explored as functional ingredients and nutraceuticals in order to tackle chronic inflammation related diseases.

Dietary sphingolipids impact circulating ceramide species: new insights using stable isotope tracing of sphingosine in Caco-2/TC7 intestinal cells

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In the frame of metabolic disease outbreak, increased circulating concentrations of specific sphingomyelin (SM) and ceramide (Cer) species, notably C18:0 Cer and C24:1 Cer, have been revealed as cardiovascular risk factors. Fish and dairy are major food sources of SM but the potential impact of dietary SM intake on circulating Cer species is scarcely known. Rodent studies showed that tritiated SM was hydrolyzed in the intestinal lumen into Cer, and further to sphingosine (SPH) and fatty acids (FA) that were absorbed by the intestine.

Our objective was to investigate in Caco-2/TC7 cells cultured on semi-permeable inserts the uptake and metabolism of SPH and/or C23:0, the main FA of milk SM also reported in some fish. Mixed micelles (MM) mimicking postprandial lipid micelles were prepared without or with SPH, SPH and C23:0 (SPH+C23:0) or C23:0. After 16h incubation of such micelles on the apical side, sphingolipides (SL) were analyzed in the basolateral medium by tandem mass spectrometry. TG secretion increased 11-fold in all MM-incubated cells compared with lipid-free medium.

Apical supply of SPH-enriched MM induced increased concentrations of total Cer in cells and addition of C23:0 in SPH-enriched MM led to a preferential increase of C23:0 Cer and C23:0 SM. Complementary experiments using deuterated SPH demonstrated that SPH-d9 was partly converted to sphingosine-1-phosphate-d9, Cer-d9 and SM-d9 within cells incubated with SPH-enriched MM. A few Cer-d9 (2% of added SPH-d9) was recovered in the basolateral medium of (MM+SPH)-incubated cells, especially C23:0 Cer-d9 in (MM+SPH+C23:0)-enriched cells associated with decreased C18:0 Cer-d9.

Altogether, MM enriched with SPH and very long-chain FA such as found in postprandial micelles formed after dietary SM ingestion, impacted directly SL endogenous metabolism in enterocytes, resulting in the secretion of TG-rich particles enriched with diet-typical Cer and potentially reduced atherogenic species. Favorable impacts on cardiovascular risk remain to be elucidated.

Quantification of pentadecanoic acid (C15:0) in marine species and dairy products

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Pentadecanoic acid (C15:0) is an odd chain fatty acid (OCFA), currently described to be mainly found in dairy products. Indeed, its synthesis by rumen bacteria from propionyl-CoA results in higher presence of C15:0 in ruminant milk (1% of total fatty acids) than in other food type. Hence, C15:0 is often considered as a plasma fatty acid biomarker of dairy consumption.

Yet, recent studies showed it may also be present in certain type of marine species, challenging the assertion of exclusivity of the presence of C15:0 in dairy fat. This study aimed to assess whether marine species can be reliable sources of C15:0 among food.

We performed investigations of dietary components to determine the presence and quantity of C15:0 in a large food panel, including dairy and marine products. Total fatty acids were extracted and analysed by gas chromatography and mass spectrometry to determine fatty acid percentage. Internal standards were used to assess real fatty acid quantities in products, as a measure expressed in mg/100 g, further related to the number of food portions consumed.

Results first confirmed that C15:0 represented on average 1.5% of total fatty acids in dairy (milk, yoghurt, cheese, cream…). In marine food, C15:0 represented between 0.3 and 1 % of total fatty acids (depending on type of sea food and species). In other types of non-dairy food (vegetable oils, seeds), C15:0 generally accounted for very small amounts or traces of total fatty acids. Considering the amount of total fat in food portion, here dairy products contain the most quantities of C15:0. Marine species don't appear as a major source of C15:0, yet still contain higher total amounts of C15:0 than that of other non-dairy food. Nonetheless, the use of fish extracted oils in food industry could account for a significant part of C15:0 amount in food.

Conflict of interest: this work received financial support from Centre National Interprofessionnel de l'Economie Laitière (CNIEL).

Lipolysis of marine liposomal solutions naturally rich in LC-PUFAs by phospholipases: a comparative in vitro gastrointestinal digestion study with marine emulsion

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Marine oils, naturally rich in n-3 long-chain polyunsaturated fatty acids (LC-PUFAs), with eicosapentaenoic acid (EPA; C20:5 n-3) and docosahexaenoic acid (DHA; C22:6 n-3) as the leading fatty acids, are strongly involved in the prevention and treatment of inflammation, hypertension, cardiovascular and neurodegenerative diseases. During digestion, the fate of these LC-PUFAs depends on their chemical form (triacylglycerols or phospholipids). Various classes of lipids were extracted through a green process from salmon heads (*Salmo salar*), concentrated, and purified to formulate triacylglycerol emulsions and liposomal solutions from phospholipids. Their lipolysis was first tested *in vitro* using individual gastrointestinal lipases and phospholipases to identify the enzymes potentially involved in the digestion of these formulations. These formulations were then subjected to the combined action of lipases *in vitro*, mimicking the physiological conditions of the gastrointestinal tract, both in the stomach and the upper small intestine, to evaluate the digestibility of TAG and liposomes. The *in vitro* results showed that the TAG emulsion was hydrolyzed by porcine pancreatic extracts (PPE) and pure pancreatic lipase (PPL) with its cofactor, colipase, and to a lesser extent by pancreatic lipase-related protein 2 (PLRP2) and a gastric extract (RGE) containing gastric lipase. No hydrolysis was observed with purified pancreatic phospholipase A2 (PLA2) and carboxyl ester hydrolase (CEH). The liposomal solution was hydrolyzed by PLA2, PPE, and PLRP2, where the phospholipase activities depended on the presence of bile salts, suggesting a combined action of these with the phospholipases. Using a two-step *in vitro* digestion model, it was shown how the fatty acids from TAG and PL formulations were released during the gastric and intestinal phases of digestion. Both substrates were found to be digested with a higher lipolysis level obtained with liposomes (around 75%) vs TAG emulsion (around 33%). The liposomes appear as a perfect carrier for delivering hydrophilic molecules in the small intestine while preserving them from gastric environment.

Evaluation of Biosolvents and Ultrasound-Assisted Extraction for Lipid Recovery from *Chlorella vulgaris*, *Fucus vesiculosus* and *Ulva sp.*: A Sustainable Approach for Food and Nutraceutical Applications

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Algae are sustainable sources of healthy and functional lipids. Food-grade solvents are required to obtain extracts suitable for food and nutraceutical industries. However, these non-toxic solvents have low lipid extraction yield and/or purity and, therefore, novel technologies have emerged to address this disadvantage.

This work aimed to evaluate the efficiency of the biosolvents ethanol (E) and ethyl acetate (EA), as well as the application of ultrasounds (UAE) when using those biosolvents, to obtain lipid extracts from *Chlorella vulgaris*, *Fucus vesiculosus* and *Ulva* sp., in comparison with the conventional Folch method. Extraction yield and lipid purity were determined by gravimetry, fatty acids (FA) were profiling by GC-MS, antioxidant capacity was evaluated by DPPH assay and anti-inflammatory potential was assessed by the cyclooxygenase (COX-2) inhibition assay.

The results showed that the extraction approach can significantly influence the extraction yield, as well as the composition of the resultant extracts, including the FA content. EA+UAE is a promising food-grade alternative to high lipid purity (>74%) ingredients with great PUFA and *n*-3 FA contents. Lipid rich extracts from *C. vulgaris* can also be produced by E+UAE. Overall, all extracts contain *n*-3 and *n*-6 FA and showed antioxidant and anti-inflammatory properties, but the degree of action depends on the algae sample and the extraction approach applied.

The produced extracts provide a sustainable and natural alternative to synthetic additives and can be used as ingredients with health promoting properties.

Lipid content in Chlorella biomass: variability related to biomass origin

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Among marine microorganisms, microalgae are well known for their potential for industrial valorization of their lipid fraction for biodiesel or food applications.

They appear as a sustainable source of lipids with valuable nutritional qualities, nevertheless very few eukaryotic microalgae species are currently allowed for human food consumption and mostly belong to the *Chlorella* genus. Therefore, there is a need for a more exhaustive characterization of its biomass and especially of the parameters leading to a variation of its content in lipids of nutritional interest.

Indeed, Chlorella biomass contains several classes of lipids including polyunsaturated fatty acids (PUFA) ω3 and ω6 which content and ratio can vary significantly depending on the species considered as well as the trophic mode used for cultivation. While the lipid content of in-lab produced *Chlorella* has been already studied, the variability of commercial biomass composition is barely described. Here we characterized the lipid classes and fatty acid profiles of six commercial biomasses of *Chlorella vulgaris* and *C. sorokiniana* as well as those of in lab-produced *C. sorokiniana*, grown in photo-autotrophy and mixotrophy conditions. Our results showed significant variations in the lipid composition of the biomasses, *i.e.* (i) ω6/ω3 ranging between 1,3 and 8.9 in commercial biomasses and 2.4- ~4 between photo-autotrophy and mixotrophy lab-produced biomasses, (ii) variable polar/apolar lipid ratios with high ratios largely dominated by bioactive polar galactolipids in photo-autotrophy, and (iii) variable free fatty acid levels in commercial and lab-produced biomasses.

All these data demonstrate how the environmental conditions of production or of post-processing are important to understand and pilot lipid quality, in order to maximize the nutritional benefits of the consumption of well controlled microalgae biomass.

POSTER SESSION 7
NEW PROGRESS IN LIPID METHODOLOGY

Genome-scale Metabolic Networks for better understanding of lipid metabolism and production

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Genome-scale Metabolic Networks (GEMs), i.e. mathematical modelling of living organisms is a powerful technology to better understand complex behaviour of living organisms and design biosolutions. Indeed, the pitfall in engineering biosolutions rises from the extreme complexity of the molecular components within the cell and the large number of interactions with its environment, like with microalgae in very diverse and adverse environments. The rational engineering using mathematical models allows an acute comprehension of the metabolic fluxes happening inside the cell, as well as derisking biotech projects and reducing the cost and time to push a product on the market.

iMEAN developed a modelling platform accelerating the reconstruction of GEMs for any type of organisms. Especially, iMEAN reconstructed *in silico* models of detailed lipid metabolism in plants and microalgae. These tools can be used to screen, *in silico*, the consequences of environmental stresses on microalgae physiological stage including lipid content.

Furthermore, modelling the metabolic and regulatory networks of microorganisms unleashes optimisation of experimental setups and metabolite production at industrial scale. iMEAN modelling platform reduces the reconstruction of GEMs by four times and delivers models of higher quality which accelerates usage of GEMs for scientific discoveries and the development of innovative products.

Ultrasonic-assisted food-grade extraction using ethanol for obtaining high-value-added lipids from *Nannochloropsis oceanica*

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Nannochloropsis oceanica is a good source of polar lipids rich in *n*-3 polyunsaturated fatty acids, mainly eicosapentaenoic acid (EPA, 20:5 *n*-3). These lipids are of high interest for the food, nutraceutical, and pharmaceutical industries. However, these applications demand the use of safe food-grade solvents. This may affect the quality of the extracted lipidome compared to classical extraction methods as the use of food-grade solvents is generally associated with low extraction yields of lipids.

In this study, the polar lipid profile of *Nannochloropsis oceanica* was characterized using hydrophilic interaction liquid chromatography coupled to high resolution mass spectrometry after lipid extraction with different solvents to provide a comprehensive overview on the impact of these solvents in the composition and antioxidant properties of the resulting lipid extracts. Chloroform/methanol (CM) was the conventional extraction approach, while dichloromethane/methanol (DM) and dichloromethane/ethanol (DE) offered a less toxic alternative to CM. Ethanol (E) was used as food-grade biosolvent, and both ultrasonic bath (E+USB) and ultrasonic probe (E+USP) were used to increase the lipid extraction yields using ethanol.

The polar lipid signature and antioxidant activity of DM, E+USB and E+USP were comparable to CM, while DE and ethanol were significantly different. These results showed that ethanol-assisted extraction provided similar extraction efficiency and shifted the polar lipidome towards a greater similarity to conventional extraction methods. This demonstrated the feasibility of E+USB and E+USP as food-grade sources of polar lipids with potential for high-end biotechnological applications.

A combined lipidomic and proteomic profiling of *Arabidopsis thaliana* plasma membrane

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The plant plasma membrane (PM) plays a key role in nutrition, cell homeostasis, perception of environmental signals, and set-up of appropriate adaptive responses. An exhaustive and quantitative description of the whole set of lipids and proteins constituting the PM is thus necessary to understand how the way these components, are organized and interact together, allow to fulfill such essential physiological functions. Here we provide by state-of-the-art approaches the first combined reference of the plant PM lipidome and proteome from *Arabidopsis thaliana* suspension cell culture. We identified a reproducible core set of 2,165 proteins which is by far the largest set of available data concerning the plant PM proteome. We combined lipidomic approaches, allowing the identification and quantification of an unprecedented repertoire of 405 molecular species of lipids. We showed that the different classes of lipids (sterols, phospholipids, and sphingolipids) are present in similar proportions in the plant PM. Within each lipid class, the precise amount of each lipid family and the relative proportion of each molecular species were further determined, allowing us to establish the complete lipidome of Arabidopsis PM, and highlighting specific characteristics of the different molecular species of lipids. Results obtained are consistent with the plant PM being an ordered mosaic of domains and point to a finely tuned adjustment of the molecular characteristics of lipids and proteins. More than a hundred proteins related to lipid metabolism, transport or signaling have been identified and put in perspective of the lipids with which they are associated. All these results provide an overall view of both the organization and the functioning of the PM.

Resolving triacylglycerol and glycerolphospholipid regioisomers of marine lipids using mass spectrometry and machine learning

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Lipids are important structural and bioactive components in cell membranes and play a vital role in the physiology of all living organisms. Marine biomass are important sources of lipids, especially rich in n-3 polyunsaturated fatty acids crucial for human nutrition and health. As the dominating lipids in most marine species, triacylglycerols (TAGs) and glycerolphospholipids (GPLs) have complex compositions resulting from both the fatty acid combinations (molecular species), and the positional distribution of the fatty acids (FA) in lipid molecules (regio- and stereoisomers). While conventional analysis has focused on the overall fatty acid composition and different lipid classes, modern lipidomics platforms have commonly reached the level of revealing molecular species of marine lipids. However, the comprehensive profiles of TAG and GPL regio- and stereoisomers have remained largely unresolved due to the lack of powerful methods.

We recently developed a novel UHPLC-ESI-MS/MS method and a tailor-designed software for quantifying regioisomers of TAGs and GPLs in complex mixtures. Using the new tools, we resolved for the first time the comprehensive profiles of TAG and GPL regioisomers of different marine species including fish, micro- and macroalgae. Lipids were first extracted using a modified Folch method and separated into TAG- and GPL-rich fractions. The lipid fractions were analysed using reverse phase UHPLC-ESI-MS/MS operated in positive (TAGs) and negative (GPLs) modes. The regioisomer composition were calculated based on the relative abundances of DAG^{+} (TAGs) and FA^{-} (GPLs) fragment ions in the MS/MS spectra using a fragmentation model created and validated using regiopure reference compounds. The novel findings on the TAG and GPL regioisomers provide new insights on the biochemistry of marine species as well as the nutritional value and bioactivities of marine lipids.

Navigating lipidomics: key challenges and perspectives in lipid nomenclature for biologists

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Lipids are key compounds in the study of metabolism and are increasingly studied in biology projects. It is a very broad family that encompasses many compounds, and the name of the same compound may vary depending on the community where they are studied. In addition, their structures are varied and complex, which complicates their analysis. Indeed, the structural resolution does not always allow a complete level of annotation so the actual compound analysed will vary from study to study and should be clearly stated. For all these reasons the identification and naming of lipids is complicated and very variable from one study to another which greatly complicates data interoperability.

In this communication we will present the complicated issues surrounding the simple name of a lipid. We will discuss the different way to name lipids (with chemoinformatic and semantic identifiers) and their importance to share lipidomic results within the community but also to map lipid data into functional networks. The correct naming and reporting of lipids identities remains complex and confusing, with many identifiers and initiatives available, which greatly complicates data sharing. It is why it needs to be harmonized so we propose to adopt the same rules and associate essential elements to all lipidomic data set.

Release of free fatty acids in Chlorella biomass during post-harvesting steps

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Within the European legislative framework, the promising market of microalgae for human consumption is dominated by products originating from “Spirulina” and “Chlorella” whole biomass. Among other properties, Chlorella is marketed for its health-promoting functional lipids such as polyunsaturated fatty acids ω3 and ω6.

While Chlorella’s biomass is mostly sold dehydrated, the drying step of the downstream processing currently consists in half of the energetical production cost emphasizing the environmental and economic interests of wet downstream processing and the need for studies determining the optimal conditions for these steps.

Nevertheless, some challenges need to be overcome. The level of free fatty acids (FFA) in the product, which can exceed 10% of total lipids (Barouh et.al. 2024) can be detrimental for the overall lipid quality, in terms of flavor and nutritional properties.

Free fatty acids can be naturally released from glycerolipids by lipases present in microalgae cells. While endogenous lipolysis is mentioned in the literature, the reaction kinetics during dark anoxic storage have not been characterized in Chlorella yet. Here, fatty acid hydrolysis has been followed during up to 120h after harvesting cells of *Chlorella sorokiniana* concentrated by centrifugation, using liquid/liquid extraction and quantification by GC-FID. Results showed that initially an average of 15% of total fatty acids were already released just after concentration. During dark anoxic storage, the level of FFA in the concentrated cells remained stable for almost 20h before increasing significantly to reach more than 80% after 28h. We also showed that increasing cells concentration during storage increased disproportionately fatty acid release and that early harvesting of cells, during culture’s growth phase vs stationary phase, resulted in limited fatty acid release. These results suggest that wet storage conditions should be further studied to limit endogenous lipolysis in microalgae biomass and to secure lipid quality in the downstream processing steps.

Metatoul-Lipidomic Facility: Cutting-Edge Lipidomics for Metabolic Research

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The MetaToul-Lipidomique Core Facility (https://www.i2mc.inserm.fr/lipidomique/) is a part of the Toulouse Metabolomic and Fluxomic Platform (MetaToul), which is one main partner of the national infrastructure MetaboHUB. It is dedicated to lipidomic analysis through analytical chemistry methods like chromatography coupled or not to mass spectrometer. We propose analysis on a wide lipidome coverage: neutral lipids, fatty acids (classical and hydroxylated), free oxylipine, sterols, oxysterols, phospholipids, sphingnolipids, polyphospho-inositide phosphate, sphingoide base, bile acid, skin ceramides and short chain fatty acid profiling. Qualitative and semi-quantitative lipids analysis can be performed on various biological samples: tissue, fluid, cells...and can be applied in different domain such cancer, inflammation, obesity. The facility is equipped with 4 liquid chromatography (LC) systems coupled to mass spectrometry (MS) with two low resolution and two high resolution system, one gas chromatography (GC) couple to MS, two GC-FID and one TECAN robot to prepare samples. The team is composed of 7 full time engineers (3 permanents, 4 non permanents), it proposes services for all lab (academic and private companies), develop new methods on demand and can be associated on grant demand. To make a request for analysis please https://mama-webapp.metabohub.fr/.

The major ongoing developments are: developing workflows for analyzing large series of samples (> 500 samples), miniaturizing the samples required for analysis (down to a few cells), implementing isotopic tracing methods for complex lipids, and establishing spatial lipidomics. They will be presented shortly on this poster.

MetaboHUB (French national infrastructure in metabolomics and fluxomics for life sciences), towards next generation metabolomics and fluxomics: from population to single cells

METABOHUB consortium, Colsh B., **Bertrand-Michel J.**

Since 2013, French National Infrastructure MetaboHUB (MTH) funded by the French National Research Agency (ANR) addresses key critical priorities in metabolomics, fluxomics and bioinformatics to (i) provide high throughput quantitative analytical technologies for biochemical phenotyping of large variety of biological samples, (ii) identify metabolites and annotate metabolomes through implementation and maintenance of reference spectra databases, (iii) develop broadband flux measurements, (iv) provide access to high added-value services to national/international scientific community and industry players, (v) attract and train a new generation of scientists and users through promotion of metabolomics in higher education and continuing education. The ambition of MTH has been to draw on its unique analytical (68 Mass spectrometers, 16 NMR and 7 robotic platforms), digital (online open services, >3000 users worldwide) and human capacity (6 national leading facilities, 150 scientists, 70 FTEs) in order to develop a technological offer through complementary skills in analytical chemistry, robotics, bioinformatics, biostatistics and biochemistry toward a wide range of academic and nonacademic scientific communities in France and worldwide. MTH develops next generation metabolomics from large-scale metabolic phenotyping to the detailed study of molecular processes (from the single cell to the individual and the population).

Of note, MTH developed methods to be able to provide metabolomics data for cohorts with more than 1000 individuals leading to key breakthroughs for disease biomarker discovery. MTH also increased the throughput of fluxomics studies to allow screening of more than 100 strains of microorganisms. Combining AI technologies and metabolic phenotyping, MTH led studies to predict plant metabolic responses to extreme climatic conditions. Finally, MTH developed a unique computational resource predicting more than a million associations between metabolites and biological concepts using literature mining. MTH is though at the forefront of metabolomics and fluxomics development providing to scientists a unique infrastructure in Europe.

Development of an in-situ monitoring system to study the rheological changes during the in vitro gastric digestion of O/W emulsions and its impact on intestinal release of free fatty acids

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As food moves through the gastrointestinal tract, its viscosity changes, affecting nutrient transport and absorption. This can be studied using *in vitro* digestion systems, but common sampling techniques (*ex-situ* analysis) are not be fully representative. This study aimed to develop an *in vitro* gastric digestion system with *in-situ* rheological measurement (iGD-isRM) and evaluate the release of intestinal free fatty acids (FFA) from O/W emulsions stabilized with saponin or lecithin and thickened with modified starch. The iGD-isRM consists of a digestion vessel connected to a cup attached to a rheometer, incorporating a recirculation mechanism that allows for the continous measurement of apparent viscosity at a shear rate of 50 s^{-1}. *In vitro* assays followed the INFOGEST protocol, with the gastric phase replicating the addition of simulated gastric fluid (SGF), pH changes, and gastric emptying. Rheological measurements of the emulsions were conducted using both the iGD-isRM and the original rheometer cup. The release of intestinal free fatty acids (FFA) was assessed using the pH-stat method. An abrupt decrease in viscosity was observed during the first 20 min, dropping from 2129 mPas for saponin and 657 mPas for lecithin. No significant differences ($p>0.05$) were found between the viscosity profiles of the chyme using SGF with and without enzymes, suggesting that the decrease was due to the dilution effect. For undigested emulsions, apparent viscosity values differed: 2494.6 mPas for saponin using the iGD-isRM compared to 1041.6 mPas with the original cup, and 551.3 mPas 173.9 mPas for lecithin, respectively. However, viscosity was observed not to depend on time for any of the profiles. The FFA release was higher for O/W emulsions stabilized by saponin (44.38%) than lecithin (37.45%) likely due to the high solubility of saponin, attributed to its glycosidic groups, and smaller oil droplet size ($d_{50saponin}$= 0.40 µm vs. $d_{50lecithin}$= 2.75 µm) inducing a larger surface area for enzyme action. The iGD-isRM enabled *in-*

situ measurement of apparent viscosity, but the cup's geometry may promote a non-uniform shear field with increased turbulence, and its material to induce a series of interactions that could alter the cup's inner surface leading to an increase in apparent viscosity.

Exploring fatty acid synthesis in alternative crops via ^{13}C-labeling approaches

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To address global issues related to food and energy security, my research group is studying alternative crops, historically considered as weeds until recently being identified as promising sources of renewable specialty fuels and other industrially-relevant chemicals. Indeed, *Physaria fendleri* (aka. lesquerella) and *Thlaspi arvense* (aka. field pennycress), winter annuals closely related to *Arabidopsis thaliana*, produce and store in their seeds unusual fatty acids (FAs) that can replace several petrochemicals currently used in industry. Our long-term goal is to advance these plants—that can be grown off-season—as dedicated bioenergy and industrial crops. However, for these oilseed crops to become economically viable sources of unusual FAs, oil synthesis needs to be improved. A lack of knowledge of the metabolic pathways underlying FA synthesis in *Physaria* and pennycress seeds presents a major constraint.

Free of toxins and rich in hydroxy fatty acids, *Physaria* is a promising alternative to imported castor oil and is on the verge of being commercialized. This study aims to identify important biochemical step(s) for oil synthesis in *Physaria*, which may serve as target(s) for future crop improvement. To advance towards this goal, the endosperm composition was analyzed by LC-MS/MS to develop and validate culture conditions that mimic the development of the embryos in planta. Using developing *Physaria* embryos in culture, we were able i) to determine the efficiency with which embryos convert substrates into biomass components, and ii) to replace the substrates by ^{13}C-labeled ones and monitor the flow of ^{13}C-carbon in central metabolic pathways leading to oil synthesis. Finally, different *Physaria* accessions with contrasting seed oil content are currently under investigation and compared to other plant embryos, including pennycress.