

Ulva

The Future Wheat of the Sea

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Final Report



COST Action CA20106 SeaWheat
ULVA: Tomorrow's "Wheat of the Sea", a Model for an Innovative Mariculture

Final Scientific Report (V1 10.2025)
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SeaWheat (CA20106) is a COST Action dedicated to advancing the sustainable development of *Ulva* cultivation as a model for an innovative mariculture system.

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SeaWheat
COST Action CA20106
TOMORROW'S 'WHEAT OF THE SEA': ULVA, A MODEL FOR AN INNOVATIVE MARICULTURE

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EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

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FOREWORD

The SeaWheat COST Action has been an extraordinary journey – one that united a diverse and passionate community around a shared vision: to advance the science, innovation, and application of *Ulva* for a sustainable blue bioeconomy.

When we launched SeaWheat in November 2021, our ambition was to create a pan-European, interdisciplinary platform that would bridge gaps between scientific disciplines, foster collaboration across borders, and connect research with society and industry. Four years later, this vision has become a reality.

The SeaWheat network brought together more than 450 participants from 53 countries, including 18 Inclusiveness Target Countries and 17 SMEs. Through six dynamic Working Groups – focusing on basic biology, aquaculture, food and feed, bioactives, ecosystem services, and regulation – we established a strong, integrated knowledge base for UL research and its applications.

Our collective achievements are remarkable. SeaWheat supported 48 peer-reviewed publications, including several influential review papers that have already shaped scientific and policy discussions. The pioneering E-ULVA initiative, led by the Universities of Ghent, Galway, and Algarve, has begun to standardise Ulva taxonomy across Europe. This milestone will serve both science and industry for years to come.

We have invested profoundly in the next generation of Ulva researchers through five Training Schools and 24 Short-Term Scientific Missions, while also ensuring that our knowledge reaches far beyond academia. From three animated films translated into 14 languages to a multilingual Ulva recipe book and 18 “Lunch with Ulva” webinars, our outreach activities have brought marine science closer to the public than ever before.

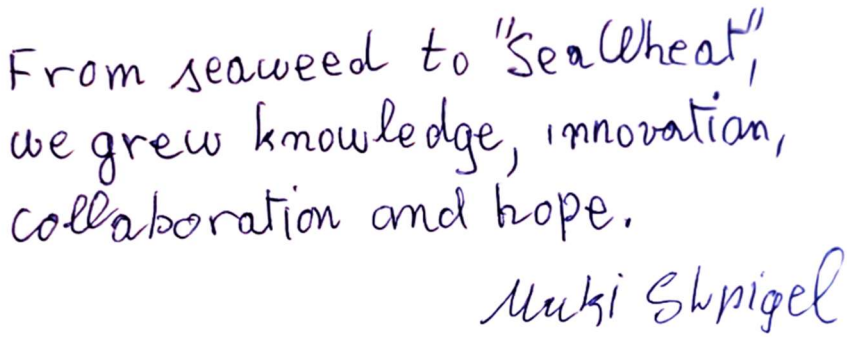
More than anything, SeaWheat has been defined by its community. Its success is rooted in the dedication of Working Group leaders, Core Group members, and countless contributors who gave their time, expertise, and enthusiasm to build something truly collaborative.

We are deeply grateful to our Vice-Chair, Prof. Bela Buck, for his wisdom and leadership, and to Dr. Leigh Livne, our Grant Holder Manager, for ensuring smooth coordination throughout these four years. Our thanks also go to our hosts, partners, and industry collaborators, who demonstrated that science and practice can thrive together.

As we close this chapter, we recognise that SeaWheat does not end here. The network, skills, and momentum built during this Action have laid the foundation for the next stage of Ulva research and innovation. Together, we have established a strong platform for a sustainable, science-based Ulva bioeconomy in Europe and beyond.

Let this final report not be a conclusion, but a launchpad for the future.

Chair of CA 20106
Muki Shpigel



INTRODUCTION AND ACTIVITIES

BIOLOGY
Dr. Ronan Sulpice

FOOD, FEED AND BIOMATERIALS
Dr. Sylvia Strauss

BIOACTIVE PRODUCTS
Dr. Thomas Wichard

SOCIAL, LEGAL AND REGULATORY ASPECTS
Dr. Celine Rebours

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Ulva

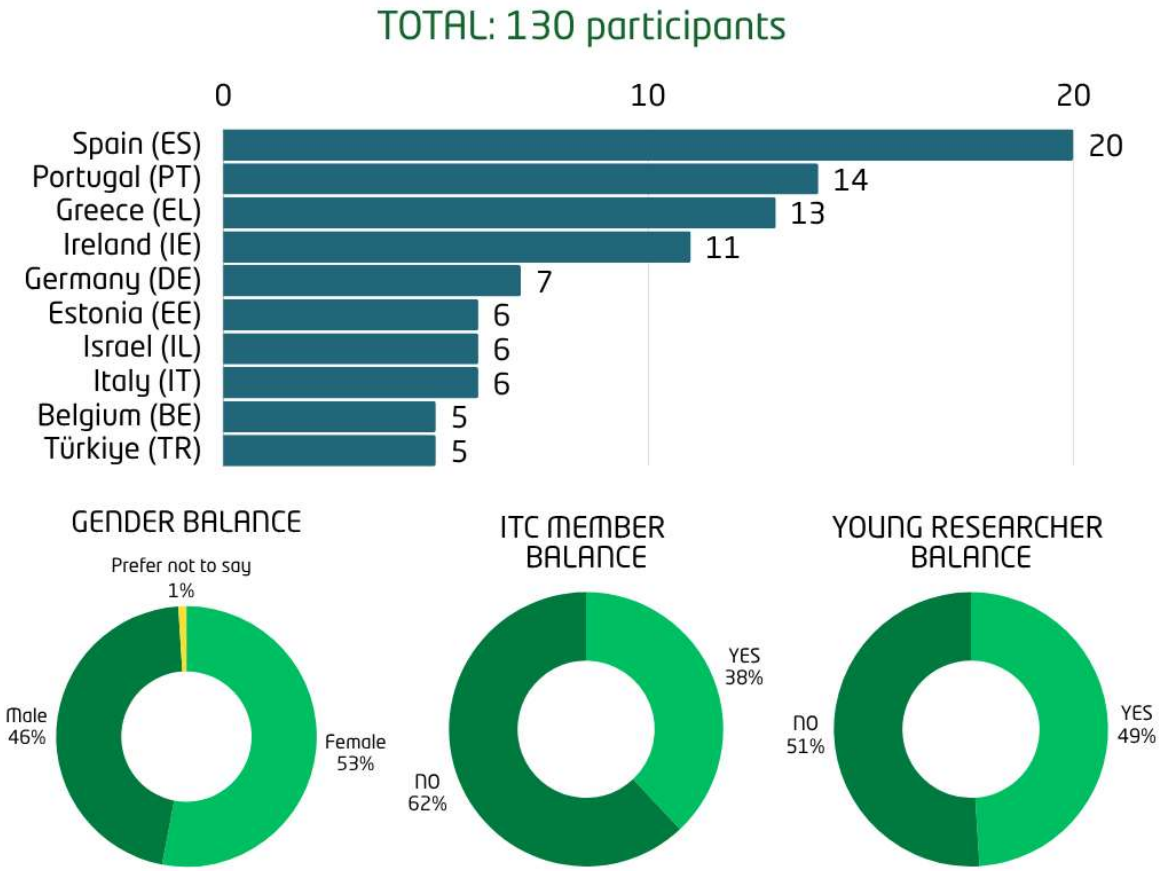
The Future Wheat of the Sea

ULVA BIOLOGY (WG1)

LEADER

Ronan Sulpice (University of Galway, IE)

PARTICIPANTS



RATIONAL AND OBJECTIVES

A growing interest in the development of seaweed aquaculture in Europe has arisen over the past decade, seeking alternative sustainable food sources and other valuable products. The WG1-Ulva Biology specifically aimed at improving the collective knowledge on the different aspects of *Ulva* biology, including systematics, genetics, reproduction, microbiome, diseases, and ecology. Such aspects are key for a sustainable and cost-effective development of *Ulva* aquaculture, but also for providing a key understanding of marine ecosystems. For example, the life cycle of the majority of *Ulva* species at the start of SEAWHEAT was not controlled, and the identity of Ulva species present along European shores remained largely unexplored. Also, variations in biochemical content of Ulva biomass, if described, were not adequately explained in terms of relative impacts of environmental factors (e.g. temperature, light, salinity, nutrient loads) and natural variation.

The main objectives of WG1-Ulva Biology have been defined to address such gaps and can be summarised as (1) provide an extensive map of Ulva spp along European shores, and even watercourses to inform coastal ecosystems comprising Ulva spp; (2) identify main factors responsible for variations in biochemical composition of Ulva biomass via collection and analysis of a large number of Ulva specimen along European shores; (3) describe variation in Ulva microbiome via analysis of Ulva microbiome of specimen collected across Europe; (4) improve our knowledge of Ulva reproduction, growth in response to temperature, nutrients etc via dedicated lab experiments, training schools, and STEM.

ACTIVITIES AND KEY FINDINGS

a. **Ulva spp. Systematics and their identification: clarifying inconsistencies afflicting traditional taxonomy.**
This deliverable provides the results for Task 1.1. The inconsistent systematics and phenotypic plasticity of the Ulva genus will be clarified. *Ulva* samples were collected across Europe by WG1 as part of the eUlva initiative led by Prof. Olivier De Clerck (University of Ghent). All samples collected were sent to Ghent for analyses (silica gel) or as DNA extracts. This initiative is the largest to date, with over 4000 samples analysed. Those results will inform potential new Ulva species, identify the main Ulva species, and particularly describe environmental conditions associated with specific Ulva species, such as salinity and temperature. The distribution of Ulva species across Europe will also be of key importance for aquaculture, informing policymakers for registration of those species as Novel foods, but also avoiding the intrusion of non-local species.
More information here: https://sites.google.com/view/eulva

b. **Ulva microbiome, interactions with bacteria on and in the seaweed fronds, affecting Ulva's growth and development.**
This deliverable provides the results for Task 1.2. Similar to task 1.1, samples were collected across Europe. In fact, samples analysed for microbiome are a sub-part of those analysed for species ID (eUlva project). Around 400 samples were collected and are currently under analysis. This work will inform microbial diversity across Europe, identify core bacteria, as well as bacteria specific to given environments and/ or species. As all efforts are provided based on volunteering labs, it is continuing even after official end of SeaWheat.
More information here: https://sites.google.com/view/eulva

c. **Environmental and seasonal environmental factors affecting Ulva strains' growth and productivity (nutrients, light, temperature, hydrodynamics, green tide events, etc.).**
Same as for microbial analyses, samples analysed for biochemical composition were also analysed for species ID. In total, close to 500 samples were analysed, in triplicate, from 14 EU countries. Significant variation was found in biochemical composition, and as environmental parameters were collected, the data are now analysed to try to disentangle the respective influences of species ID, natural variation and environment.
Also, a number of lab-based studies were performed (e.g. via STEM) to identify the influence of temperature and salinity on biochemical composition of the biomass, as well as on growth.
More information here: https://sites.google.com/view/eulva

FUTURE DIRECTIONS & RECOMMENDATIONS

WG1 activities are still ongoing, and several additional publications are being drafted, e.g. European Ulva spp diversity, microbial diversity and biochemical composition. Progress was made on those gaps. Also, efforts were made regarding the control of the life cycle (Bibi et al., in preparation).
However, some gaps remain, and future efforts should focus on:

1. Elucidation of metabolic pathways in Uva spp at the molecular level. Seawheat focused on describing the large extent of biochemical natural variation in Ulva spp, but the genes involved remain largely unknown. Such knowledge could allow the creation of tailored varieties for defined applications, e.g. via CRISPR-Cas9 technology.
2. Functional studies of the microbiome associated with Ulva spp. At present, the focus has been mainly on the identification of the different bacterial species associated with Ulva. However, a better understanding of the Ulva holobiont should include metagenomics and whole genome metabolic networks to understand the cross-talks between Ulva spp and its associated microbiome.
3. Control of sporulation and life cycle of Ulva. Progress was made during Seawheat, and induction of sporulation for several Ulva spp species has been achieved (Bibi et al., unpublished). However, the molecular mechanisms are not understood. A better understanding would allow us to develop better sporulation protocols. Also, it could allow the creation of sterile varieties, which would be of intense interest for the cultivation of Ulva in ponds.

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ULVA IN AQUACULTURE (WG2)

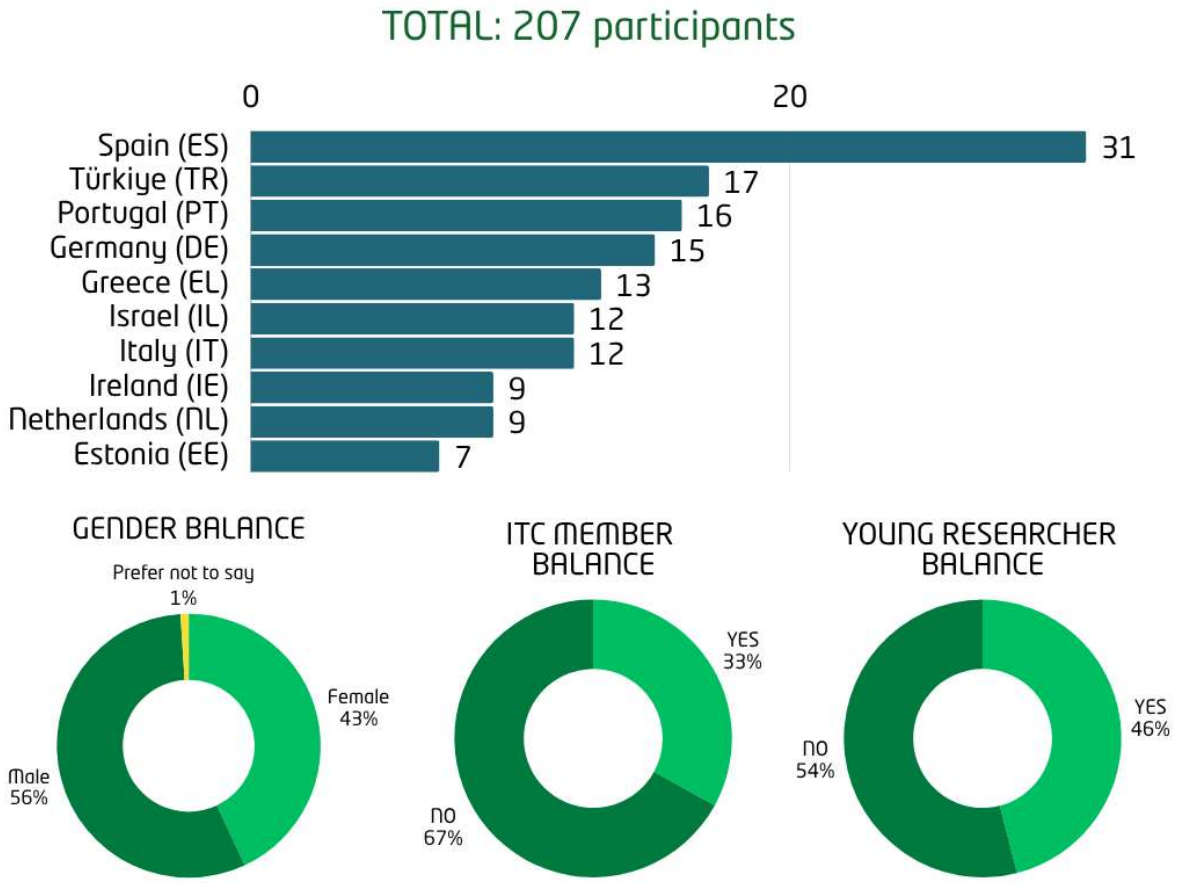
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RATIONAL AND OBJECTIVES

On a global scale, macroalgae production (~30 × 10^6 tons Fresh Weight (FW)) represents only a small percentage of the annually produced 16 × 10^{11} tons of terrestrial crops, grasses, and forests (~16 × 10^{11} tons). The global production of *Ulva* spp. (including the former genus *Enteromorpha*), accounted for 3,737 tons FW in 2021 (FAO 2022; Hofmann et al., 2024), of which only about 50 Mt FW are produced in Europe in land-based (24%) and sea-based (76%) systems (Araújo et al., 2021).

The strong interest in *Ulva* as a sustainable marine resource is reflected in the rapid increase in publications with *Ulva* as a keyword in the title since 2000 (from 17 in 2000 to 239 in 2024, Scopus search). Further interest underlying the exploitation of Ulva biomass is demonstrated by the formation of the of the COST Seawheat Action in 2022, a network of researchers (early career and established), students, and industry partners from 27 European countries and inclusiveness target countries (ITCs) whose mission was to make a step-change towards a green economy based on *Ulva* mass production and utilization within the European community and beyond. The traditional use of *Ulva* spp. for food, their fast growth rates, nutritional value, cosmopolitan nature and the potential to provide ecosystem services present *Ulva* spp. as excellent candidates for aquaculture. In some geographic regions, *Ulva* can be cultivated year-round. Scaling up the production of *Ulva*, or “sea lettuce”, could lead to the establishment of sea lettuce as a valuable component in the future diet of coastal and inland

populations. Moreover, their rapid acclimatisation to environmental changes and strong capacity for nitrogen and phosphorus uptake have led to the identification of *Ulva* spp. as ideal biofilters that can be integrated into different types of polyculture or integrated multi–trophic aquaculture (IMTA) approaches.

Despite the stark increase in interest in the cultivation and exploitation of *Ulva* spp., only 10-15 commercial enterprises currently produce *Ulva* spp. in Europe (Araújo et al., 2021; Vázquez Calderon & Sanchez Lopez, 2022). This lack of growth in the industry is partly due to the remaining existence of bottlenecks and constraints in the commercial-scale cultivation, harvest and utilisation of *Ulva* in different industries. Often, these constraints and bottlenecks are not reported in the literature because they are associated with negative results (Rostenthal 1979), which can create strong publication bias. Therefore, the objective of this WG2 is to shed light on the constraints and challenges connected with the implementation and scaling-up of *Ulva* cultivation along the entire supply chain, from production to harvesting and processing utilisation.

Alongside Seawheat annual meetings and workshops addressing "*Ulva* aquaculture for food and feed production: state-of-the-art, bottlenecks, risks and gaps", unified experts from European and associated countries, including affiliated SMEs and academic institutions, to promote a closer cooperation between *Ulva* experts and stakeholders in the food and feed sector. The participants focused on identifying bottlenecks, challenges, and proposals for solutions. Based on the results of these meetings, our own research experience, and evidence from the literature, we present the most relevant challenges that occur along the supply chain of *Ulva* production, including cultivation, harvesting, processing, and post-processing (utilisation), and provide potential solutions.

The biomass of the green seaweed *Ulva* is a valuable source of an innumerable range of products that can be harnessed for promoting health and well-being. However, the production of large quantities of *Ulva* biomass, whether achieved through land- or sea-based settings, in a sustainable manner, is a significant challenge. The specific objectives of SeaWheat COST Action, WG-2, aimed to identified and summarized from available knowledge the necessary actions to be taken to (1) engineering concepts for large-scale cultivation in land- and sea-based facilities, (2) suggesting cultivation protocol for spore collection and *Ulva* preservation, seeding, cultivation, and harvesting in land- and sea-based facilities, and (3) describe the potential impediments (biological, technological, economic, and political) for a potential large-scale *Ulva* biomass production.

ACTIVITIES AND KEY FINDINGS

a. **Protocol: Guideline of engineering concepts for large-scale cultivation in land- and sea-based facilities.**

   Several discussion moments during Seawheat served as the basis for the definition of the contents of this document. Work was carried out during the Seawheat conferences and during the workshop organised in Lisbon in May 2023. It was decided that the guideline would go beyond the engineering concepts, to include also information on the biological and environmental challenges of such cultivation units.

   In brief, the guideline contents are as follows: **Section 1** on land-based cultivation. This section covers topics such as when to select land-based technologies, considering it is ideal for more controlled production environments, suitable for regions with limited access to clean coastal waters, preferred when consistent biomass quality and biosecurity are priorities, and it enables year-round production, less dependent on seasonal changes. Then proceeds with sub-sections with more practical information on "Comparison of land-based technologies", "Abiotic factors", "Biotic factors", "Contaminations", "Biomass quality and safety", "Biomass harvesting", "Biomass processing techniques". **Section 2** is focused on sea-based cultivation. It also includes an initial sub-section on when to select sea-based technologies. In this case, one of the main advantages is the fact that this approach can be more cost-effective for large-scale operations. It is also suitable for integrated aquaculture in the same environments, with a much large potential for bioremediation impacts than its land-based equivalent. It does, however, request access to suitable areas with clean, nutrient-rich coastal waters. In this respect, maritime planning, licensing and social acceptability may play an essential role in the success of this kind of technology. Then, similarly to Section 1, different sub-sections on the same topics will deal with particularities for sea-based units. While some of these sub-sections include information that is common between land-based and sea-based, others, like biomass harvesting, are significantly different. The final version of this document will become available at the Seawheat website upon its publication in an open-access format.

More information here: to be added in future

b. **Protocol: Cultivation protocol for spore collection and *Ulva* preservation, seeding, cultivation, and harvesting in land- and sea-based facilities.**

One of the challenges for European *Ulva* cultivation is the reduction of the cultivation cost. This relates to all parts of the production phase and all types of production systems, whether at sea or on land. Sporulation is one of the key parts of the production cycle. Manual seeding of *Ulva* fragments on longlines might work as a strategy in low-salary countries, but is considered too labour-intensive for Europe. On the other hand, spontaneous sporulation, fragmentation or protoplast formation might be an essential biomass loss factor, depending on the species or strain under cultivation. Hence, achieving control of the sporulation process, or the production of other initial phases of individuals, such as biomass, is of paramount importance for upscaling commercial *Ulva* cultivation.

The life-cycle of *Ulva* is not very complex; however, many shortcuts and alternative routes have been observed in laboratory situations. Some of those may be species or even strain-specific, and for most of them, it is entirely unknown what the triggers are. In this contribution, the state of the art of knowledge of reproduction control of *Ulva* will be summarised and discussed. The primary focus will be on the role of sporulation-inhibiting factors, their identification, and what we know about their regulation. Based on this knowledge, developed in the last two decades, a general protocol for sporulation has been developed, and some specific methods have been identified for individual species or strains, which is part of the objective for task 2.2 of WP2 “Ulva in Aquaculture” of this COST action. In addition, potential alternatives to sporulation, such as protoplast development (incl. spontaneous events of protoplast development) will be discussed in this deliverable.

The document starts with some general considerations, considering that two stages of sporulation or gamete formation can be distinguished in Ulva: 1st, sporangia/gametangia formation and subsequent spore/gametangia formation, and 2nd, release of the sporangia/gametangia. It has been known since the 1970s that certain extracellular compounds play an essential role in the control of these two processes (Nilsen & Nordby 1975, Nordby 1977). However, few studies have been carried out on the subject. Only in 1996 did the amount and action of these substances start to become clearer with the work of Stratmann et al. (1996). In recent years, work on the reproduction and morphogenesis of *Ulva* and other algal species, and their specific and non-specific interaction with their epiphytic microflora, has intensified. A move towards practical applications has been made (Wichard & Oertel 2010, Spoerner et al. 2012, Oertel et al. 2015, Vesty et al. 2015, Wichard 2015, Wichard et al. 2015, Grueneberg et al. 2016, Gao et al. 2017, Weiss et al. 2017). For these reasons, the proposed protocol is essentially based on the elimination of inhibitory factors, fragmentation and incubation in a culture medium high in nutrients and, if needed, application of cold shock. The protocol will be made available through the Seawheat website.

More information here: to be added in future

c. **Report**: **Potential impediments for *Ulva* biomass production.**

As a result of the several discussion moments throughout this COST Action, namely the general conferences and the workshop organised by WG02 and WG03, in May 2023, the experts involved identified four major categories of constraints or impediments for the sustainable cultivation of *Ulva*. More information can be found in the report of the workshop. Nevertheless, those constraints or impediments are summarised in the following tables, along with potential solutions.

Biological Limitations	Proposed Solutions
Limited taxonomical knowledge due to the lack of morphological identification traits	A quick, easy method for identifying cryptic species must be developed.
Differences in culture conditions between species and strains	Careful strain selection and characterisation, preservation and documentation of characterised strains in biobanks
Controlling the life cycle (sporulation control)	Standardised, species-specific protocols for inducing and prohibiting sporulation
Seasonality limits constant product quality.	Controlled, land-based cultivation and/or automated monitoring for quality control
Technological Limitations	**Proposed Solutions**
Many different cultivation systems have pros and cons	Tested, optimised and standardised culture system design for each species and region
Scaling-up production	Contributions from large investors, or the development of subsidies and/or cooperatives or associations of producers
Biomass quality control	Automated monitoring
Harvesting & seeding technology	Automated seeding and harvesting technology
Economic Limitations	**Proposed Solutions**
High production costs	Automation throughout the production chain, improved IMTA, sharing & economising of processing facilities
High energy requirements	Innovative drying technologies must be developed (e.g. microwave-assisted drying)
Consumer acceptance	Improve education, communication & marketing, conduct consumer surveys, and emphasise “grown & produced in Europe”
Product development	Professional business strategies are needed, development of a “common brand” (e.g. Sea Greens), offering high-quality and diverse products.
Political Limitations	**Proposed Solutions**
Competition for space	Multi-use scenarios, IMTA, eco-tourism
Licensing	Standardised and streamlined licensing procedures for producers
Taxonomic changes in *Ulva* species conflict with European food regulations	Better communication between scientists and the EC, flexibility and quick response in regulations
Harvesting & seeding technology	Automated seeding and harvesting technology

FUTURE DIRECTIONS & RECOMMENDATIONS

To tackle the biological limitations of *Ulva* production in Europe, an agronomical approach should be adopted. This should include careful screening and characterisation of identified seaweed strains, followed by their deposition and documentation in public germplasm banks that provide potential users with information about their commercial traits of interest (e.g. growth and nutrient uptake rates, temperature and salinity optimum). Germplasm banking of genetic material is a well-established concept for preserving plant genetic diversity, thus contributing to food security, conservation and restoration. However, macroalgae currently represent a tiny percentage of strains in publicly accessible germplasm banks, and these are lacking strategic international coordination, which could have severe ecological and economic implications as species and their genetic

diversity disappear rapidly due to local and global environmental stressors (Hofmann & Brakel et al., 2025). A large-scale genetic barcoding and eco-physiological assessment is needed for deposited seaweed strains of commercial interest. Furthermore, to overcome cryptic diversity among *Ulva* spp., a fast, reliable method for species identification that is cost-efficient is necessary to confirm the species that are being produced. Further studies are also needed for the development of standardised, species-specific protocols for inducing and prohibiting sporulation to improve productivity during the seeding, nursery and cultivation phases.

While many technological developments have been made over the past few decades for seaweed cultivation, each system has its pros and cons. To optimise production of Ulva in Europe, we need tested, optimised and standardised culture system designs for each species and region. Furthermore, automation technology for seeding, monitoring biomass quality during production, and harvesting is essential to overcome technological challenges and improve production efficiency. The incorporation of artificial intelligence into these processes could help improve production efficiency. Finally, in order to scale-up production to levels that would meet European goals (e.g. the very lofty goal of 8 million tons of European seaweed by 2030), a combination of contributions from large investors with the development of subsidies and or/cooperatives and associations of producers could be a good path forward.

To reduce the high production costs of *Ulva* in Europe, not only are automated processes throughout the production chain necessary, as mentioned above, but also the improvement and broader acceptance of integrated multi-trophic aquaculture (IMTA) systems and the sharing and economising of processing facilities. To improve consumer acceptance, more education and communication surrounding macroalgae products are necessary, and SeaWheat has already made significant contributions in this direction. Better business and marketing strategies are necessary, and producers should conduct consumer surveys to determine what products consumers want. Finally, the development of a familiar brand for European seaweed (e.g. Sea Greens is used in Norway), combined with increased emphasis on seaweed grown and produced in Europe, can contribute to improved consumer acceptance and faster product development.

To avoid competition for space with other industries, multi-use scenarios where diverse products are produced in a shared space (e.g. using wind farms for seaweed and mussel aquaculture) provide a good solution. The same is true for land-based IMTA systems. Standardised and streamlined licensing procedures for European producers are necessary to enable more producers to become active. In parallel, better communication between scientists and the European Commission, particularly regarding rapid taxonomic changes in *Ulva* spp., could help overcome issues with food regulations. Flexibility in these regulations will be necessary if the name of cultivated *Ulva* spp changes often due to new progress in taxonomical research. In fact, flexibility in regulations should also extend to other areas, considering that the seaweed industry is novel, falling, from the production perspective, somewhere between “aquaculture” and “agriculture”. Then, from an application perspective, the sector can fit the *exciting* biotechnology sector as well as traditional areas such as food, feed, chemical, materials, etc., to name a few.

Despite the currently small market size, it is clear that consumer acceptance is increasing and will continue to increase in the coming years, also as a result of projects and initiatives sponsored by national and European entities. If there is adequate political action, following up on political will, there is no doubt that the seaweed industry will develop significantly in Europe, contributing in several ways to the welfare of the European population.

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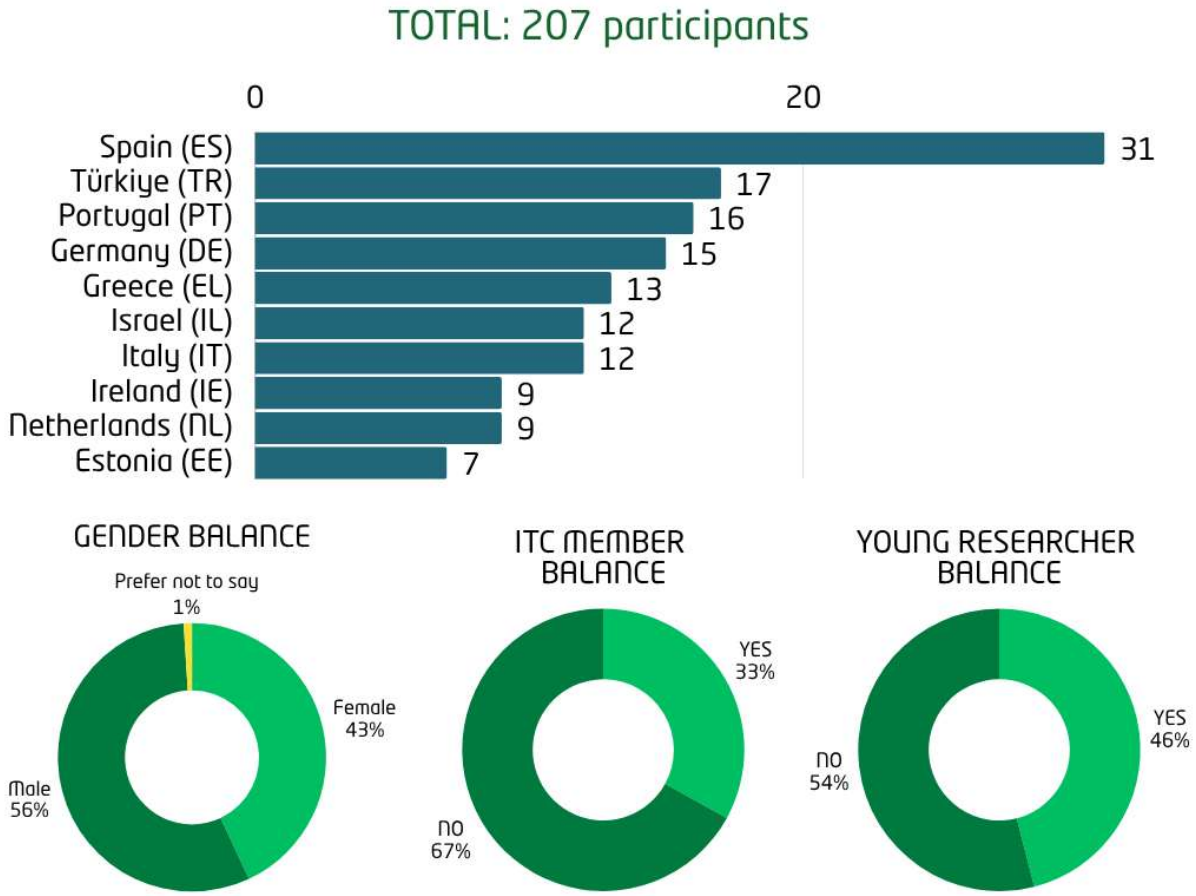
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ULVA AS FOOD, FEED AND BEYOND (WG3)

LEADER

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PARTICIPANTS



RATIONAL AND OBJECTIVES

With a rapidly growing world population, the agricultural food gap will increase due to climate change-induced constraints on natural resources, i.e., fresh water and farmland. Consequently, ensuring food security has become a global imperative. Thereby, the oceans will play an increasingly important role in providing food, animal feed and other valuable, sustainable biomaterials. However, marine wild stocks cannot meet the increasing demand for fish or other biomass sources, including macroalgae. Therefore, seaweed cultivation may be essential for contributing to food security by provisioning food or feed ingredients (Araújo et al. 2022; Forster and Radulovich 2015; Radulovich et al. 2015). Several distinct characteristics indicate an immense potential for green algae in the genus *Ulva* to play a central role in the European seaweed aquaculture industry. The most notable characteristics of *Ulva* include worldwide coastal distribution, fast growth rates, relatively simple life cycle, ease of culture, historical use in food and feed, documented bioactivity and efficiency as a biological filter (Mata et al., 2010; Lawton et al., 2013; Sebök & Hanelt, 2023; Al-Hafedh et al., 2014; Stedt et al., 2022; Shahar et al., 2020; Bao et al., 022; Kirst 1990; Steinhagen et al., 2021).

From a food and feed perspective, *Ulva* species contain suitable levels of proteins, vitamins, trace minerals, and dietary fibres for human and animal consumption (Toth et al. 2020; Trigo et al. 2021; Stedt, Trigo, et al. 2022; Stedt, Toth, et al. 2022; Steinhagen, Larsson, et al. 2022; Steinhagen, Enge, et al. 2021; Taboada et al., 2009). With an amino acid composition comparable to soy or egg protein, and including all essential amino acids (except tryptophan), selected strains of Ulva bearing high protein contents can partially substitute less sustainable protein sources (Dominguez & Loret 2019). In addition, high contents of essential dietary fibre and

other bioactive substances such as phenolics, vitamins, minerals, fatty acids, and pigments render it a beneficial food item providing health and functional advantages (Rajapakse and Kim 2011; Holdt and Kraan 2011; Lopes et al. 2019; Moreira et al. 2022; Qi et al. 2005).

The sustainable exploitation of Ulva as food and feed can therefore contribute to the increasing demand for renewable and novel nutritious food sources, emphasised by the UNSDGs (United Nations, 2015; Faber et al., 2021). The currently increasing trends for health-promoting foods and lifestyles and the recommended consumption of plant (and algae)-based foods (Willett et al. 2019) imply essential opportunities for the food industry to develop seaweed-based products.

The tasks for this WG3, *Ulva as food, feed and beyond have been defined as follows:**

The collective knowledge on *Ulva* food for human consumption, animal feed, and use of *Ulva* as a source of biomaterials will be consolidated. The nutritional value of *Ulva* and its safety as food will be validated. The existing knowledge of *Ulva* biology and mariculture (WG 1, 2) will be identified concerning the food and feed production industries. This will fulfil challenge **(b**), namely developing commercial applications in food and feed industries. The WG activities include the following tasks:

Task 3.1 The applications and nutritional values of *Ulva* spp. in humans and animal nutrition, including food processing, will be identified; **D 3.1.**

Task 3.2 *Ulva* as a source of new biomaterials will be investigated; **D 3.1**

***The following task adjustments had been made:**

At the beginning of the Action period, a better distinction between WG3 and WG4 tasks had been defined to avoid overlap of topics. We defined WG3 to deal with *Ulva* only as a raw source for food and feed as well as biomaterials beyond for non-food applications, e.g. as bioplastics, packaging or biostimulants. In contrast, WG4 was clearly focusing on the chemistry of extractable substances from *Ulva* and extracted bioactives of processed *Ulva* as well as *Ulva*-associated microbial metabolites. Extracts would then be applicable for e.g. health supplements, nutraceuticals, food additives.

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ACTIVITIES AND KEY FINDINGS

Co-initiation of EULVA project

(https://sites.google.com/view/eulva , see in detail under WG1 and WG4)

Ulva Taxonomy: Why Identification Complexity Impacts Nutritional Assessment

Ulva species exhibit simple morphologies (mostly tube or foliose forms) that are challenging to identify, particularly due to phenotypic plasticity —meaning the physical form of one species can vary widely with environmental conditions and its algal microbiome - making traditional morphological identification methods on the species level unreliable. However, most of the existing *Ulva* literature to date is based on morphological identification, rendering species-level identification data questionable. The correct identification of both wild stocks and cultivated *Ulva* spp. biomass is necessary, as the traits vary between the species, and is particularly important due to its prevalent application in commercial projects and industrial product labelling. In order to identify species and strains of particular commercial value, for example, using species selection criteria, molecular identification methods such as DNA barcoding must be consistently used in future.

The aim of the EULVA taxonomy initiative of WG1 and WG4, which was co-initiated by WG3 members, was to identify and specify species distribution across Europe and combine the data with biochemical and nutritional characteristics and microbiome data. This was initiated in order to clarify common taxonomy issues with *Ulva* (see below) and to identify and characterise wild and cultivated Ulva strains for a potential future selection for cultivation purposes. EULVA results will be reported by the respective WG1 and WG4. However, the intended initially separate nutritional analysis of cultivated *Ulva* strains remained unsuccessful due to insufficient available sample material, lack of participating producers, and technical issues in the volunteering laboratory.

a., b. Report on *Ulva* for human consumption, animal feed and use of inedible fractions as a source of biomaterials.

To improve awareness of the seaweed *Ulva* in food, feed, and beyond, and to disseminate the latest knowledge, 27 authors from the COST consortium contributed to this comprehensive review publication (Hofmann et al. 2025; https://doi.org/10.1080/10408398.2024.2370489). The paper provides a critical review of the current status, challenges and opportunities of incorporating this genus into the mainstream so that it may become tomorrow's "wheat of the sea". It includes chapters on *Ulva* in aquaculture, human consumption, aquafeed, terrestrial feed, and applications beyond food and feed.

Of course, consumption of seaweeds in general and *Ulva* in particular also raises safety concerns as it may entail microbiological or chemical risks, in particular by accumulating harmful heavy metals. In a separate review, Vargas-Murga et al. (2025) https://doi.org/10.1016/j.foodchem.2025.145941 critically analysed 176 peer-reviewed papers to evaluate metal(oid) accumulation in *Ulva*. This paper revealed substantial variability in the essential and non-essential element content due to environmental conditions, geographic regions, morphological forms, and analytical methods used in both wild and cultivated *Ulva*. Based on these findings, the review identified and outlined key areas requiring attention to ensure the safe and effective use of *Ulva*. Standardised analytical protocols are needed to improve consistency and comparability across studies and to enable accurate detection of toxic element forms.

FUTURE DIRECTIONS & RECOMMENDATIONS

Below are key areas where further work is needed and recommendations for advancing the science and applications of *Ulva* in food, feed and biomaterials.

1. Species identification, genetic diversity and strain selection

One of the foremost gaps is still in reliable species identification and characterisation of *Ulva* strains. Many cultivated *Ulva* may not have definitive taxonomic identity, which complicates comparisons between studies, reproducibility, and optimisation (e.g. in strain-specific performance, nutritional profiles, growth rates). Consistent and standardised molecular identification is needed to characterise and share suitable *Ulva* strains for particular end-uses (food, feed, biomaterials).

2. Nutritional composition and safety

While *Ulva* has favourable nutritional potential (notably in protein, vitamins, antioxidants, fibres), there remains incomplete understanding of how composition varies with species, growth conditions (light, nutrients, temperature), cultivation system, harvest time, and post-harvest processing. Therefore, there is a need to prioritize a systematic nutritional profiling under standardised conditions, including assessment of how processing (e.g. washing, blanching, drying, fermentation) can affect nutritional value and safety.

3. Processing, value-added products and ingredient development

There is a gap in understanding and employing suitable processing methods for *Ulva* to create value-added, sensory attractive ingredients for food and feed. Furthermore, scalable, cost-efficient processing pipelines that preserve *Ulva*'s functional compounds (proteins, ulvan polysaccharides, pigments, bioactives) are still lacking. More research could ensure that the resulting ingredients meet regulatory, sensory, and functional standards. In addition, durable biomaterials (films, gels, coatings) need better characterization of mechanical, chemical, and biodegradability properties, and how these are influenced by *Ulva* species and treatment.

4. Safety, regulatory and consumer acceptance

Ulva-based foods, feeds, and biomaterials must undergo rigorous safety assessment, including contaminants (pathogens, heavy metals, environmental pollutants), allergenicity, and long-term exposure. Regulatory frameworks at national and European levels are, in many cases, not yet fully adapted to *Ulva*-derived ingredients, which creates uncertainty for producers and consumers. Moreover, consumer perception and acceptance of *Ulva* in food products need to be better addressed by specific marketing strategies and improved communication. Social science research should further be involved for acceptance, labelling, taste, texture, and cultural compatibility.

5. Economic feasibility, scaling up and life cycle assessment

Many promising studies are small-scale. However, translating yields and value-added product development into economically viable large-scale production remains a challenge. Cost of cultivation (mainly in offshore or land-based systems), harvesting, transport, drying, processing, and downstream product formulation need better cost modelling. Moreover, life cycle assessment (LCA) and environmental impact (energy, carbon, water,

nutrient budgets) are under-represented. More economic and environmental analyses should be integrated alongside technical development to ensure that *Ulva* applications are sustainable at scale.

6. Functional performance in end-use applications

For future-proof *Ulva*-derived products, more testing in real end-use settings is recommended. For feed, that means trials with different classes of livestock, aquaculture species, with attention to performance metrics, gut health, immune responses, and microbiome. For human food, above all, more sensory trials and competent product development are required, considering the special requirements of algal ingredients. For biomaterials, tests on durability, stability, and regulatory compliance in intended applications.

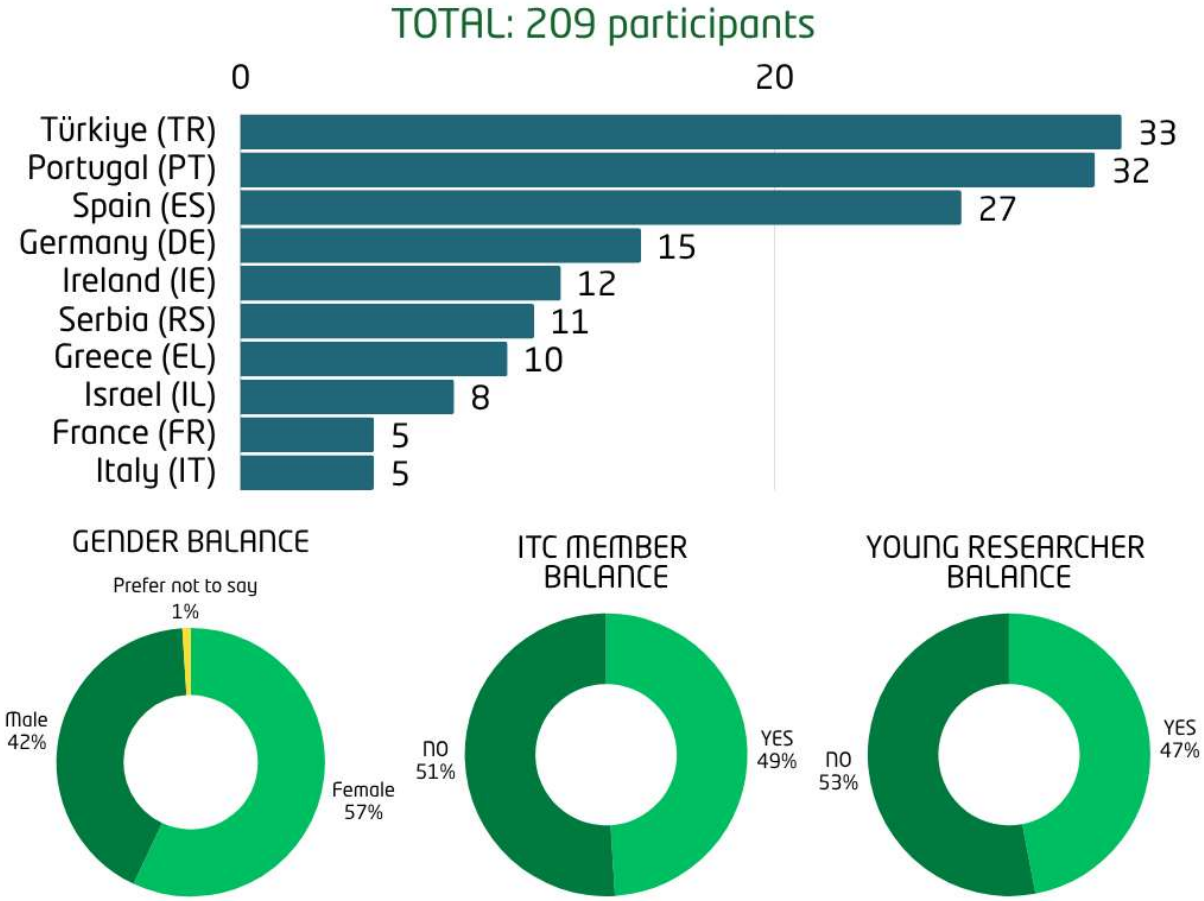
In summary, while *Ulva* offers great promise as a sustainable marine SeaWheat resource for food, feed and beyond, realising its full potential demands interdisciplinary coordinated research to fill critical gaps in taxonomy, nutrition and safety, processing and material science, scaling and economics, and consumer acceptance.

BIOACTIVE CHEMICALS AND *ULVA*-ASSOCIATED MICROORGANISMS (WG4)

LEADER

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PARTICIPANTS



RATIONAL AND OBJECTIVES

The green seaweed *Ulva* (commonly known as sea lettuce) is a widely distributed green macroalga with high ecological and industrial importance. *Ulva* is recognized as a valuable source of bioactive compounds, including polysaccharides (ulvans), proteins, fatty acids, sterols, pigments, and vitamins. These metabolites are of interest for applications in food, feed, nutraceuticals, cosmetics, pharmaceuticals, and as biostimulants in agriculture (Ozogul et al. 2024). The COST Action CA20106 (SeaWheat) and its Working Group 4 (WG4: Bioactive Products) have aimed to explore, characterize, and valorise bioactive metabolites from *Ulva*.

The main objectives include: (**i**) Identifying and characterizing extractable metabolites from *Ulva* relevant for industrial uses by the participating research groups. (**ii**) Developing analytical and biorefinery approaches to optimize extraction and applications within workshop and training schools. (**iii**) Evaluating the antioxidant, antibacterial, antiviral, and other bioactivities of *Ulva* metabolites and their associated microbiome. (**iv**) Promoting collaboration among European and international laboratories to build a comprehensive understanding of *Ulva*'s bioactive potential through STSMs, newly established collaboration for the preparation of a review article, and presenting our results at conferences.

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ACTIVITIES AND KEY FINDINGS

Working Group 4 (WG4) has made substantial progress in exploring the bioactive potential of ***Ulva*** species, focusing on both the diversity of extractable compounds and their functional applications. Research under this WG has revealed a broad array of substances with relevance to the food, cosmetic, pharmaceutical, and other industrial sectors. The deliverables D 4.1 report on the following topics:

a. **Extractable substances from Ulva, utilised as food additives, in cosmetics and for various other purposes (D4.1a).**
   The bioactive potential of Ulva species was thoroughly investigated by *WG4*, with an emphasis on chemicals that can be extracted sustainably and used in a variety of industries. The discovery and characterisation of multiple classes of extractable compounds with great potential for use in food, cosmetics, medicines, and biotechnological applications was one of the group's *significant* accomplishments. Specifically, a WG4 core team has written a review article outlining the potential of *bioactives* obtained from Ulva (Wichard et al. 2025, to be submitted).
   (1) Ulva was found to contain a diverse profile of lipids and fatty acids, including omega-3 polyunsaturated fatty acids (PUFAs), glycolipids, and phospholipids (Hoffman et al. 2024; Wichard et al. 2025). These compounds are of particular interest to the nutraceutical and cosmetic industries, where they are associated with anti-inflammatory, skin barrier-supporting, and cardiovascular health-promoting properties (Table 1). A dedicated lipidomics training, hosted in Aveiro in 2024, enabled WG4 participants to deepen their expertise in advanced analytical techniques and to *harmonise* lipid profiling approaches across laboratories. The workshop also facilitated collaboration with external experts and supported early-career researchers in developing *specialised* skills (Monteiro et al. 2022).
   (2) Further investigation led to the identification of various sterols with potential implications for cholesterol metabolism (Mulder et al. 2025, presentation on COST Action conference, Bremerhaven, Germany), as well as photosynthetic pigments such as chlorophylls and carotenoids. These pigments were confirmed to have antioxidant activity, reinforcing their value as natural *colourants* and functional additives in food and cosmetic products.
   (3) Another promising category of bioactives found in Ulva includes proteins and bioactive peptides. Preliminary screening has shown that Ulva-derived peptides may support nutritional enhancement, making them attractive candidates for use in protein-enriched or functional food products (Steinhagen et al. 2025). Ongoing studies are investigating their digestibility, bioavailability, and potential health claims.
   (4) The significance of these findings was further emphasised during the 2024 WG4 Workshop and Training School in Aveiro (Portugal, see reports), where researchers evaluated and practised extraction technologies and discussed economic and environmental considerations for market entry. Discussions also touched on the potential application and integration of Ulva-based compounds into existing product pipelines and the potential for co-products in circular bioeconomy models and scalable biorefinery pipelines for the production of algae-based bioactive *compounds* (presented by H. Pereira of the GreenCoLab, Portugal).
   (5) In a collaborative effort with WG1, Ulva samples were collected across Europe by WG1 as part of the eUlva initiative (O. *De* Clerck) (https://sites.google.com/view/eulva) for microbiome analysis, while WG4 focused on the quantification of biochemical constituents. This newly established and ongoing network aims to correlate Ulva biodiversity with its associated microbiome and biochemical composition, offering an integrated perspective on ecological and functional diversity.
   In summary, WG4's work has created the framework for Ulva's holistic valorisation plan, which will use its biochemical variety to support innovation across different sectors. The enhanced extraction techniques (for example, in lipidomics and thallusin quantification), knowledge transfer efforts during training schools and STMS, and scientific publications produced during the COST Action all contribute to the widespread dissemination of this knowledge.

Table 1: Overview of extractable compounds in *Ulva* and their bioactive potential (**Wichard et al. 2025**; review of WG4 to be submitted)

Compound Group	Examples	Bioactives
Polysaccharides	Ulvan	Antioxidant, antiviral, anticoagulant

Phenolics	Flavonoids, phenolic acids	Antioxidant, anti-inflammatory
Proteins/peptides	ACE-inhibitory peptides	Antihypertensive, antimicrobial
Fatty acids	αLEA	Anti-inflammatory, cardioprotective
Pigments	Carotenoids	Antioxidant, anticancer
Vitamins	Vitamins A, C, E	General health, metabolism support
Sterols	Stigmasterol	Anticancer
Minerals	Ca, Fe	General health

b. The potential of *Ulva* strain extracts as natural antioxidants, antibacterial and antiviral activities and isolation of bioactive molecules (D4.1b).

In parallel with the compositional analysis of *Ulva*, Working Group 4 (WG4) placed significant emphasis on investigating the biological activity of its extracts and individual compounds in multiple research groups (Ulrich et al. 2022; Palou et al. 2023; Gnayem et al. 2024; Schultze et al. 2024).

(1) New identification techniques were tested, such as Raman spectroscopy or near infrared spectroscopy (Palou et al. 2023 and Schultze et al. 2024). These bioactivity studies formed a crucial part of the group's contribution to the COST Action, supporting the identification of functional properties that could translate into the identification of new substances, and which might be applied across pharmaceuticals, nutraceuticals, cosmetics, and sustainable health products.

(2) One of the most consistently observed properties was antioxidant activity. Extracts from multiple *Ulva* strains exhibited high levels of radical-scavenging capacity, suggesting strong potential for preventing oxidative stress and associated cellular damage. Importantly, WG4's research demonstrated that antioxidant potency was not uniform across strains or cultivation conditions. Instead, it was influenced by a range of environmental and physiological variables, including light intensity, seasonal changes, nutrient availability, and even cultivation method (e.g., wild-harvested vs. aquaculture-grown). These findings, supported by work such as Cardoso et al. (2025), point to the need for strain-specific selection and optimised cultivation strategies when targeting antioxidant functionality for commercial applications.

(3) Another promising avenue of investigation involved the antibacterial and antiviral properties of *Ulva*-derived compounds. In vitro studies carried out across partner laboratories showed that ulvan-rich extracts and specific lipid fractions demonstrated inhibitory activity against a range of bacterial and viral pathogens. These bioactivities were especially relevant in the context of developing eco-friendly alternatives to synthetic preservatives in cosmetics and bioactive ingredients for dermaceutical or topical pharmaceutical formulations. For instance, ulvans can be versatile bioactive building blocks for the development of hybrid biomaterials for biomedical applications (presented by V. Roussis of the University of Athen, Greece)

The antimicrobial properties of *Ulva* also hold promise for incorporation into food packaging and coatings, aligning with EU goals for reducing chemical preservatives and promoting biobased materials.

(4) A third significant development emerged from WG4's work on the isolation and characterisation of specific bioactive molecules also derived from bacteria associated with the seaweed host. Notably, progress was made in identifying and isolating rare compounds such as morphogenetic substances (including thallusin), which play roles in algal development and interspecies signalling (Ulrich et al. 2022; ongoing collaborations within the COST Action network). These compounds have sparked interest for their potential in plant health, aquaculture, and microbial community management. In addition, the discovery of novel sterols opens new directions for research into cholesterol-lowering nutraceuticals and cell membrane-targeting therapies. These achievements were supported by methodological advances shared through WG4 training activities, which introduced improved chromatographic and spectrometric techniques tailored to marine matrices.

(5) The cumulative outcomes of these efforts highlight the multifunctional potential of *Ulva* as a bioactive resource. WG4 demonstrated antioxidant, antimicrobial, and biochemical properties make it uniquely positioned to address multiple market needs with a single, sustainable raw material. WG4 has ensured that this knowledge is not only documented through scientific publications but also accessible through open-access and was taught in the training school. The group's targeted capacity-building activities, including short-term scientific missions and hands-on training schools (**see report**), have helped train the next generation of researchers and enhanced research capabilities across the COST network.

In conclusion, the bioactivity profile of *Ulva* confirmed during the Action reaffirms its value as a renewable marine resource. These findings serve as a springboard for continued exploration, product development, and cross-sectoral innovation in the blue bioeconomy.

FUTURE DIRECTIONS & RECOMMENDATIONS

Prospective Directions and Suggestions: The results and accomplishments of WG4 in the COST Action on marine bioactives highlight the significant potential of *Ulva* species as a sustainable supply of valuable bioactive chemicals. Based on the advancements achieved throughout the Action, many prospective directions and proposals arise to inform ongoing research, implementation, and policy formulation (WG5).

Enhancing Bioprospecting and Functional Characterization: Future research should broaden the scope of bioprospecting to encompass a wider variety and a defined range of *Ulva* strains and growth conditions. Environmental factors, including salinity, light intensity, and nutrient availability, substantially impact the metabolite profiles of Ulva, hence altering the yield and efficacy of bioactive chemicals (e.g., Gnayem et al. 2024). Targeted omics methodologies, such as transcriptomics, metabolomics, and lipidomics, as well as mechanistic studies, will be crucial for elucidating the molecular mechanisms underlying these variances to overcome the descriptive approaches in phycology. However, the data-driven approach will facilitate the discovery of new chemicals with unexploited commercial or medicinal potential.

Standardization and Scalability of Extraction Protocols: Although WG4 achieved notable advancements in optimizing extraction procedures in collaboration with WG1 and due to training schools, further efforts are needed to standardize these protocols across laboratories and guarantee consistency. Special emphasis must be placed on the environmental and economic sustainability of extraction methods, prioritising green chemistry principles and scalable procedures as reviewed in our WG achievement (Wichard et al., 2025) submitted. Joint pilot initiatives with industry collaborators can assess the techno-economic viability of scaling up *Ulva*-derived product lines, including nutraceuticals, cosmeceuticals, and bioplastics.

Enhancing Intersectoral Cooperation: The communication established between academia and industry (SME) during workshops in Lisbon and Aveiro (Portugal) in 2023 and 2024 set up new networks and collaborations. These networks will now enable technology transfer, regulatory compliance, and market access. Promoting interdisciplinary collaboration among marine ecologists, chemical engineers, and regulatory specialists would expedite the creation of commercially feasible solutions.

Regulatory Alignment and Policy Support: The rapid commercialisation of *Ulva*-derived goods necessitates strict adherence to food safety, pharmaceutical, and cosmetic standards. It is advisable to engage with regulatory authorities at both national and EU levels to ensure that innovative *Ulva*-based products comply with safety and efficacy standards. Additionally, policy frameworks must facilitate the sustainable cultivation of macroalgae, encompassing provisions for integrated multi-trophic aquaculture (IMTA). The uptake and sequestration of metals and micropollutants should be particularly monitored (Hardegen et al. 2025, Vargas-Murga et al. 2025)

Instruction and teaching the next generation of (applied) researchers: The efficacy of COST Action initiatives, especially short-term scientific missions and training schools, underscores the importance of investing in early career researchers and fostering multidisciplinary skill enhancement. Future projects ought to enhance accessibility to practical training in sophisticated analytical techniques, bioinformatics, and process engineering. Although the overall importance of seaweed research is acknowledged, the involved and funded scientists are a small group in the scientific landscape.

Conclusion: The encouraging outcomes produced by WG4 establish a robust basis for ongoing study, innovation, and valorisation of *Ulva* bioactives and particular new networks of scientists who are now seeking Horizon Europe-based funding. Here, future initiatives must emphasise ecological sustainability, technical scalability, and regulatory integration, while enhancing cross-sector collaboration and education. By utilising these guidelines, *Ulva* can transform from a still underexploited marine resource into a fundamental component of the blue bioeconomy in Europe.

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ULVA ECOSYSTEM SERVICES (WG5)

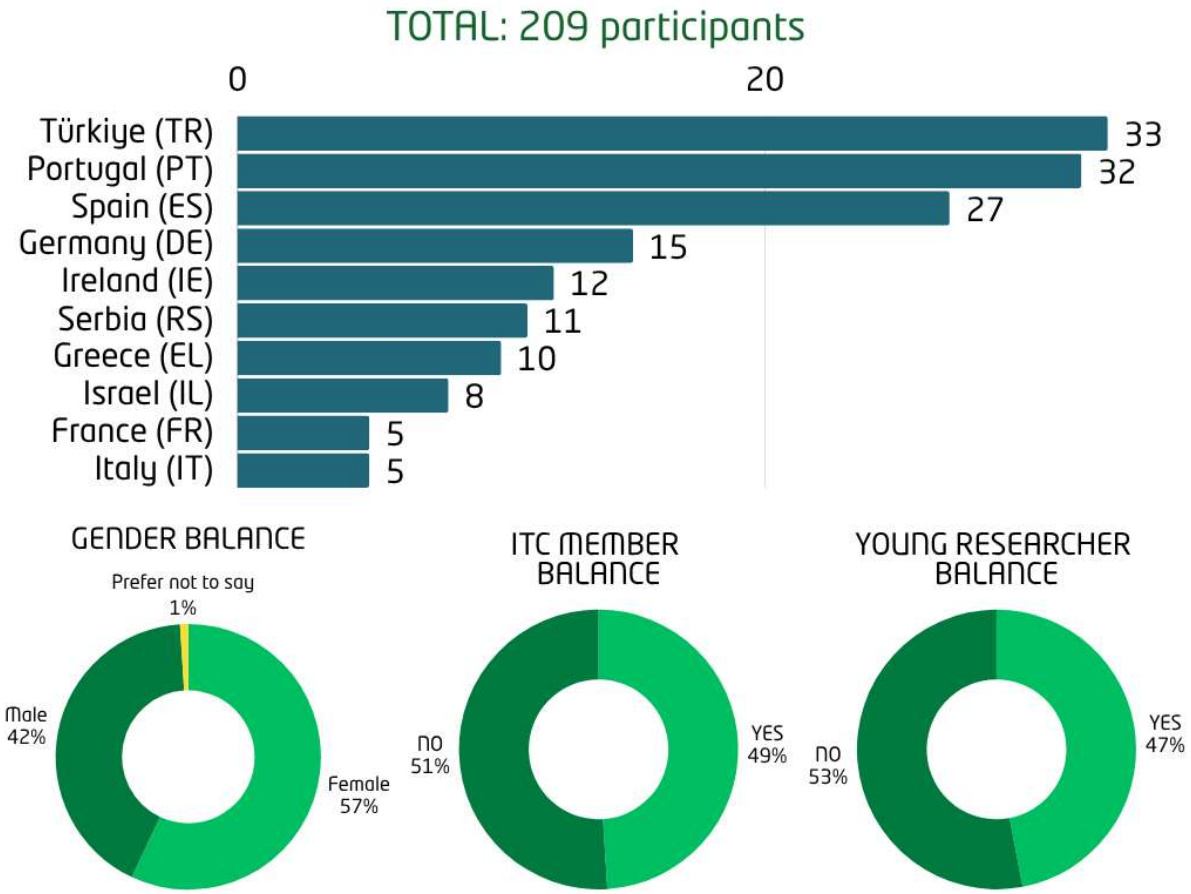
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RATIONAL AND OBJECTIVES

The green seaweed Ulva is a cosmopolitan genus growing in all marine and brackish waters around the globe. Wherever the species grow, they provide ecosystem services (Haines-Young and Potschin 2017) or ecosystem disservices (Lyytimäki 2015)*, being part of the biogeochemical cycles of nutrients, carbon, sulphur and other minerals*, affecting the composition of the surrounding water, the atmosphere and the associated biota. In balanced ecosystems, Ulva provides feed, shelter and oxygen for other marine organisms (Clemente and Thomsen 2024), whereas in eutrophic waters, Ulva grows out of control, forming excessive mass blooms, also called green tides (Smetacek and Zingone 2013; Ye et al. 2011). In either case, the harvest of Ulva provides a biomass to be used for food, feed, biostimulants, materials and more (Bikker et al. 2016; Juul et al. 2024; Hofmann et al. 2025). Ulva can also be cultivated in systems both on land and in the sea, as a more controlled means of emission capture and utilisation – enabling the re-use of nutrients and carbon from waste streams or lost to sea (Christiansen 2018; Larsen-Ledet et al. 2024; Mazarrasa et al. 2014; Sode et al. 2013; Zollmann et al. 2021; Neori 2008). The ecosystem services and disservices of Ulva species have yet to be fully described and mapped to gain sufficient knowledge to actively make use of the ecosystem services for improved environmental conditions and livelihood (Fricke et al. 2025).

WG5 has worked to increase and disseminate knowledge on Ulva ecosystem services and disservices, educate early career researchers in the concepts, potentials and methodologies of Ulva ecosystem services and disservices, and formulate recommendations for policies strengthening the active applications of Ulva Ecosystem services in a European and global context.

ACTIVITIES AND KEY FINDINGS

The following activities have been carried out as part of WG5:

WG meetings (Knowledge transfer): 14 WG meetings have been accomplished with online participation of WG5 members. Here, knowledge and research have been shared between group members.

Workshop (Knowledge transfer): A workshop was organised in Estonia in May 2024 in collaboration with WG6.

Training school (Knowledge transfer, career development): A training school was organised in Estonia in connection with the workshop with the participation of early career scientists and SME representatives.

Short Term Scientific Missions (STSM) (Knowledge transfer, carrier development): WG5 has supported a minimum of 4 STSMs, and connections have been made for transfers related to, but directly funded by, SeaWheat, i.e. as part of the SeaChem project (ECR from University of Galway to SME' PureAlgae (Denmark).

Publications (Knowledge creation): Several research papers connected to WG5 have been published by members of WG5. In addition, joint papers are in preparation based on the WG5 work: One systematic literature review summarising Ulva Ecosystem Services and Disservices (Deliverable D5.1a+b), one experimental research paper based on the WG5+6 training school in Estonia and one joint paper summarising the cost-benefits and life cycle assessment of harvesting Ulva for nature regeneration and provision of green protein (D5.1c). In addition to the peer-reviewed publications published, submitted and in preparation for submission within 2025, three master's theses and one bachelor's thesis have been finalised as part of WG5.

The WG5 MoU deliverable (5.1) has been achieved in the form of two joint peer-reviewed papers:

1. Ulva Ecosystem Services - A Systematic literature review of the global ecosystem services and disservices of Ulva according to the CICES framework, to be submitted to Science of the Total Environment. This paper summarises tasks 5.1-5.4 and concludes parts a and b of the Deliverable 5.1:
   a. **The providing and supporting ecosystem services of *Ulva* spp., nutrient and carbon removal and recycling.**
   b. **The regulating services, the effect of biomass culture on reducing climate gas emissions and mitigating ocean acidification.**

   The preliminary results show that Ulva species deliver Ecosystem Services around the globe, with studies and descriptions from all continents and all climate zones. Most dominant are the Maintenance and regulation Ecosystem services with 'Filtration/storage/sequestration', 'bioremediation of toxic substances or waste', and 'Regulation of chemical conditions of salt water being the most dominant categories. However, also 'Regulation of chemical composition of atmosphere and ocean', 'Maintaining nursery (habitat, feed)', 'Control of erosion', 'Control of hydrological cycle', 'Pest control (incl. *Invasive species and Disease controls are among the* Maintenance and regulating ecosystem services described for Ulva. For the provisioning *of Ecosystem Services, the* provision of nutrition (food and feed) and materials dominated together with Materials, whereas energy was represented at a lower level. Here*, we also demonstrate a movement with time in the Ulva utilisation focus, from energy towards higher value,* such as nutrition. Regarding the cultural ecosystem services, Ulva was strongly represented under the 'Characteristics of living systems that enable scientific investigation or the creation of traditional ecological knowledge, as all registered studies represented the *creation* of knowledge.

   The review documents the importance and at the same time under-acknowledgement of Ulva Ecosystem Services and emphasises the need for identifying, quantifying, acknowledging and valorising the Ulva Ecosystem Services to actively use them to support the UN SDGs (Duarte et al. 2022) and for progressing towards safe operating space within planetary boundaries (Richardson et al. 2023).

   More information here: link will be added after publishing

2. Towards Restorative and Regenerative Aquaculture: Sustainable Seaweed Production for Ecosystem Health and Blue Growth to be submitted to Science of the Total Enviroment. This paper summarises tasks 5.5 and concludes the part c of the Deliverable 5.1:

   c. **An economic cost-benefit analysis of biomass production and harvesting of Ulva spp. for ecosystem services**

   This case study, situated within Denmark's scientific–industrial R&D landscape, investigated the dual benefits of protein extraction and water quality improvement, alongside climate change mitigation, through the gentle harvesting of Ulva spp., a green seaweed prevalent in green tide events. The biobased product–service system (BPSS) was evaluated using life cycle assessment (LCA) and net present value (NPV) methodologies to assess environmental sustainability and economic viability.

   The LCA quantified the environmental impacts of harvesting and processing 1 ton of Ulva spp., under system boundaries that either included or excluded (i) emission capture and utilisation (ECU) of surplus aquatic nutrients and CO_2, and (ii) footprint savings from substitution. Scenario analysis compared the current Danish electricity mix with a future wind-powered energy system, covering climate change (CC), freshwater eutrophication (FE), marine eutrophication (ME), and land use (LU). Across scenarios, system-level footprints ranged from 124–145 kg CO_2 eq. (CC), 0.05–0.07 kg P eq. (FE), 0.006–0.007 kg N eq. (ME), and 3.8–8.4 m² (LU). Energy-related emissions accounted for only 15–20% of total GHGs, though the Danish mix was 38% more emission-intensive than wind. When ECU and substitution effects were included, the BPSS achieved net-negative footprints, ranging from –144 to –122 kg CO_2 eq. (CC), –0.36 to –0.34 kg P eq. (FE), –4.1 to –4.0 kg N eq. (ME), and –132 to –128 m² (LU).

   Economic feasibility was assessed for two habitat restoration scenarios: harvesting 4.5 kilotons annually (base case) and 11 kilotons (extended case). Without revenues from environmental savings, NPVs were –0.6 million and 3.3 million €, respectively. Including environmental savings increased NPVs to 1.9 million and 10 million €, with payback periods of 3 years (base case) and 1 year (extended case). The rapid return on investment was driven by the availability of wild-harvested Ulva spp. from green tide events and the improved utilisation of processing capacity at scale, which together reduced the cost per tonne and enhanced overall economic performance.

   More information here: link will be added after publishing

DIRECTIONS AND RECOMMENDATIONS

Future efforts should focus on integrating *Ulva* cultivation into environmental management and circular bioeconomy strategies. Legislation should pave the way for expanding the use of *Ulva* in nutrient-rich waste streams and eutrophied waters, enabling dual benefits of improving marine water quality—thereby contributing to the objectives of the EU Water Framework Directive—and providing biomass that complements land-based production systems. Establishing food-grade certification standards for *Ulva* cultivated in food system waste streams is essential to ensure safety, traceability, and consumer confidence.

To mitigate the environmental and climate impacts of uncontrolled *Ulva* blooms, gentle and environmentally sound harvesting methods should be developed. Such approaches can enhance environmental quality, prevent greenhouse gas emissions from decomposing biomass, and generate valuable raw material for bio-based industries. In parallel, the introduction of a nutrient credit system is recommended to acknowledge and incentivise companies that cultivate or harvest *Ulva* for ecosystem regeneration and alternative biomass production, aligning economic mechanisms with environmental goals.

Preventive actions are equally critical. Nutrient emissions to coastal waters should be limited through the integration of *Ulva* cultivation in wastewater treatment and aquaculture effluent systems, capturing nutrients before they contribute to eutrophication and green tide formation.

Despite progress in understanding *Ulva* ecology and utilisation, key knowledge gaps remain. The global extent and economic consequences of *Ulva* green tides are still poorly quantified. Addressing these gaps through coordinated monitoring, remote sensing, and economic assessment will provide the evidence base needed for effective policymaking, valorisation strategies, and sustainable integration of *Ulva* into the blue bioeconomy.

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SOCIAL, LEGAL AND REGULATORY ASPECTS (WG6)

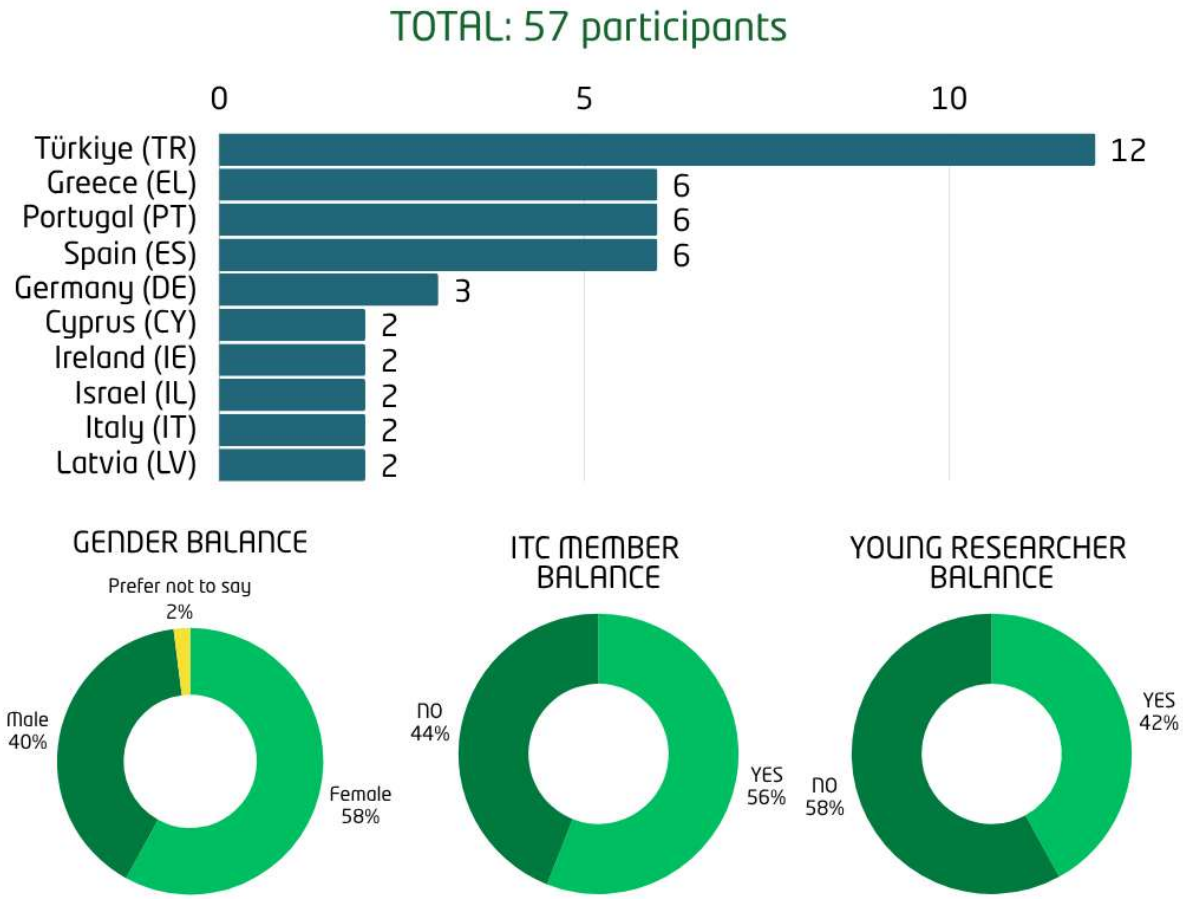
LEADER

Céline Rebours (Møreforsking AS, NO)

CO-LEADER

Pierantonio Addis (Cagliari University, IT)

PARTICIPANTS



RATIONAL AND OBJECTIVES

Blue Biotechnology (BB) is a multidisciplinary, knowledge- and capital-intensive technological sector, essential for innovation under the European Sustainable Blue Economy (SBE) (COM(2021) 240) and Green Deal (GD) Goals (COM/2019/640). The BB sector in Europe includes non-traditionally commercially exploited marine organisms and their biomass applications in pharmaceutical, medical, cosmeceutical, nutraceutical, aquacultural, agricultural, and energy sectors. Under the Farm to Fork strategy (COM/2020/381 final), the algae biomass is identified as one of the most valuable sources of structurally diverse bioactive compounds among the marine organisms, and its utilisation would support society to gain various environmental and health benefits. Therefore, in 2022, the European Commission (EC) also launched a proposal titled "Towards a Strong and Sustainable EU Algae Sector", also called the “EU algae initiative” (COM/2022/592 final), highlighting the importance of utilising algae as a renewable resource in Europe to ensure a secure supply of raw materials and energy.

As a result, the European Maritime, Fisheries and Aquaculture Fund (EMFAF) with the Blue Invest fund, already supported 97 companies in the algae business with access to finance for early-stage businesses, SMEs and scale-up, for which 39 received technical assistance from the Blue Invest team and 26 reached a technology readiness (TRL)-9 (Carboni et al 2025). Out of the 116 projects about algae financed by Horizon 2020 between

2014 and 2021 for a total of € 273.1 million, 25 projects deal with macroalgae for biofertilizers and bio-feed, biofuels and bioplastics, and biorefineries. Besides, 11 projects deal with algae in combination with other organisms such as molluscs, echinoderms, shellfish, finfish, and other sea invertebrates (Hranilovic et al., 2023). Nevertheless, from the total macroalgae produced in the EU-27 in 2022 (94.5 thousand tonnes w.w.), only 0.7% (704 t W.W.) was produced by aquaculture. The macroalgae farmed in the EU represent 0.00193% of the global seaweed aquaculture (375 t w.w. or 0.001% in 2021), while the rest of European countries contributed with an additional 336 t w.w. or 0.0009% (Rebours & Sánchez López, 2025). On the contrary, the production volume of the seaweed industry is reported to have increased worldwide since 1950 (Rebours & Sánchez López, 2025).
Despite these trends of increased demand for algae biomass worldwide and efforts from the EC to support its development, the macroalgae cultivation in Europe is still at an early stage of development, with limited volume being produced and even the lowest quantity reaching the market. In the last 20 years, the efforts have mainly focused on kelp species due to the relative ease of controlling their reproduction cycles and producing large volumes directly at sea (limiting land use and moving the production to the Ocean). However, these brown macroalgae face challenges in penetrating the market, making it difficult for the European seaweed industry to answer the existing demand for marine biomass for the production of food and feed ingredients with a low carbon footprint, calling for a need to diversify the cultivated species.
Building on the successes of previous EU and pan-European projects, and due to its unique characteristics, the green macroalgae of the genus *Ulva* (Linnaeus, 1753) were identified as the most suitable candidates and model organisms for a novel European mariculture (Hofmann et al., 2025). The species of *Ulva* can effectively assimilate dissolved nutrients with high efficiency and biomass yield (e.g. Lubsch & Timmermans, 2018). Only a limited number of Ulva species are currently cultivated on a small commercial scale in Southern Europe, predominantly in land-based systems, and in some cases under Integrated Multitrophic Aquaculture (IMTA) systems (e.g. Neori et al., 2003). In northern Europe, high biomass yields in land-based systems have also been demonstrated with *Ulva* biomass that can be valorised as food and feed ingredient (e.g. Bruhn et al., 2011).
Worldwide, *Ulva spp*. Production is estimated at 3699 t.w.w. for a value of 1,074,400 EUR with an average price of 290.41 EUR per t.w.w. (Rebours & Sánchez López, 2025), While in Europe, Ulva production remains very low, and the total production numbers are difficult to estimate. Today, the production in terms of volume and value is reported to national authorities and further communicated to Eurostat and FAO databases. In both cases, the species are not accurately named, making it difficult to oversee the size of the Ulva industry in Europe. Seaweed industry (incl. Ulva) also faces the challenge of being a plural sectoral activity, forcing entrepreneurs to navigate a complex regulatory framework governed by a multitude of agencies both nationally and at the European level. This multi-sectoral distribution of the business also makes it challenging to identify the actors across Europe and to understand if segment(s) of the value chain are still missing to reach the existing market. Furthermore, even seaweed (incl. Ulva) extracts are today already used in a large variety of products across Europe, this ingredient is still perceived as novel by consumers and may result in a large portion of the adult population having low acceptance (neophobia) in consuming seaweed (Losada-Lopez et al, 2021).
Therefore, the WG6 examined the regulatory framework relevant to Ulva production at a large scale, investigated the acceptance of Ulva products by society, and the economic aspects, including risk assessment in different scenarios and geographical locations. This WG fulfilled the Cost Action challenge “e” about enhancing acceptance of Ulva as food, feed and source of bioactive products by society, and drawing recommendations for amendment or reinforcement of the current regulatory framework for Ulva-based industries.
Under **Task 6.1**, the European regulations related to the Ulva mass production (land- and sea-based) for the market of food, feed (incl. potentially highly valuable secondary metabolites) were reviewed to understand the regulatory framework that applies to food and feed safety and quality was reviewed and analysed to identify the gaps to support the development of a sustainable European Ulva industry. The existing national legislations and the implementation of these EU regulations by member states and associated EU countries were also investigated by national contact representatives (a total of 27 and their deputies) through desk study and, when possible or needed, with consultations of national authorities, NGOs, industry and SMEs across Europe. The regulation framework addressing the environmental impact and evaluation of the Ecosystem services was done in collaboration with WG5. Collaborations with WG1, 2 and 3 were also established to increase knowledge and

understanding of the regulations and governance that apply to their respective segments of the value chain (e.g. WG1: Nagoya protocol and Material Transfer Agreement; WG2: EU Aquaculture Assistance Mechanism/Eu4Algae licensing toolbox; WG3: food, feed, cosmetics labelling).

Under **Task 6.2,** several surveys and face-to-face consultations were completed to better understand the impact of Ulva cultivation, processing and marketing on various communities. Some surveys were directed to SMEs to collect their knowledge relative to, e.g. their consumers' acceptance of Ulva as food and their impact on job creation in their community. Other surveys were directed to the participants of some of the Training School (TS) and Workshop (WS) in order to assess better the level of knowledge, know-how and education of the participants (preselection of young employees or entrepreneurs in the Ulva industry). TS and WS were also designed to increase the level of competence of these populations. In collaboration with WG7, videos were created to increase various stakeholders ' knowledge, including the general public, about the Ulva industry and the potential benefits of consuming Ulva. Under WG7, a cookbook was also published to encourage potential consumers to try Ulva in their daily food preparation.

Under **Task 6.3 and 6.4**, a survey was conducted to assess the risks (e.g. Legal/Licensing, Financial / Budget, Chain of supply, Production/Manufacturing, Personnel, Biological risks, Health and Safety Issues, Environmental/Weather/Nature, Marketing/Access to Market) related to any segment of the land and sea-based Ulva industry (e,g, culture, processing, biorefineries, marketing). Participants to the survey were also invited to indicate their countermeasures. When existing, respondents were invited to report any conflicts of interest incl. competition of at sea and land space for large scale production of Ulva. The result of this survey will be transferred to related governments with suggestions for possible amendments.

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ACTIVITIES AND KEY FINDINGS

The WG 6 Deliverable D 6.1 (a, b, c) aimed at proposing recommendations for updating policies about seaweed production (incl. culture) regarding environmental aspects and to address eventual conflict of interests. D6.1 ambition to report on the following topics:

a. National regulations on seaweed mass production and its marketing as food, feed and valuable secondary metabolites.

b. The impact of Ulva cultivation on various communities concerning social aspects.

c. Ulva as food and feed, nutrition, job creation, community income and education.

Therefore, following the investigations described under tasks 1 to 4, all the reports below were completed and fed into the deliverable 6.1 (D6.1).

a. Policy Brief

This deliverable provides the overall results for D 6.1. The document gave a short description of the Ulva industry, the existing EU strategic roadmap for a sustainable seaweed industry and then provided the recommendations for improving regulation frameworks and a list of the support tools necessary to develop a sustainable Ulva industry in Europe further.

More information here: link to be added

b. Summary of the EU policy and governance for Ulva production

This deliverable provides the results for D 6.1. The document is designed to guide young entrepreneurs through the existing EU strategies, documents, regulatory framework and support mechanisms already available in Europe for the seaweed industry. Supplementary materials were also provided through presentations from DG MARE, DG SANTE, consumers and the EU4Algae licensing tool kit. These presentations are also made publicly available.

More information here: link to be added

c. **Overview of the National Legislation relevant to Ulva industry.**

This deliverable provides the results for D 6.1a. The tables 1 to 4 provide information from 20 EU27 member states (Belgium, Bulgaria, Croatia, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Latvia, Malta, Netherlands, Portugal, Romania, Spain, Sweden), 3 Countries under the stabilisation agreement (EU candidate countries, Moldova, Montenegro, Turkey) and 4 Countries with close relationship with the EU and bilateral agreements (Israel, Norway, U.K., South Africa). Each table lists the regulations that apply to the Ulva industry in each country, as well as indicates the responsible agency, the corresponding EU and /or international regulations implemented nationally. Table 1 focuses on the General legislations (e.g. Marine Spatial planning), table 2 on legislations to addressed at the planning stage (e.g. licensing), table 3 on legislation that need to be addressed when the production is in operation (e.g. hygiene practices) and table 4 presents the legislations that applies depending of the market (e.g. food, feed, biomaterials, cosmetics, pharmaceutics, agriculture and others). These four tables are planned to be published as supplementary materials in the peer-reviewed paper under preparation for the Journal of Environmental Science and Policy, and will be confidential until publication.

More information here: link to be added after publishing

d. SME platform

This deliverable provides the results for D 6.1b and c. During the project an SME platform was created to facilitate the consultation of the stakeholders and increase communication and potential collaboration between the various SME throughout the value chain. The goals of the SME platform were i. identify the scientific bottlenecks and regulatory issues facing the industry, ii. facilitate the knowledge transfer from the Research entities to the industry, iii. Promote the creation of a network with other companies and research institutes to establish a consortium for future EU projects, iv. promote European Citizens' Initiative (ECI) and v. connect Ph.D. and young scientists to industry.

More information here: link to be added

e. Ulva industry value chain

This deliverable provides the results for D 6.1b and c. During the project, a stakeholder analysis was conducted to identify the companies explicitly involved in the Ulva industry. 64 Companies were reported to work with Ulva spp, but only 17 participated in the CA SeaWheat. There were 21 aquaculture producers: (9 sea-based, 13 land-based, 6 IMTA); 33 harvesters: 33 (wild collection, blooms), five that both conducted aquaculture & harvesting operations, 32 processors: (extracts, food preparations), 39 B2B - sellers (raw & processed), 29 B2C – Retailers: (processed mainly) and 12 associations such as tech providers, Algaebase, R&D, stakeholder platforms, Observers. This list was used to understand better the value chain and their representatives were invited to participate in the different consultations and surveys organised under the CA SEAWHEAT. The structure of the value chain was presented at the Final SEAWHEAT conference in Bremerhaven in September 2025.

More information here: link to be added

f. Online Stakeholders' surveys

This deliverable provides the results for D 6.1b and c. During the course of the project, three surveys were conducted. The first one was done during the application of the Responsible Research and Innovation (RRI) principles after a short presentation kick-off conference meeting in Brussels (Belgium) with online participants. to understand the profile of the participants in the CA SEAWHEAT project and their level of awareness, and to introduce the RRI, Convention of Biological Diversity (CBD), Nagoya protocol (NP), Access and Benefit sharing Clearing house (ABSCH)that apply to Marine biotechnology. There were 54 respondents. The results of this survey were presented during the first SEAWHEAT conference in Cadiz (Spain) in September 2022. They were further used to design the training courses, workshops and conference meeting presentations to increase awareness and understanding of RRI, CBD, NP and the need to negotiate the access right with the primary owner and the use of Material transfer agreements to avoid future conflicts of interest.

More information here: link to be added

g. Two face-to-face stakeholders' consultation

This deliverable provides the results for D 6.1b and c. Face-to-face stakeholders' consultations were designed to understand the challenges faced by the SME and industry interested in the seaweed or Ulva sectors in developing a sustainable seaweed (Ulva) industry in Europe. The first-round table consultation, which was conducted in Brussels (Belgium) in February 2023, highlighted the low acceptance of European consumers for seaweed products and the difficulties in designing suitable and cost-efficient production/engineering systems. During the second-round table in Galway (Ireland), 3 main issues were identified: 1. the negative perception of the European public on seaweed, 2. the cost of production, and 3. legislation and regulatory issues. The results of the surveys were presented at the Final SEAWHEAT conference in Bremerhaven in September 2025. Further, based on these results, efforts were made through out the project period to increase understanding of the existing regulatory framework by presenting the European regulatory framework that apply when producing Ulva for a variety of markets (e.g. food, feed, cosmetics, fertilisers) to various audiences during SEAWHEAT workshops and conference but also outside the SEAWHEAT consortium during the Nordic Seaweed Conference in Greena (Denmark) in October 2024 and the International Seaweed Symposium (Victoria, Canada) in Mai 2025. A special session was also organised during the Danish stakeholders meeting (Erhvervsnetværksmøde) in Greena (Denmark) to reinforce the understanding of the steps to achieve legal, equal, and ethical sharing of genetic resources in Blue Biotechnology early in the process of innovation in the seaweed (Ulva) sector, to avoid future conflict of interest.

h. Risk assessment online Survey

This deliverable provides the results for D 6.1b and c. A final survey targeted specifically the industry stakeholders throughout the value chain to complete a risk analysis in order to understand better the challenges faced by the Ulva industry in Europe. Participants are invited to identify potential risks and suggest possible countermeasures. There were 28 respondents by the 23rd of September 2025. Nevertheless, to increase participation, the survey deadline is extended to the 15th of October 2025. The results will then be included in the peer-reviewed paper under preparation and will contribute to the development of science-based guidelines for decision-makers and support future technological advances and interdisciplinary research.

More information here: link to be added

i. Consultations of TS and WS participants

This deliverable provides the overall results for D 6.1c. During this exercise, the participants in the TS were invited to self-evaluate their knowledge, know-how, and gaps in knowledge before and after the training/teaching session. They could also propose what could be done to improve the training session. Even though the overall satisfaction was very high, the intensity (short time and a lot of information to be able to cover the proposed topics) was the main negative issue reported, and this could be limiting the complete understanding or retention of the information by the student. In future training initiatives, allocating more time may be the solution. The results of these consultations were presented during the Management Committee meeting of the SEAWHEAT.

j. Collaboration with other EU-commissioned groups

This deliverable provides the results for D 6.1 to ensure that the results are further communicated to the EU Commission through its diverse working group.

- ESPP (European Sustainable Phosphorus Platform)
- EU4Algae – Licensing tool kit
- EABA – Novel food (Algae Taxonomy Changes)
- EU tender (example: Contract CINEA/2023/OP/0006 “Study to support Sustainable EU Algae Industry”)
- JRC/EMODnet

FUTURE DIRECTIONS & RECOMMENDATIONS

The EU Governance and Strategy roadmaps illustrated that the European Commission (EC) is supportive of the development of the Algae industry.

- The Green Deal COM/2019/640
- The Clean Planet for all strategy COM/2018/773
- The European Climate Law Regulation (EU) 2021/1119
- The Blue Growth Strategy COM/2021/240
- The Farm to Fork strategy COM(2020) 381
- The Sustainable Development of Aquaculture Strategy COM(2021) 236
- The Organic Production Action Plan COM(2021) 141 final/2
- The Strong and Sustainable EU Algae Sector Strategy COM/2022/592
- The Circular Economy Action Plan For a cleaner and more competitive Europe COM(2020) 98 final (under revision)

However, the regulatory framework that applies to the seaweed (Ulva) industry is complex as this industry expands over a variety of sectors of activities, each of which has already developed its own regulatory framework. Therefore, to further support the sustainable development of the seaweed (and in particular Ulva) based industry, the CA SEAWHEAT WG6 participants would like to propose the following recommendations:

1. Develop a unified regulatory framework for seaweed products to consolidate the Common (internal) European market and clarify the rules of access of the EU27 market by non-EU member states that want to import by developing the concept of Fair Trade (COM/99/0619 final).
2. Ensure traceability of the seaweed ingredients for food and feed by ensuring that products claimed as European products are produced in Europe, not only packaged in Europe, by amending Regulation (EU) 1169/2011 and (EC) 767/2009.
3. Homogenise the reporting of seaweed production data across Europe to increase the accuracy of the EUROSTAT (Regulation (EC) 762/2008): quantity and value reported should be done per species and location.
4. Define a standard method for identification of *Ulva* species to ensure correct naming of the species in the policies and reporting frameworks (incl. Recommendation (EU) 464/2018).
5. Correction of species name in the Novel Food Regulation (EU) 2015/2283.
6. Increase knowledge and data about *Ulva* (seaweed production systems and products) to facilitate carbon accounting in the Regulation (EU) 2024/3012.
7. Support the concept of Integrated Aquaculture/agriculture by including *Ulva* species as a tool for water quality restoration in the:
   a. Water Policy Framework Directive 2000/60/EC
   b. Wastewater treatment and sewage sludge Directive 86/278/EEC*
   c. Urban Wastewater Treatment Directive (EU) 2024/3019
   d. Nitrate Directive 91/676/EEC

Further to support both the Ulva sector young entrepreneurs in developing their economic activity and, the National and European authorities in understanding better the need of this industry and oversee its activities, the CA SEAWHEAT WG6 participants would like to propose the following tools to be established at national or European levels through close collaboration with the industry and in some case with the participation of the research community:

1. Appoint national contact points to navigate and increase knowledge about the existing national and European regulatory framework.
2. Include production data per location and species in the EMODnet platform.
3. Define standards for production and products in the common EU markets: e.g. Management plan for disease control (e.g. bacteria, viruses), traceability, risk assessment, food and feed safety documentation, environmental requirements (including reporting methods).
4. Complete the EU Aquaculture Assistance Mechanism: information about the national licensing system for all European countries and production protocols to support start-up.
5. Complete the features in the Blue Invest platform with information to support Ulva (Seaweed) industrial development as sustainable solutions for almost all the identified blue sectors.
6. Increase portfolio for the establishment of a sustainable Ulva (Seaweed) aquaculture under the European Maritime, Fisheries and Aquaculture Fund (EMFAF).
7. Allocate Research and Innovation Fundings to increase scientific understanding and documentation of Ulva aquaculture as an agent for bioremediation of nitrogen, phosphorus emissions and ecosystem services.

Finally, the WG 6 will work beyond the CA SEAWHEAT project to prepare a peer-reviewed publication that will present the results of this study. This scientific publication will be proposed to various working groups commissioned by EC, in particular, some of the data collected about national licensing are already in discussion to be added to the EU4Algae licensing toolkit.

SME PLATFORM

LEADER

Ioannis TZOVENIS (Microphykos, GR)

RATIONAL AND OBJECTIVES

The SME platform includes universities, R&D institutes, SMEs, large companies, NGOs and government departments which run public services. The SMEs partners involved belong to the aquaculture sector (fish and seaweed) and seaweed-related industries (food, feed, bioactive products) and marketing. Throughout the COST Action timeline, stakeholders had active roles in conferences, workshops, STSMs and TS. These activities brought together academia and industry sectors for knowledge exchange and its rapid transfer to industrial end-users. The SME platform was used to introduce ECIs to the industries and foster possible careers. This was achieved by actively involving SME partners in SeaWheat activities, so SME partners related to seaweed industries can present their activities to future scientists and leaders of relevant sectors.

In the Cadiz Conference (SEP 2022) the objectives of the SME Platform were framed and set: To bring together all the leading players of the *Ulva* industries to:

- Identify the scientific bottlenecks and regulatory issues facing the industry
- Facilitate the transfer of knowledge from the Research entities to the industry
- Promote the creation of a network with other companies and research institutes to establish a consortium for future EU projects
- Promote ECI and connect PhD and young scientists to industry

As SME Leader was approved after volunteering Dr Paul Kleidal (Denmark). However, he stepped down in late 2022 as he left his organisation. Dr Ioannis Tzovenis was approved in February 2023 after volunteering to take up the position.

ACTIVITIES AND KEY FINDINGS

Meetings

1. At the Cadiz Conference (Sep 2022), objectives for the SME Platform were established.
2. The main issues were outlined at the Brussels SME meeting (Feb 2023).
3. In Riga (Sep 2023), topics included public perception, an animated clip presentation, licensing issues, sustainable seaweed cultivation, collaborations, R&D opportunities, the creation of a private LinkedIn channel, marketing strategies, and insights from a US professional.

- In Brussels (Apr 2024), SMEs presented, with participation from representatives across several countries. A cookbook was published with an introduction by Prof. Dr. Shpigel.
- In Galway (Sep 2024), knowledge gaps in industrial applications were identified, and the LinkedIn and WhatsApp group "Seawheat SMEs" was launched.
- In Cagliari (Jun 2025), a questionnaire on SME Platform performance and a detailed Ulva industry survey were presented.
- The Final Conference in Bremerhaven (Sep 2025) featured a session on the Ulva industry, keynote addresses on aquaculture trends, EU sector lessons, Danish seaweed efforts, support for algaepreneurs, and a review of the SeaWheat SME Platform, followed by a panel discussion on the industry's future.

Training Schools

- April 25-28, 2023: Training School for 14 ECIs (including industry participants) at Fisheries Research Institute, Kavala, Greece.
- June 26-29, 2023: Training School for 17 ECIs (9 from industry) at Grice Hutchinson Experimental Station, Malaga, Spain.
- May 14-17, 2024: Training School for 16 ECIs (3 from industry) at Koiguste Marine Field Station, Saarema, Estonia.
- June 26-28, 2024: Training School for 16 ECIs (2 from industry) at University of Aveiro, Portugal.
- April 1-4, 2025: Training School for 15 ECIs (with industry representation) at Fisheries Research Institute, Kavala, Greece.

Key topics include consumer acceptance, production challenges, negative public perception of seaweed in Europe, high production costs and regulatory hurdles.
Ulva industry structure:

- 64 companies (17 in SeaWheat)
- 21 aquaculture producers (9 sea-based, 13 land-based, 6 IMTA)
- 33 harvesters (wild collection, blooms)
- 5 engage in both aquaculture & harvesting
- 32 processors (extracts, food preparations)
- 39 B2B sellers (raw & processed)
- 29 B2C retailers (mainly processed)
- 12 other roles (tech, R&D, platforms)

Challenges:

- Immature European sector: small size, limited collaboration, low awareness and demand, complex regulations, negative image
- Scale-up difficulties: high costs, fragmented value chain, stringent regulations
- Environmental risks: invasive species, nutrient depletion, monoculture vulnerabilities
- Climate change: increased Ulva blooms, risk of anoxia, worsened perceptions
- Socio-economic conflicts with fisheries and tourism, requiring multidisciplinary approaches

ACHIEVEMENTS

At the start of the Action, there was minimal communication between academia and the Ulva industry. Through the SeaWheat COST Action, we identified value chain stakeholders, facilitated meetings and training, and created tools like the SEEK search engine and online groups (LinkedIn, WhatsApp) to support collaboration. Large conferences further connected academia and industry, driving new ideas and projects. Ongoing engagement through surveys and discussions mapped industry needs and opportunities, leading to collaborative initiatives such as NOVAFOODIES at the EU level and multiple national projects, with more proposals underway.

FUTURE DIRECTIONS & RECOMMENDATIONS

The thorough discussions and surveys between the industrial, academic and policy making stakeholders highlighted the following points for future progress:

- Selective breeding,
- Biotechnology,
- Microbiome engineering, quorum quenching,
- Circular economy practices,
- IMTA,
- Enable year-round production

Although the EU goal for 2030, i.e. to reach 8 M tonnes of seaweed, develop 85,000 jobs and bring in € 9 B in value, seems highly optimistic and challenging, the industry and the other stakeholders truly believe in the goal of Ulva spp. to be tomorrow's Wheat of the Sea.



Science Communication

Ulva

The Future Wheat of the Sea

Publications

THE GREEN SEAWEED ULVA: TOMORROW'S "WHEAT OF THE SEA" IN FOODS, FEEDS, NUTRITION, AND BIOMATERIALS

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ABSTRACT

Ulva, a genus of green macroalgae commonly known as sea lettuce, has long been recognized for its nutritional benefits for food and feed. As the demand for sustainable food and feed sources continues to grow, so does the interest in alternative, plant-based protein sources. With its abundance along coastal waters and high protein content, *Ulva* spp. have emerged as promising candidates. While the use of *Ulva* in food and feed has its challenges, the utilization of *Ulva* in other industries, including in biomaterials, biostimulants, and biorefineries, has been growing. This review aims to provide a comprehensive overview of the current status, challenges and opportunities associated with using *Ulva* in food, feed, and beyond. Drawing on the expertise of leading researchers and industry professionals, it explores the latest knowledge on *Ulva*'s nutritional value, processing methods, and potential benefits for human nutrition, aquaculture feeds, terrestrial feeds, biomaterials, biostimulants and biorefineries. In addition, it examines the economic feasibility of incorporating *Ulva* into aquafeed. Through its comprehensive and insightful analysis, including a critical review of the challenges and future research needs, this review will be a valuable resource for anyone interested in sustainable aquaculture and *Ulva*'s role in food, feed, biomaterials, biostimulants and beyond.

GREEN SEAWEED ULVA SPP. BIOPROCESSING: BIOACTIVE MOLECULES, EMERGING EXTRACTION TECHNOLOGIES, AND INDUSTRIAL APPLICATIONS IN THE CIRCULAR BIOECONOMY

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ABSTRACT

Ulva, commonly known as “sea lettuce,” is a resilient green macroalga and a promising sustainable source of bioactive compounds. Thriving in nutrient-rich waters and amenable to cultivation, *Ulva* is abundant in coastal ecosystems and well positioned to support marine circular economies. This review critically evaluates its chemical composition, bioactivities, extraction technologies, and industrial applications relevant to the food, pharmaceutical, cosmetic, and agricultural sectors. Ulva biomass contains polysaccharides (notably ulvan), proteins, essential amino acids, polyunsaturated fatty acids, polar lipids, vitamins, minerals, carotenoids, and phenolic compounds. These constituents are associated with diverse health-promoting properties, including antioxidant, anti-inflammatory, antimicrobial, neuroprotective, and antihyperlipidemic activities. Advances in extraction are expanding the potential for scalable, efficient recovery of high-value compounds. Emerging methods include ultrasound-assisted extraction, microwave-assisted extraction, enzyme-assisted extraction, pressurized liquid extraction, and supercritical fluid extraction. Complementary developments in green solvents, such as bio-based reagents, deep eutectic solvents, and ionic liquids, offer environmentally friendly alternatives to conventional solvents. The range of potential applications is broad, encompassing functional foods, dietary supplements, animal feed, bio-based packaging, cosmetics, and pharmaceuticals. Integrating *Ulva* into sustainable value chains directly supports the United Nations Sustainable Development Goals , particularly in relation to health, resource efficiency, and environmental protection. Despite significant progress, challenges remain in taxonomic resolution, biomass standardization, large-scale biorefinery implementation, and regulatory harmonization. We conclude that *Ulva* holds strong promise as a flagship marine bioresource, provided that advances in biotechnology, aquaculture engineering, and green chemistry are coupled with enabling policies that facilitate its full integration into the emerging blue bioeconomy.

METAL(OID)S IN ULVA – SHOULD WE BE WORRIED?

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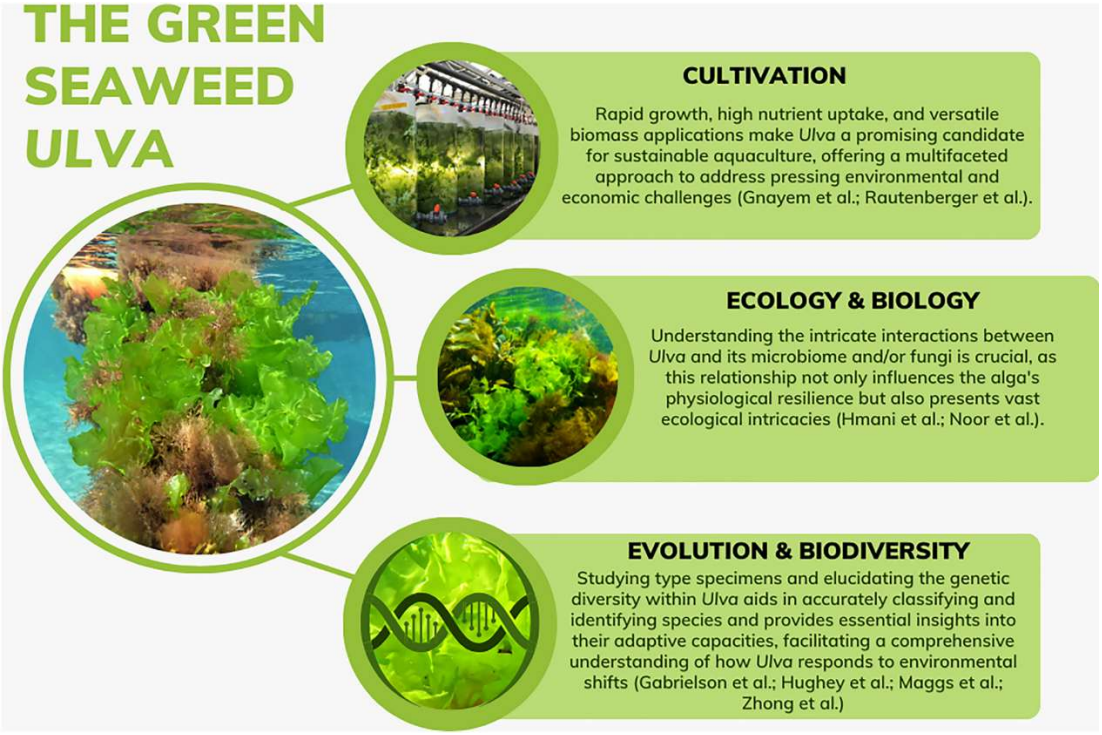
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ABSTRACT

Ulva spp. are promising food resources owing to their nutritional richness and beneficial properties. However, it accumulates potentially toxic trace elements, raising health safety concerns and proving useful for biomonitoring studies. In response to this concern, this review, conducted in collaboration with the EU-COST Action CA 20106 network, critically analysed 176 peer-reviewed papers to evaluate metal(oid) accumulation in *Ulva*. This study revealed substantial variability in the essential and non-essential element content due to environmental conditions, geographic regions, morphological forms, and analytical methods used in both wild and cultivated *Ulva*. The analysis was based on gross morphology (tube or foliose) rather than species-level identification. The identification of toxic forms, such as methylmercury and inorganic arsenic, remains limited, highlighting the need for element speciation to more accurate assess safety. Based on these findings, the review identified and outlined key areas requiring attention to ensure the safe and effective use of *Ulva*. Standardised analytical protocols are needed to improve consistency and comparability across studies and to enable accurate detection of toxic element forms. Improved taxonomic resolution, using molecular tools, is essential for distinguishing species-specific accumulation patterns. Expanding research into understudied geographic regions will help capture global variability in environmental influences on trace element uptake. Finally, standardised cultivation parameters are crucial to control elemental composition in farmed *Ulva* and to ensure its suitability for human consumption and commercial applications.

SPECIAL ISSUE: PHYLOGENY AND ECOLOGY OF ULVA. PART I

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Ralf Rautenberger
Germanium dioxide as agent to control the biofouling diatom *Fragilariopsis oceanica* for the cultivation of *Ulva fenestrata* (Chlorophyta)
https://doi.org/10.1515/bot-2023-0075
Research article: Germanium dioxide (GeO_2) inhibits the proliferation of the biofouling marine diatom *Fragilariopsis oceanica*, while photosynthesis and growth of the green macroalga *Ulva fenestrata* remain unaffected.
Keywords: *Ulva*; macroalgal aquaculture; diatoms; biofouling; photosynthesis

Nabeel Gnayem, Razan Unis, Rima Gnaim, Alexander Chemodanov, Álvaro Israel, Jallal Gnaim and Alexander Golberg
Seasonal and culture period variations in the lipid and fatty acid content of *Ulva lactuca* cultivated in Mikhmoret onshore (Israel)
https://doi.org/10.1515/bot-2023-0027
Research article: A quantitative examination of the lipid and fatty acid content in *Ulva lactuca* in different harvest seasons revealed that the maximum content of lipids was in the summer, and that of polyunsaturated fatty acids (PUFA) was in the winter.
Keywords: green seaweed; *Ulva lactuca*; seasonal variation; culture period variation; lipids; fatty acids

Sadia Noor, Mst. Nadira Begum, Satyajit Roy Rony, Mohammad Zashim Uddin, Md. Hossain Sohrab and Md. Abdul Mazid
Bioactivity and chemical screening of endophytic fungi associated with the seaweed *Ulva* sp. of the Bay of Bengal, Bangladesh
https://doi.org/10.1515/bot-2023-0040
Research article: Endophytic fungi, isolated from the marine green macroalga *Ulva* sp. of the Bay of Bengal of

Bangladesh, were identified using morphological and phylogenetic analyses. These fungi produce bioactive metabolites with antimicrobial activity, DPPH free radical scavenging activity, and cytotoxic potential.
Keywords: seaweed; marine endophytic fungi; antimicrobial; DPPH scavenging activity; bioassay

Imen Hmani, Fatemeh Ghaderiardakani, Leila Ktari, Monia El Bour and Thomas Wichard
High-temperature stress induces bacteria-specific adverse and reversible effects on *Ulva* (Chlorophyta) growth and its chemosphere in a reductionist model system
https://doi.org/10.1515/bot-2023-0053
Research article: Essential bacteria provide algal growth and morphogenesis factors to the seaweed *Ulva*. As a result, both bacteria and algae must adequately respond to heat stress. Temperature affects bacteria-specific effects on propagule growth.
Keywords: algae–bacteria interactions; metabolome; morphogenesis; *Rathayibacter*; *Ulva*

Kai-Le Zhong, Masanori Hiraoka, Xu Gao, Bayden Russell, Zi-Min Hu, Weizhou Chen, Ju-Hyoung Kim, Norishige Yotsukura, Hikaru Endo, Naohiro Oka, Shinya Yoshikawa and Juan Diego Gaitan-Espitia
Environmental gradients influence geographic differentiation and low genetic diversity of morphologically similar *Ulva* species in the Northwest Pacific
https://doi.org/10.1515/bot-2023-0073
Research article: Molecular markers allowed the detection of eight morphologically similar *Ulva* species along the northwest Pacific region. These species show different biogeographic patterns and low genetic variation that are likely explained by gradients in temperature and oceanic currents.
Keywords: biodiversity; China; genetic diversity; green tides; seaweed

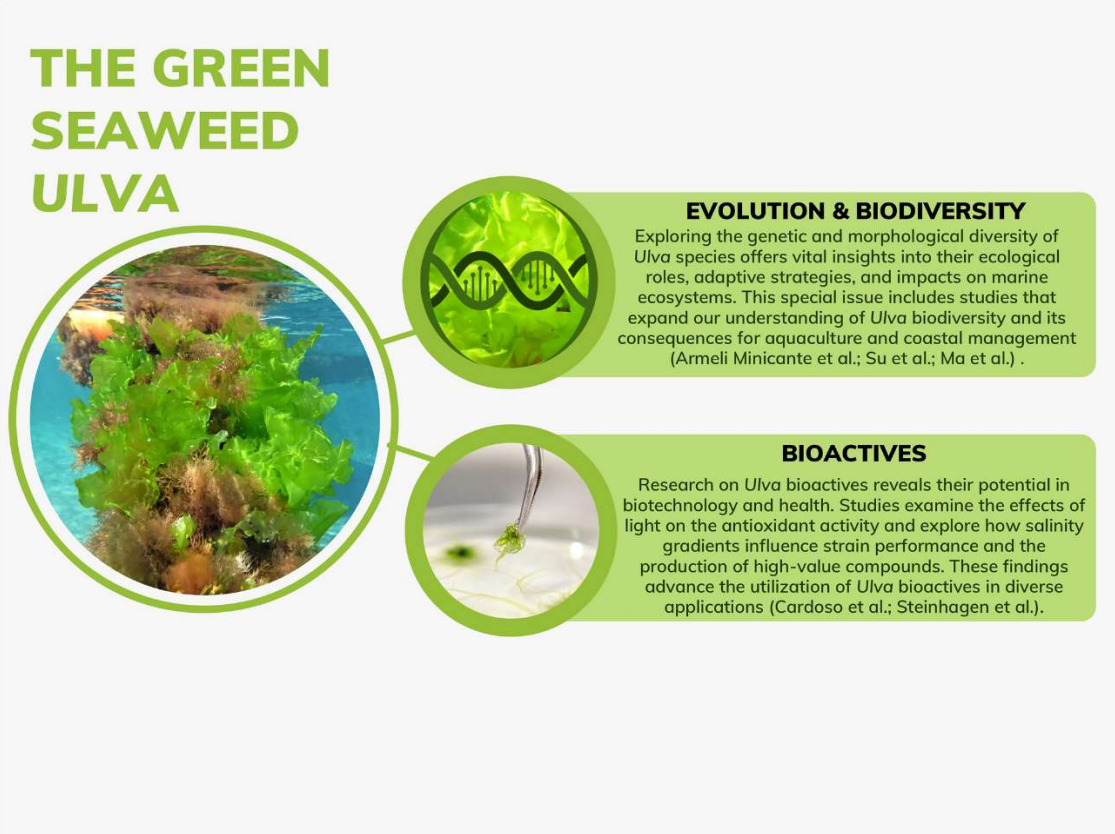
Paul W. Gabrielson, Anna Claire Smith, John F. Bruno, Todd J. Vision and Margarita Brandt
Taxonomic assessment of blade-forming *Ulva* species (Ulvales, Chlorophyta) in the Galápagos Archipelago, Ecuador using DNA sequencing
https://doi.org/10.1515/bot-2023-0079
Research article: DNA barcoding, the only reliable method to identify blade-forming *Ulva* species worldwide, found four species in the Galápagos Archipelago: *Ulva adhaerens*, the first report of this species in the southeast Pacific Ocean, *U. lactuca*, *U. ohnoi*, and *U. tanneri*.
Keywords: DNA barcoding; ITS; rbcL; tufA; *Ulva adhaerens*; *U. lactuca*; *U. ohnoi*; *U. tanneri*

Jeffery R. Hughey, Kathy Ann Miller and Paul W. Gabrielson
Genetic analysis of *Ulva* (Ulvaceae, Chlorophyta) type specimens resolves northeast Pacific blade-forming species
https://doi.org/10.1515/bot-2023-0072
Research article: DNA sequencing of northeast Pacific *Ulva* type specimens revealed that *U. californica*, *U. dactylifera*, *U. stenophylla*, *U. taeniata* and *U. tanneri* are distinct species and that some GenBank sequences labeled as these species are misidentified.
Keywords: *Ulva californica*; *Ulva dactylifera*; *Ulva stenophylla*; *Ulva taeniata*; *Ulva tanneri*

Christine A. Maggs, Anne R. Bunker, Francis Bunker, David Harries, John Kelly, Frédéric Mineur, Jaanika Blomster, Pilar Diaz-Tapia, Paul W. Gabrielson, Jeffery R. Hughey and Juliet A. Brodie
Updating the Ulvaceae in the green seaweeds of Britain and Ireland
https://doi.org/10.1515/bot-2023-0078
Research article: The Ulvaceae flora of Britain and Ireland is revised using DNA sequences that separate two species of *Ulvaria* and provide correct names for several *Ulva* species with reference to type materials.
Keywords: DNA sequences; taxonomy; *Ulva*; *Ulvaria*

SPECIAL ISSUE: PHYLOGENY AND ECOLOGY OF ULVA. PART II

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Chun-Yu Su, Silvia Fontana and Shao-Lun Liu
Unforeseen green tide of floating tubular *Ulva meridionalis*, a lethal threat to oyster farming, along the west coast of Taiwan: tracking its origin and ecophysiological insights
https://doi.org/10.1515/bot-2024-0006
Research article: Floating tubular *Ulva meridionalis* caused an unprecedented green tide along Taiwan's west coast, likely originating from China's north coast. Our study found that Taiwan's nutrient-rich estuarine environments promote its growth, leading to the mortality of farmed oysters.
Keywords: green tide; ecophysiology; oyster farming; Taiwan; *Ulva meridionalis*

Yingying Ma, Jin Zhao, Fanzhou Kong, Tian Yan and Peng Jiang
Morphological discrimination of fouling *Ulva* and *Blidingia* species on *Pyropia* rafts in Rudong, China: an in-field protocol for early monitoring of algae forming the Yellow Sea green tide
https://doi.org/10.1515/bot-2023-0077
Research article: A morphological method, convenient for field-use with large sample sizes, was developed to discriminate five species that were confirmed as predominant fouling seaweeds over the past decade on nori cultivation rafts in the Subei radial sand ridges.
Keywords: green tide; morphological identification; *Pyropia* raft; the Yellow Sea; *Ulva*

Simona Armeli Minicante, James T. Melton III, Damiano Spagnuolo, Antonio Manghisi, Giuseppa Genovese, Marina Morabito and Juan Lopez-Bautista
A DNA barcode inventory of the genus *Ulva* (Chlorophyta) along two Italian regions: updates and considerations

https://doi.org/10.1515/bot-2023-0071
Research article: Preserved specimens of institutional herbaria are a fundamental tool for monitoring environmental variations and taxonomic changes. In this study, the results obtained constitute an update of the DNA barcode inventory of *Ulva* species along Italian and Mediterranean coasts.
Keywords: *Ulva*; Mediterranean Sea; *Ulva chaugulei*; tufA; LTER-Italy

Isabel Cardoso, Anneke Meißner and Laurie C. Hofmann
The effect of irradiance versus light dose on the antioxidant activity of two strains of *Ulva lacinulata*
https://doi.org/10.1515/bot-2023-0064
Research article: The antioxidant and photosynthetic activities of two *Ulva lacinulata* strains were evaluated under different irradiances with the same light dose. Different responses between strains suggest that light dose rather than irradiance may impact antioxidant activity more strongly in certain *Ulva* spp.
Keywords: ABTS assay; antioxidants; irradiance; daily light integral; *Ulva*

Sophie Steinhagen, Ida Johansson, Julia Specht, Swantje Enge, Karin Larsson, Ingrid Undeland and Gunilla B. Toth
Unlocking economic potential of the *Ulva* crop for low salinity environments: exploring the effect of salinity gradients on the performance and valuable compounds of Baltic Sea strains
https://doi.org/10.1515/bot-2024-0079
Research article: This study investigates the effects of long-term low salinity on *Ulva* strains, revealing enhanced growth and protein content across most strains, with strain-specific pigment responses. Results support targeted strain selection to optimize Baltic Sea aquaculture for the Blue Economy.
Keywords: adaptation; aquaculture; Blue Economy; crude protein

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Ulva

The Future Wheat of the Sea

Lunch with Ulva



2023













2024

















2025











HOST

Karina Balina (University of Latvia, LV)

OBJECTIVE:

Lunch with Ulva is an online seminar series created to enhance knowledge exchange, collaboration, and visibility among scientists, industry representatives, and policymakers working in the field of Ulva and the blue bioeconomy. The main goal is to strengthen the SeaWheat community and highlight Ulva as a promising biomass for food, materials, and biostimulant applications, promoting sustainable and circular approaches.

FORMAT

Each session takes place online (via Zoom) and lasts approximately 60 minutes. It includes a 30–40 minute presentation by an invited speaker, followed by open discussion and Q&A. The sessions are held in an informal and engaging atmosphere to foster interaction and exchange among participants.

ACTIVITIES AND RESULTS (UP TO 2025):

- More than **twenty online sessions** have been organised, covering topics such as seaweed cultivation, biorefinery approaches, sustainability assessment, and market perspectives.
- Each session attracted on average **40–70 participants** from over 20 countries.
- Webinars were promoted through e-mail and LinkedIn.
- *Lunch with Ulva* has become a recognizable and successful SeaWheat communication brand, attracting new audiences and increasing engagement, especially among early-career researchers and SMEs.

Lunch with Ulva has significantly contributed to community building within the SeaWheat network, strengthened international collaboration, and provided a dynamic platform for sharing knowledge on *Ulva* cultivation, applications, and sustainability. It serves as a best-practice example of effective science communication in the European seaweed research community.

2023	
27.04.2023	**Prof Antonio Melendez** **Carotenoids: versatile compounds for the sustainable production of health-promoting products**
Abstract:	Carotenoids are versatile isoprenoid compounds biosynthesized by photosynthetic organisms as well as by some bacteria, fungi and arthropods. Most animals can incorporate them through the diet. They play key roles in photosynthesis and ecology that make them key for food security. They can also be metabolized into many compounds including phytohormones and other signals that play important roles in plants, some of them of great importance for their development and resilience and therefore also for food security. Their interest in foods is due to their roles as pigments, vitamin A precursors (some of them) and also as health-promoting compounds. Recently there is a renewed interest in their use with cosmetic purposes. Ulva is a good source of carotenoids, including lutein and β-carotene, which are among the main dietary carotenoids. In this contribution, the importance of carotenoids in agro-food and health in the context of food security, sustainability and health promotion is summarized. Some research needs in relation to Ulva and seaweed carotenoids will be pinpointed.

29.06.2023.	**Dr. Thomas Wichard** **The green macroalga Ulva - a model system in chemical ecology and its potential applications in aquaculture**
Abstract:	This talk focuses on the research conducted in recent years and discusses how U. mutabilis has developed into a model organism with the specific perspective that a reductionist model system allows. The field of systems biology will achieve a comprehensive, quantitative understanding of the dynamic interactions between Ulva and its associated bacteria to better predict the behaviour of the system. For instance, gardening the microbiome in aquaculture reduces the operational taxonomic units in cultivated compared to freshly isolated Ulva with its native microbiome. U. mutabilis, which is conspecific with the cosmopolitan Ulva compressa, also serves as a unique model system to investigate the requirements for withstanding environmental stresses, such as cold temperatures or micropollutants.
27.07.2023.	**Dr José Pintado** **Microbial manipulation of the Ulva holobiont as a strategy for disease control in aquaculture**
Abstract:	In IMTA-RAS, macroalgae (e.g. Ulva spp) act as a biofilter by reducing the inorganic components (N, P and CO2) issued from the metabolic activity of fish. Moreover, macroalgae can contribute to the microbial balance of the systems and disease prevention. The webinar will present research focused microbial management strategies in IMTA-RAS, based on Ulva-epiphytic bacteria such as those from the Phaeobacter genus that produce tropodithietic acid (TDA) with antagonistic activity against pathogens. The research approach involves from field sampling to aquaria experiments and metagenomic and metabolomic techniques.
28.09.2023.	**PhD Isabel Cardoso** **OVERCOMING BOTTLENECKS IN SEAWEED CULTIVATION - Natural protoplast production in Ulva lacinulata**
Abstract:	Naturally occurring protoplasts were observed in a culture of Ulva lacinulata collected two years prior. This strain has been cultivated under laboratory conditions and after many attempts at induction of reproduction, no method described in the literature showed any success. Moreover, degradation is known to occur often in this species, and during one of these occurrences, we closely observed the thalli, the tissue degradation, and cells that were found being released into the water. We confirmed that these cells were naturally occurring protoplasts and found that the initial partially degraded thalli could recover and grow after the natural production and release of protoplasts. Our observations of natural protoplast production during tissue degradation in U. lacinulata close an important knowledge gap in understanding this species' reproductive cycle, which is one of the most cultivated Ulva spp. Additionally, the production of natural protoplasts can potentially be exploited to improve the efficiency of Ulva cultivation methods in the future.
25.10.2023.	**Prof. Muki Spiegel** **The Use of *Ulva* in Integrated Multi-trophic Aquaculture (IMTA), an Ecological Approach For A Sustainable Aquaculture.**
Abstract:	The scarcity of freshwater, overfishing and declining ocean biodiversity, marine eutrophication by anthropogenic activities, and the increasing demand for seafood have all required attention from a more comprehensive, global perspective. Moving from conventional aquaculture toward an ecological approach to developing and managing a sustainable aquaculture that cares for environmental and sociological aspects can relieve at least some of these problematic issues. Nutrient removal using Integrated Multi-Trophic Aquaculture (IMTA) Systems is a promising ecological approach for sustainable aquaculture. The

	rationale behind the IMTA systems is to convert the excretions of the organisms cultured upstream into valuable food for the organisms cultured downstream. In marine IMTA systems, seaweed has a high capacity for nutrient uptake per unit of culture area and can be an essential additional valuable product. In addition to nutrient removal by the green seaweed Ulva lactuca, the IMTA system proved to be a reliable source of sustainable biomass for human consumption, animal feed and high-value by-products for the food additive industries. IMTA systems in offshore cage cultures and land-based facilities will improve the food conversion rate (FCR) and diversify the mariculture products, ultimately increasing profit for the farmer. In addition, they will often create additional jobs and, no less importantly, will reduce environmental pollution.
14.12.2023.	**Dr. Alexander Golberg** **Process development for *Ulva* sp. biorefinery: high-voltage pulsed electric field, hydrothermal liquefaction and open fermentation**
Abstract:	*Ulva* sp. is an emerging biomass to produce food, chemicals and fuels. However, such a biomass processing is challenging, and often new equipment and protocols are needed. Specific challenges include large amounts of intracellular salts and different from lignocellulose biomass cell wall. In this talk I will discuss 1) equipment and process development for extraction of phytochemicals from the *Ulva* sp. biomass using high voltage pulsed electric field preprocessing, 2) hydrothermal biomass processing to produce bio-crude and biochar and 3) halophytic fermentation of Ulva sp. biomass to produce polyhydroxyalkanoates, promising substitutes of polyesters.

2024	
29.02.2024.	**Prof. Félix López Figueroa** **The use of solar energy : photosynthesis , photomorphogenesis and bioactive compounds in Ulva spp. (Chlorophyta)**
Abstract:	Photosynthesis is the key physicochemical process of the Life through which solar energy is transformed in chemical energy (ATP) and reducing potential (NADPH) to be used in assimilation process of inorganic carbon, nitrogen or sulphur. Photosynthesis can be measured as gas evolution i.e oxygen evolution or carbon assimilation or by in vivo chlorophyll a fluorescence used to estimate productivity of Ulva spp. In addition, light is source of environmental information detected by non-photosynthetic photoreceptor (UVA/Blue, green red/far red) and through a transduction chain, the expression of genes can be modulated controlling growth and the synthesis of bioactive compounds i.e. B light stimulates N metabolism and R light the C metabolism.
25.04.2024	**Dr. Erik-jan Malta** **Ulva production in photobioreactors: (dis)advantages, issues and potential for upscaling**
Abstract:	I will discuss the production of Ulva sp. in photobioreactor systems, in particular for the production of interesting biochemical compounds for various applications. Results will be presented on Ulva growth in the reactors and their chemical composition from my own research and from the literature. Attention will be paid to dynamics in biomass, potential causes, microbiome of the algae, knowledge gaps (in particular with respect to collapses) and potential for upscaling.
27.06.2024	**Dr. Olivier De Clerk** **Ulva biology, morphology and microbiome dynamics.**
Abstract:	Olivier De Clerck is a phycologist. His research focuses on genetics, diversity and distributions of marine macroalgae. A substantial part of his research involves culturing seaweed strains to study development, morphogenesis and reproduction of various seaweed species. In many cases such experiments are linked to genetic/genomic studies. The latter research bridges his academic interests with applied phycology aimed at cultivating seaweeds (strain selection and ecophysiology) and developing a European seaweed aquaculture sector. He currently supervises a team of five senior researchers, eight doctoral students and three technicians. According to Google Scholar, he has (co)authored more than 250 papers in peer reviewed journals, with >13.800 citations and an h-index of 56. Olivier is a member of the steering group of the European Marine Biological Research Centre (EMBRC) and president of the International Phycological Society. He coordinated the Ulva genome project in collaboration with several European research institutes and acted as group or work package leader in several European research projects (COST Phycomorph and Seawheat, Horizon Europe United, Ultfarms, Algae Pro Banos and iCulture).
25.07.2024	**Dr. Yossi Tal** **Removal of nitrates from brine sea water effluent of desalination plant**

Abstract:	Sustainable marine aquaculture; Seaweed cultivation and processing in land based systems. Applied marine biotechnology; Microbial nutrients cycles in marine aquaculture environments; Mass cultivation of Macro alga, vertebrate and invertebrate marine species; Molecular microbiology; Ecology of marine environments; Biology and engineering of marine recirculating aquaculture system; Seaweed cultivation and processing; Business development for aquaculture farms: Large projects management in marine aquaculture.
29.08.2024	**Dr. Lior Guttman** **Ulva for improving aquafeed sustainability: insights from IMTA system at the NCM and a bacterial-biotechnological approach for improving seaweed's digestibility and nutritional value.**
Abstract:	Ulva is one of the most promising candidates for replacing unsustainable ingredients like animal and plant meals and oils in aquafeeds. Studying Ulva's dual use in an integrated multi-trophic aquaculture system (IMTA) in water purification and as a protein-rich nutritious ingredient highlights this potential. Still, the high content of indigestible polysaccharides reduces Ulva's nutritional value and digestibility. It is assumed that genomic knowledge on the ecology of the gut microbiome of an algal-fed sea urchin is essential for developing a bacterial-based process for the decomposition of the algal polysaccharides. For this purpose, research was performed to investigate the structure and functionality of bacterial communities along the digestive tract of the algivorous sea urchin Tripneustes gratilla elatensis. The study combines 16S rRNA amplicon sequencing, bioinformatics, and in vitro trials.
26.09.2024	**Dr. Annette Bruhn** **Harvest of Ulva as a tool for habitat restauration supporting the blue bioeconomy**
Abstract:	Sea lettuce (*Ulva* spp.) forms massive green tides in nutrient-rich water bodies around the globe. The decomposing of *Ulva* biomass reduces environmental quality and recreative value. Harvest of *Ulva* prior to decomposition is of increasing interest as a tool to improve environmental quality and contribute to the circular bioeconomy. In a science-industrial Danish case-study, the extent of *Ulva* green tides was documented between 2018 and 2024 using drone and image technologies. Gentle mechanised harvest of the *Ulva* biomass was tested and documented over three seasons, registering environmental impacts and *Ulva* biomass quality for food and feed purposes. Harvest of *Ulva* can be used as a tool to improve environmental quality in nutrient-rich water bodies, however improving harvest method and post-harvest processing is needed for utilising the biomass for food and feed.
24.10.2024	**Dr. Stefan Kraan** **ULVA: Tomorrow's "Wheat of the sea" but when is tomorrow?**
Abstract:	Population growth combined with increasingly limited resources of arable land and freshwater has resulted in a need for alternative protein sources, as the way we produce and consume protein is no longer sustainable. Therefore, unconventional protein sources and production methods are necessary to fulfil the global protein requirement. Ulva sp. a green macrophyte has an excellent amino acid profile yet extraction of protein from seaweeds has been notoriously

	difficult and will take at least another decade to be optimised and made economically feasible. Nevertheless, one way to start this development and support the protein transition is the use of whole Ulva seaweed to replace many carbon intensive food ingredients . However, the full acceptance of seaweed and in particular Ulva still hasn't happened despite all the efforts. Some successes and failures will be discussed and a strategy presented what the next steps should be in order to make tomorrow really "tomorrow".
28.11.2024	**Dr. Ronan Sulpice** **Ulva spp. is described as a fast growing seaweed, but what are the mechanisms behind?**
	Ulva spp. is a fast growing green seaweed, and can achieve up to 40% daily growth rate. Growth is usually assessed via determination of increases over time in surface area, fresh and dry weight, and is the result of two different mechanisms, i.e. cell division and cell expansion. Both mechanisms are expected to require the synthesis of structural compounds and soluble compounds to maintain cell turgor, those compounds deriving from photosynthates. It has been previously shown that Ulva spp. has a distinctive diurnal growth pattern, with higher rates of tissue expansion at night than in daytime, contrary to land plants. However, the pattern of the synthesis of structural compounds has not been addressed. To this aim, via use of 13C labelling, we assessed the diurnal patterns of the synthesis of photosynthates/soluble metabolites, starch, structural compounds and compared it to tissue expansion. Our results show a disconnection between tissue expansion and biomass accumulation, with structural compounds being mostly made in daylight, while tissue expansion mostly occurred at night. This strategy is likely allowing for a reduction of the energy costs required for the synthesis of structural compounds, as energy produced by the light reactions of photosynthesis could be directly used for the process, instead of relying upon respiration following starch degradation. Such strategy might explain the high growth rates of Ulva spp and future work will focus on further deciphering the primary metabolism of this group of species, including protein biosynthesis and the interconnected processes of cell division and expansion.

2025	
26.06.2025	**Dr. Alvaro Israel** **A recount of Ulva (Chlorophyta) species of potential use in the environmentally challenging Levantine basin, eastern Mediterranean Sea**
Abstract:	The genus Ulva (Chlorophyta) has an estimated 102 recognized species, and a large number of additional taxonomical identities that include subspecies, varieties and formae. The Levantine basin in the easternmost Mediterranean Sea is a constraining marine environment presenting challenging conditions for marine life, including seaweeds. This talk will present a revision of Ulva species described in the various coastlines of the Levantine eco-region, together with cultivation efforts known for the area. There are tentatively c.a. 18 Ulva species in the Levant, which is remarkably biodiverse compared with the estimated 39 species for the whole Mediterranean Sea. Contrary to frequent blooms seen in recent years for other seaweed species, Ulva has not been reported to do so, except for periods of high productivity, particularly during springtime.
31.07.2025	**Dr. Sotiris Orfanidis** **Aegean Ulva ecophysiology: From natural habitats to cultivation**
Abstract:	The genus Ulva, commonly known as sea lettuce, is a widespread green macroalga with significant ecological and economic importance. In the Aegean Sea, various Ulva species, particularly in eutrophic, sheltered bays, exhibit distinct ecophysiological characteristics, making them promising candidates for cultivation. Successful cultivation requires clean starting material (biobanking) and optimizing temperature, light, and nutrient supply (phenotyping) to maximize growth and desired biochemical composition, mirroring the ecophysiological factors that govern its growth in natural habitats. How close are we to developing region-specific and tailored protocols for Ulva cultivation?
28.08.2025	**Prof. Marianne Thomsen** **Seaweed and Food Systems in the Context of Absolute Sustainability**
Abstract:	This presentation will reflect on the role of seaweed in food systems within the context of absolute sustainability. Seaweed not only provides nutrient-rich food but also delivers ecosystem services such as carbon and nutrient capture that can support climate mitigation and water quality restoration. Using examples from recent Ulva studies, I will exemplify how choices around system boundaries and accounting methods influence sustainability assessments. The focus will be on the farm-to-fork perspective, with the waste sector kept out of scope in line with conventional food LCAs. Rather than giving final answers, the talk will invite reflection on whether and how to account for emission capture in food LCAs, and how ecosystem service delivery can be integrated into absolute sustainability frameworks. The aim is to encourage discussion on seaweed's contribution to regenerative and resilient blue food economies.
25.09.2025	**Dr. Céline Rebours** **Regulations and policies for the Ulva production in Europe: Responsible Research and Innovation, Convention of Biological Diversity, Nagoya protocol and Environmental Economic Accounts**
Abstract:	Blue Biotechnology (BB) is a multidisciplinary, knowledge- and capital-intensive technological area that significantly contributes to innovations in the pharmaceutical, medical, cosmeceutical, nutraceutical, aquacultural, agricultural, and energy sectors. The BB sector in Europe includes non-traditionally commercially exploited marine organisms and their biomass applications. Seaweeds are identified as one of the valuable sources of structurally diverse

	bioactive compounds among the marine organisms. Seaweed cultivation in Europe is still in its early stages, with a main emphasis on kelp species which faces the challenges in penetrating the market, calling for a need to diversify the cultivated species. The green species of the genus Ulva can effectively assimilate dissolved nutrients with high efficiency and biomass yield. Only a limited number of Ulva species are currently cultivated on a pilot scale, predominantly in land-based systems, and in some cases under IMTA systems. Worldwide, Ulva spp. production is estimated at 3699 t.w.w. for a value of 1 074 400 EUR with an average price of 290.41 EUR per t.w.w. 1, while in Europe Ulva production remains very low and the total production number are difficult to estimate. In addition, the species of seaweed are not accurately named during production reporting making it difficult to oversee the size of the Ulva industry in Europe. Seaweed industry (incl. Ulva) in general faces the challenge to be a plural sectorial activity, forcing entrepreneur to navigate a complex regulatory framework governed by a multitude of agencies both nationally and at the European level. This multi-sectorial distribution of the business makes it also difficult to identify the actors across Europe and to understand if segment(s) of the value chain are still missing to reach the existing market. Therefore, the Working Group 6 under the CA20103 SEAWHEAT project examined the regulatory framework relevant to Ulva production at a large scale, investigated the acceptance of Ulva products by society, and the economic aspects, including risk assessment in different scenarios and geographical locations. This presentation aims to i. present important EU regulations and policies that apply to Ulva production; ii. highlight the importance of the application of the RRI principles and Nagoya protocol in biodiscovery, and iii. suggest recommendations for amendment or reinforcement of the current regulatory framework for Ulva-based industries.
30.10.2025	**Luna van der Loos**
Abstract:	Coming…



Conferences



From Ulva aquaculture to food and feed production: state-of-the-art, bottlenecks, risks and gaps

Cadiz, Spain

September 13-15, 2022

Ulva

The Future Wheat of the Sea

TWO DECADES OF COMMERCIAL INTEGRATED AQUACULTURE OF ULVA WITH ABALONE IN SOUTH AFRICA: FEED AND MUCH MORE

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Figure 1 Buffeljags Abalone (VIKING aquaculture)

South Africa is one of the world's main commercial producers of aquacultured *Ulva* (over 2000t yr^{-1} and increasing). Large-scale integrated cultivation of *Haliotis midae* with *Ulva lacinulata* began in 2002. Paddle-raceways (ca. 30m long) are used to grow *Ulva* in abalone effluent, with the *Ulva* fed back to the abalone. In addition, two farms operate on a partial recirculation regime, using *Ulva* for bioremediation, enabling large savings in pumping costs. We are studying the operation of one of these farms, Buffeljags Abalone Farm (Viking Aquaculture), as partners in the EU H2020 ASTRAL programme. On this farm ca. 10-15t of abalone in 42 raceway tanks is integrated with ca. 1-2t of *Ulva* in a single raceway.

Supply of 'natural feed' was the original driver of *Ulva* production and is critical to the operation at the warm temperature limit of the commercial species, *Haliotis midae*. In other farms, where natural kelp is available as feed addition, farm design is based on *Ulva*-enabled recirculation. Data will be presented on the effects of *Ulva* on ammonia removal and daily rhythms of oxygen and pH, as well as the delicate balance between pH and toxicity of free ammonia nitrogen (FAN) at different recirculation rates (50%, 75% and 100%). Experimentally, dietary supplementation with 25% of fresh *Ulva* (FU) added to formulated feed (AB) significantly improved total feed consumption by ca. 90%, compared with abalone fed AB or FU alone. No significant differences in SGR or Condition Factor (CF) were recorded between treatments in the farm trial, suggesting as much as 60% of formulated feed can be substituted with FU without negatively affecting SGR and CF. Bacterial community structure analyses revealed that abalone fed FU, and its components, produced significant associations in their intestinal microbiome, suggesting specific bacteria are selected for and are associated with the digestive tract of abalone fed *Ulva* or components of *Ulva*. Several differentially abundant amplicon sequence variants (ASVs) were identified across dietary treatments with various bacterial genera, including members of the genus *Vibrio*, found to be less abundant in the gut of abalone fed FU-supplemented diets compared to AB alone. The inclusion of *Ulva* can save ca. 40% of farm pumping costs, protect against HABs by enabling short-term 100% recirculation, reduce an abalone producer's reliance on fishmeal-based dry formulated feeds and have several other health benefits.

Professor John Bolton

Emeritus Prof John J. Bolton Department of Biological Sciences, University of Cape Town, South Africa



John J Bolton has been a leading marine plant scientist in South Africa for more than three decades. Work of his research group has greatly increased our knowledge of the biodiversity and taxonomy, biogeography, ecology, and resource use of South African seaweeds, and provided scientific backing to the implementation of an internationally innovative and successful aquaculture industry, growing seaweeds in integrated systems with marine invertebrates. He has also published on seaweed diversity and use in several countries in the region, including Angola, Namibia, Mauritius, Reunion, the Eparses Islands, Madagascar and Kenya. He is the only scientist from Africa to have been President of the International Phycological Society and has twice been President of the Phycological Society of Southern Africa. He has over 160 peer-reviewed journal publications and has supervised 21 successful PhD students (4 currently registered).

He was awarded the Gilchrist Medal in 2017 by the South African Council for Oceanographic Research (SANCOR) “In recognition of the contributions of a distinguished scientist to marine science, to further stimulate excellence in South African marine science and to focus attention on South Africa’s marine and coastal environments” He is the only marine plant scientist to be awarded the Gilchrist Medal. The citation also states: “Prof Bolton has had a profound effect on the lives of more than just his students, but also on the research community as a whole, the general public, and in some instances the potentially marginalised coastal communities.”

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BAD *ULVA* VS. GOOD *ULVA*: GREEN TIDES AND NUTRIENT BIOEXTRACTION

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Recent years, increased anthropogenic activities and climate change have resulted in the formation of massive blooms of macroalgae, which have caused huge environmental problems in coastal areas. Green tides have received attention since 1970s when *Ulva* grew excessively and became a disaster along coastal areas worldwide. Since 2007, the world's largest *Ulva* blooms have been observed every summer in the Yellow Sea with distribution areas ranging from 13,000 to 30,000 km2. *Ulva* blooms have also occurred in Korea during the same periods. The amount of annual biomass produced from *Ulva* blooms in Korea was about 10,000 tons. This is much smaller than those in the Yellow Sea, China, where the biomass was as high as about 3.5×106 tons. The successive occurrence of green tides in these regions have posed significant threats to the health of marine ecosystems and lead to serious economic losses in aquaculture and coastal managements. The massive outbreak of *Ulva* has also changed marine community structures and functions due to shading, biomass decomposition and anoxia.

These green tides should also accumulate carbon, nitrogen and phosphorus in their tissues as they photosynthesize and grow. It suggests that the green tide may have a potential capacity for bioremediation if the biomass can be harvested prior to the onset of their death phase. In addition, added values may be possible if the biomass can be harvested and utilized. According to a recent market analysis, the cost of harvesting *Ulva* is about US$4050 per ton (dry weight). The nitrogen reduction cost at sewage treatment plants was estimated about US$ 825 per 1 kg ammonia-nitrogen by ozonation. Therefore, the economic benefits of removal of nitrogen if the green tide was harvested could have been US$ 5.78 billion in the Yellow Sea. By selling algae products, the net estimated profit of harvesting the green tide can be about US$450 per ton (dry weight). At the conference, I will present the issues related to *Ulva* blooms in Korea and China and the potential opportunities utilizing the biomass for ecosystem services and value-added products.

Dr. Jang Kyun Kim

Department of Marine Science, Incheon National University



Dr. Jang K. Kim is an Associate Professor of Marine Science at Incheon National University, Korea. During the past 25 years, his research has focused on interactions between marine algae and their environment. He is most interested in seaweed blooms and seaweed aquaculture and their applications, environmentally sustainable aquaculture, and ecosystem services provided by seaweeds.

Dr. Kim has received numerous research projects from international and domestic funding agencies, including, National Research Foundation of Korea, Mistry of Oceans and Fisheries of Korea, Ministry of Environment, National Fisheries Research Institute, USEPA, USDA, NOAA, Sea Grant, etc. Through these projects, he developed technologies of open water and land-based seaweed aquaculture, integrated multi-trophic aquaculture (IMTA), a technology of integrating seaweed into a biofloc system, and seaweed-based carbon dioxide removal. He also evaluated ecosystem functions of seaweeds. Dr. Kim has published over 40 papers during the past 5 years in peer reviewed journals, including several review papers in seaweed aquaculture, IMTA and ecosystem services. Dr. Kim has served on various international, national, provincial and municipal committees including, UNDP/GEF YSLME Steering Committee member, Advisory Committee Member of Yellow Sea Peace Forum, Coastal Management Planning Committee of Korea, Coastal Management Council of Incheon, Incheon Fisheries Forum, etc. Dr. Kim is also a member of Editorial Boards of SCI or SCOPUS journals, including Algae, Journal of the World Aquaculture Society, Fisheries and Aquatic Sciences, and Toxicology and Environmental Health Sciences.

ULVA AQUACULTURE: OPPORTUNITIES, EXPLOITATION, PRODUCTS AND GAPS

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Any one with a social media account must have noticed the immense interest generated in seaweed over the last 5 years . From climate change solution, carbon sequestration, protein to feed the world and many more overhyped slogans while in reality we have just started in earnst to look at what is possible in respect of cultivation, what species are suitable and foremost what can one do with the generated biomass. *Ulva* in that respect is no different with massive green tides in several parts of the world but of poor-quality vs high quality protein rich, tank cultivated *Ulva* in small quantities. *Ulva* has a lot of interesting bioactive molecules amongst them ulvan a suphated polysaccharide and e.g. an excellent amino acid profile to name a few. So from a food perspective *Ulva* is very interesting, however our relationship with food has become a very precarious one. After decades of manipulating and optimising production and farming systems including animal dietary needs in parallel with the temptation of processed and fast food it seems that the human population has run into some major health and disease issues. On the other hand food has long been used to improve our health and knowledge of the relationship between food components and health is now being used to improve food. This has created an unprecedented opportunity to address public health issues through diet and lifestyle in which *Ulva* can play an interesting role. However, value proposition vs cost of cultivation and reaching the general public is of crucial importance. *Ulva* or their constituents have great potential as products in the food, functional food markets and as feed ingredient and several novel products based on *Ulva* have entered the market in recent years. An overview of potential seaweed bioactives together with current macroalgae functional food products and applications in feed will be presented addressing the gaps currently preventing *Ulva* as seaweed to become a mainstream food ingredient.

Dr. Stephan Kraan

The Seaweed Company Ltd Mausrevagh, Kilcoona, Headford Ireland



Born in The Netherlands he graduated with a M.Sc. degree in Marine Biology at National University of Groningen, The Netherlands. He moved to Ireland to pursue a PhD on phylogenetics and aquaculture of edible seaweeds at the National University of Ireland, Galway in 1998. He became manager of the Irish Seaweed Industry Organisation in 1998 and finished his PhD in 2001 with as subject phylogenetics, hybridisation and aquaculture of the edible brown seaweed Alaria esculenta. He established the Irish Seaweed Centre in 2001, a dedicated R&D centre for seaweed-based research and development, which was launched in 2001. After managing the seaweed centre for 9 years, Dr Kraan resigned from University life in 2009 to set up Ocean Harvest Technology Ltd to pursue and develop some commercial ideas using seaweeds for a variety of purposes amongst them functional food ingredients for fish farming and novel algae cultivation systems for biofuel production. After 8 years he resigned and co-founded The Seaweed Company I n 2018. A company with complete horizontal integration developing the whole seaweed value chain from cultivation to biostimulant feed, food, biomaterials and Pharma. Furthermore, he is involved in the development of large-scale seaweed biomass cultivation programs. Dr Kraan was President of the International Seaweed Association (2016-2019) and is the incoming President for the International Society for Applied Phycology (2023-2026). Currently Dr Kraan is also a business coach of 14 Indonesian seaweed companies under the Dutch Ministry of Economics and Climate Policy's development program (CBI/RVO) to help diversify and increase export to Europe. His main fields of expertise are aquaculture of seaweeds, sustainable development of algal resources, industrial applications of seaweeds and usage of seaweeds in animal feed, aquaculture, biotechnology and biomedicine.

ECOSYSTEM SERVICES OF ULVA

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Green macroalgae species belonging to the *Ulva* genus provide a range of ecosystem services (ESS) worldwide – categorised as 'regulation and maintenance', 'provisioning' and 'cultural' ESS, adapting the Common International Classification of Ecosystem Services (2018). The ESS derive from 1) *Ulva* being part of natural balanced ecosystems, 2) *Ulva* being harvested and removed from unbalanced ecosystems and 3) *Ulva* being cultivated as a bioresource, and/or as a mitigation or restorative ecological engineering tool capturing and utilising carbon and nutrients. The ESS provided all contribute to achieving several UN Sustainable Development Goals – directly and in an integrative manner of which SDG 3, 6, 12, 13, 14 and 15 are closely related to the planetary boundaries quantifying the ecological ceiling of the the safe operating space of humanity.
In the natural ecosystems, *Ulva* provide regulation and maintenance ESS such as photosynthesis, nutrient cycling, food and habitat. *Ulva* harvested from unbalanced ecosystems along with the cultivated *Ulva* allow for bringing into play the regulation and maintenance ESS as mitigation or restorative ecological engineering tools capturing and utilising carbon and nutrients, while providing a bioresource for food, feed, nutraceuticals and fibres.
In general, cultural ESS are provided by *Ulva* through its contribution to science and education, and by improving recreational value of coastal areas through harvest.
In EU alone, 50 tonnes of cultivated *Ulva* and 217 tonnes of harvested *Ulva* is annually (2019) used for food, feed, fertiliser, biomolecules with focus on both complementary protein sources and unique fibres, such as Ulvane. The corresponding biorefinery industry is progressing.
Challenges remain in several parts of the value chain, but *Ulva* and it's related ESS offer multiple global green solutions contributing to a succesful green transition, restoring ecosystems and supporting a circular bioeconomy. Integrating efforts between authorities, industry and science instiutitutions will accellerate innovation and development, while ensuring sustainability.

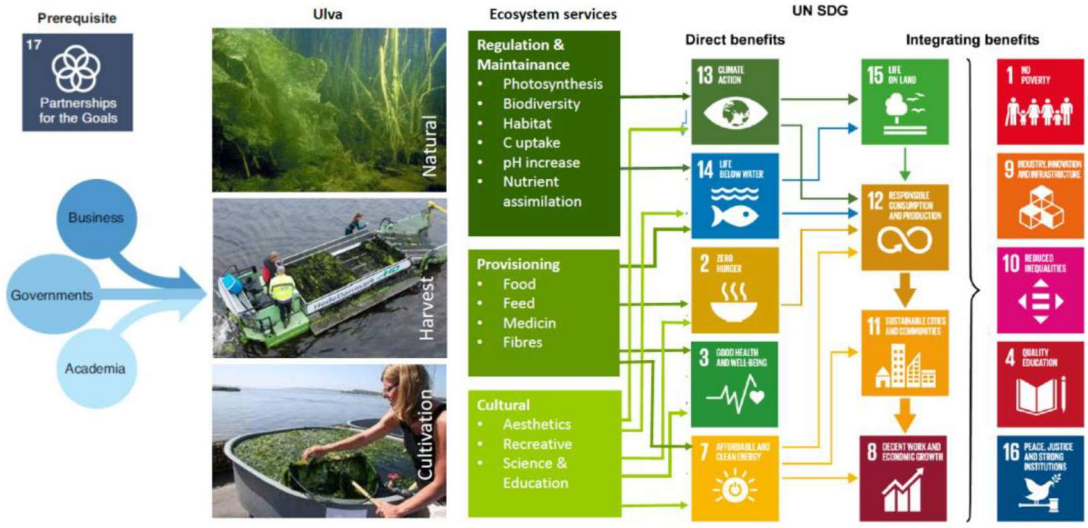


Figure 2 Ulva Ecosystem Services and their relations to the UN Sustainable Development Goals

Annette Bruhn

Annette Bruhn (AB) works as senior researcher at the Department of Ecoscience at Aarhus University, Denmark. Her research focus is cultivation of macroalgae as a bio-resource and a nature-based solution for mitigating eutrophication and climate change. AB works in the cross field between biological oceanography and macroalgae ecophysiology, and always in transdisciplinary teams in cooperation with authorities and industry/SMEs. AB is co-founder of AlgaeCenter Denmark and Nordic Seaweed Conference.

ULVA PRODUCTION AT COMMERCIAL SCALE, ENGINEERING AND BEYOND…

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Producing *Ulva* for commercial applications, irrespectively of the scale of production, as many more challenges than engineering. In fact, planning your *Ulva* farm in an "engineer manner" is always a good practice, but can become more critical after a certain scale of production and depending on the degree of control you want or, more important, that you need. Depending on what your target customers wants or your legislator requires, whatever is more tough, that will define your level of control and, most likely, the importance of engineering.
Ulva production can also be performed without a high degree of engineering, in very simple systems, depending on where you are based, how much you know about the Biology of your *Ulva* and and how much money you can obtain per kg produced. In this case, there is much more Biology and Finances involved than Engineering. In any case, a good dosis of "home-made engineering" can be useful. At least in Europe, considering the opex costs and the current market values, *Ulva* producers need to develop cost effective production methods and work to meet the requirements of the customers willing to pay more for the same kind of biomass. There will be challenges associated with legal aspects and challenges associated with market requirements. Legal aspects can be related with licenses (different types) wages and all associated taxes, work safety, environmental safety, HACCP (if producing food), human resources (including training), packaging and labelling rules, etc. just to name a few. Market requirements can refer to certifications of quality, certification of production method (ex. Biological production), other kinds of certification (depending on destination country, religion, etc.), format of your product (fresh, dried, whole, milled), visual quality of your product, etc., just to name a few.
In summary, this presentation will discuss some challenges of an *Ulva* farm. Some of those challenges can be solved through good planning/engineering solutions, some solutions might even exist already. Some of those challenges are beyond engineering… unfortunately this talk cannot give you solutions for all these chalenges, but it is just as important to know they exist.

Rui Pereira

After completing my PhD in 2004 I followed the usual pathway in academic research. Completed 2 post-docs working between Portugal (CIIMAR) and USA (UCONN), worked as research assistant at the University of Connecticut and teaching in the same University and at a Vocational Technical School (BRASTEC). In 2012 I decided to leave the Academic world and co-founded ALGAplus, which was the first Portuguese company producing seaweed in aquaculture and become an European reference as the first one to commercialize seaweed produced in land-based IMTA. In 2020 I started to work at A4F - Algae for Future, a Portuguese SME dealing with algae biotechnology, acting as Head of the Seaweed Division and contributing for developing the sector.

MARINE BIOTECHNOLOGY: RRI, CONVENTION OF BIOLOGICAL DIVERSITY, NAGOYA PROTOCOL, ACCESS AND BENEFIT SHARING CLEARING HOUSE.

Céline Rebours[1]*, Xenia T. Schneider[2], Belma Kalamujić Stroil[3], Christiana Tourapi[4], Susana P. Gaudêncio[5,6], Lucie Novoveska[7] and Marlen I. Vasquez[3]

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Blue Biotechnology (BB) is a multidisciplinary, knowledge- and capital-intensive technological area that significantly contributes to innovations in the pharmaceutical, medical, cosmeceutical, nutraceutical, aquacultural, agricultural, and energy sectors. The BB sector in Europe includes non-traditionally commercially exploited marine organisms and their biomass applications. Seaweeds are identified as one of the more valuable sources of structurally diverse bioactive compounds among the marine organisms, and their utilization would support society to gain various health benefits. As the quest for such marine-derived compounds with pharmacological and biotechnological potential upsurges, the importance of following regulations and applying Responsible Research and Innovation (RRI) principles also increases. This presentation aims at: 1) presenting important regulations and policies that apply to the BB sector at the international and EU level while demonstrating their variability in their implementation; 2) highlighting the importance of the application of the RRI principles in biodiscovery, and 3) identifying gaps and providing recommendations on how to improve market acceptability and compliance of novel BB compounds with guidelines on ethical and benefit-sharing of genetic resources.

Three qualitative surveys were conducted under the Working Group 4 “Legal aspects, IPR and Ethics” of the COST Action CA18238 Ocean4Biotech, a network of more than 140 Marine Biotechnology scientists and practitioners from 37 countries. These surveys (“Understanding of the Responsible Research and Innovation concept”, “Application of the Nagoya Protocol in Your Research", and “Brief Survey about the experiences regarding the Nagoya Protocol") indicate awareness and application gaps of RRI, the Nagoya Protocol, and the current status of EU policies relating to BB sector1[1]. The results of the qualitative survey conducted at the 2nd MC meeting of the CA20106 consortium will specifically address the level of awareness and implementation of the RRI, the Nagoya Protocol and Access, Benefit-sharing (ABS) legislation in the *Ulva* biotechnology community. The Presentation will then categorise the identified gaps into five main categories (awareness, understanding, education, implementation, and enforcement of the Nagoya Protocol) and provide recommendations for mitigating them at the European, National, and organisational levels.

Céline Rebours

Dr. Céline Rebours worked on production and products development for over the last 25 years both in the industry and academia. She has interest in environmental, economic, and social assessments of aquaculture production systems with minimum negative risks on ecosystems and low carbon footprint. Her main expertise lies in low trophic level organisms (e.g. algae, echinoderms) and the study of integrated sea and land-based production systems. A selection of recent journal publications, including book and report experts:

https://wo.cristin.no/as/WebObjects/cristin.woa/wa/personVis?type=PERSON&pnr=385879&inst=7443

[1] Marine Drugs | Free Full-Text | Responsible Research and Innovation Framework, the Nagoya Protocol and Other European Blue Biotechnology Strategies and Regulations: Gaps Analysis and Recommendations for Increased Knowledge in the Marine Biotechnology Community (mdpi.com)

OPPORTUNITIES FOR *ULVA* AS SUSTAINABLE CROP IN HUMAN AND ANIMAL CONSUMPTION

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Growing vegetable biomass from the sea has become a promising option to fulfil the urgent need for new sustainable crops. An ideal candidate for such an alternative, marine crop is cultivated Ulva. The fact that *Ulva* is an opportunistic and cosmopolitan species with high nutritional value and an array of beneficial bioactive compounds justifies each effort to become tomorrow's 'wheat of the sea'. New insights from *Ulva* research help us to better understand its biology, growth & reproduction which are prerequisites to improve its cultivation conditions. This will further allow to develop large-scale cultivation of suitable, selected strains and finally to make use of this biomass for human and animal consumption. A wide range of food products can be enriched with *Ulva* as ingredient, such as bread, meat products, cheese, pasta, snacks and other processed food. The health-conscious and vegan food sector could also benefit from *Ulva's* nutritional profile. Seaweeds are interesting candidates for novel proteins as they contain all essential amino acids.

Ulva's nutritious wealth includes high iron and magnesium, combined with a favourable fatty acid profile. A low iodine content allows applications without intake restrictions. Bioactive compounds exert health benefits such as ulvan fibres improving gut health, or carotenoids and polyphenols acting as antioxidants and anti-inflammatory agents. Livestock can also profit from these health benefits when fed with *Ulva*. Numerous studies on poultry, swine and ruminants have shown positive effects of seaweed in feed formulas on animal health, welfare, growth performance and meat quality. In aquaculture, feed containing *Ulva* is already being used in finfish, abalone and shrimp farming with positive results.

The main challenges of using *Ulva* in food and feed, however, are still to overcome the bottleneck of a reliable supply of *Ulva* for the food & feed manufacturer, but also the low acceptance of algae consumption in the Western society and therefore the willingness of the industry to invest in R&D of seaweed food applications.

Sylvia Strauss

Dr. Sylvia Strauss currently works as a scientific officer & food expert for The Seaweed Company in the development of high nutrition food and health supplements based on seaweed. In addition to a master in biology, she holds a PhD in neuroscience of Alzheimer's disease (University of Freiburg, Germany) as well as a recent master in Marine & Lacustrine Science and Management (ULB Brussels, UGent & UAntwerp). Her thesis on the reproduction of *Ulva* finally connected her to seaweed mariculture. This broad scientific interest & expertise combines well with her comprehensive know-how in culinary science, which made her a passionate promotor of seaweed as a sustainable resource for healthy food and beneficial bioactive compounds. The Seaweed Company is integrating seaweed cultivation with product development for food & health supplements as well as animal feed, crop stimulants & soil improvement.

ULVA BREEDING: A HUGE POTENTIAL FOR INCREASED YIELDS, BUT CARE SHOULD BE TAKEN!

Antoine Fort[1,2], Clara Simon[1], Alisha Nelly[1], Masami Inaba[1], Kevin Cascella[3], Philippe Potin[3], Marcus McHale[1] & Ronan Sulpice[1]*

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Sea lettuce (*Ulva spp.*) is recognised for its potential in food, pharmaceutical, nutraceutical, biorefinery and bioremediation industries and is increasingly being cultivated in aquaculture. The demands of industrial applications vary widely in terms of biomass composition and cultivation requirements. To tackle the large variety in industrial demand, adjustments in cultivation processes should be considered, but species and even strain selection are also part of the solution. In a large set of around 250 strains, we have found in average 4 to 5 fold variations in most biochemical compounds, and even in daily growth rates. This variation should be harnessed for improving the productivity of seaweed aquaculture, and the quality of the biomass produced. For example, some *Ulva* strains contain close to 40% proteins, and could be considered as alternative to soymeal for animal feed. However, care should be taken to avoid a decrease in genetic diversity among *Ulva* genus, as witnessed for all our major land crops. Thus, large scale cultivation of few elite strains should be avoided and local selection prioritised. To this purpose, easy, cheap and reliable strain selection methodologies are necessary.

In our laboratory we tested two methodologies, i.e. a high throughput phenotyping platform allowing to screen around 20 strains per week, and we also investigated the potential of garden experiments. For the later, we have characterised species composition over time among foliose strains cultivated in seawater and brackish waters, the latter being suited for bioremediation of land-based wastewaters. Our findings reflect the competitive advantage of strains displaying fastest growth in both environments. Interestingly, growth rates after a month were very similar, suggesting that selected strains cope equally well in either media. Further, we found significant variation in the composition of the biomass produced in both conditions, in particular protein and carbohydrate content. The established bulk-selection protocol provides a distinct advantage in efficiently screening large numbers of strains for their suitability to a target application, and is complementary to the high throughput phenotyping platform.

Ronan Sulpice

Dr. Ronan Sulpice is a Lecturer in the School of Biological and Chemical Sciences at NUI Galway (Ireland) since 2012. He obtained his PhD from the University of Rennes in 2000. This was followed by Researcher positions at the National Institute for Basic Biology in Japan, and then in Germany at the Technical University of Munich, and the Max Planck Institute of Molecular Plant Physiology where he stayed 9 years before joining Ireland. During his career, Dr. Sulpice interests have focused at elucidating in photosynthetic organisms the cross talk between metabolism and growth, with special emphasis at how environmental cues modulate them. He has reported those biochemical and molecular mechanisms allowing plants to fine-tune their resources to cope with environmental instability to maximise growth. To answer this question, his lab has developed state of the art technologies for phenotyping, metabolite profiling and molecular studies. His work originally was mostly about land plants, but in the last 5 years, seaweeds, and in particular *Ulva* have become the main photosynthetic organisms he is working on, with the aim at understanding how seaweeds are functioning at physiological and molecular levels, towards supporting seaweed breeding programmes for aquaculture and bioremediation.

THE GREEN MACROALGA *ULVA* – A MODEL SYSTEM IN CHEMICAL ECOLOGY AND ITS POTENTIAL APPLICATIONS IN AQUACULTURE

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The marine green macroalga *Ulva* (Chlorophyta, Ulvales) coexists with a diverse microbiome. Many *Ulva* species proliferate in nature and form green tides, which can occur when nutrient-rich wastewater is flushed into the sea. Bacteria are necessary for the adhesion of *Ulva* to its substrate, its growth, and the development of its blade morphology. In the absence of certain bacteria, *Ulva mutabilis* develops into a callus-like morphotype. However, with the addition of the necessary marine bacteria, the entire morphogenesis can be restored. To study the bacteria-induced morphogenesis, a reductionist system of a tripartite community was established. While specific bacterial strains cause algal blade cell division, bacteria of the genus Maribacter promote the differentiation of basal cells into a rhizoid and support cell wall formation because of the release of low concentration of the morphogen thallusin. This talk focuses on the research conducted in the recent years and discusses how *U. mutabilis* has developed into a model organism with the specific perspective that a reductionist model system allows. The field of systems biology will achieve a comprehensive, quantitative understanding of the dynamic interactions between *Ulva* and its associated bacteria to better predict the behaviour of the system as a whole. For instance, gardening the microbiome in aquaculture reduces the operational taxonomic units in cultivated compared to freshly isolated Ulva.
Up to now, the reductionist approach has enabled the study of the bacteria-induced morphogenesis of *Ulva* (Graphical Abstract). Importantly, specific questions regarding the optimization of cultivation conditions and the yield of raw materials for the food and animal feed industries can be now answered in the laboratory and through applied science. Genome sequencing, the improvement of genetic engineering tools, and the first promising attempts to leverage macroalgae–microbe interactions in aquaculture make this model organism, which has a comparatively short parthenogenetic life cycle, attractive for both fundamental and applied research, which is presented here in examples.

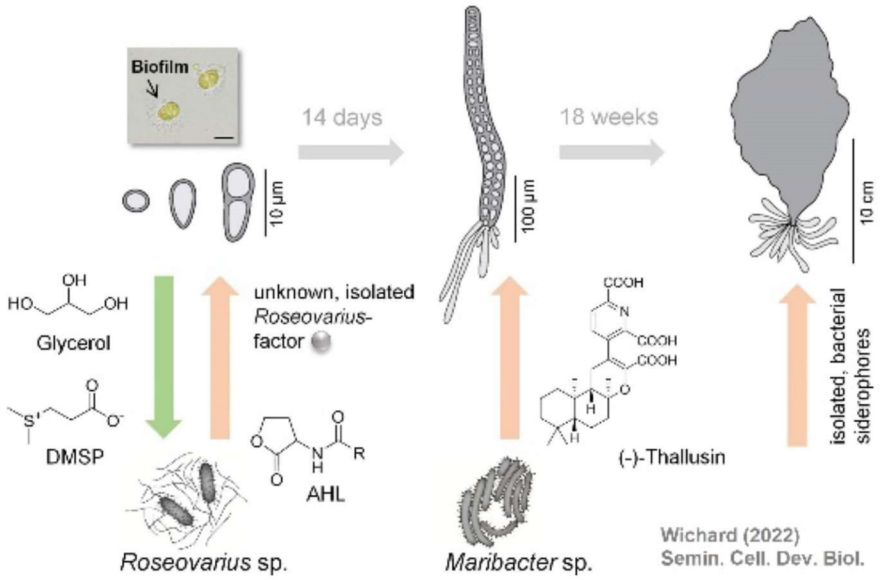


Figure 3 Ulva model system

Thomas Wichard

Thomas Wichard is the head of the research group “Chemical Ecology of Cross- Kingdom Interactions” and a lecturer in Analytical Chemistry at the Institute for Inorganic and Analytical Chemistry of the Friedrich Schiller University Jena (Germany). He earned a PhD in Biochemistry for his work on chemical defence strategies of diatoms against herbivores at the Max Planck Institute for Chemical Ecology (Germany) in 2006. After a short postdoctoral period at the École Polytechnique Fédérale de Lausanne (Switzerland), he conducted research on metal recruitment of nitrogen fixers through the production of metallophores at the Princeton Environmental Institute (USA). Since 2008, his research team has been primarily focused on the understanding the mutualistic relationships between bacteria and the marine green macroalga *Ulva* ("cross-kingdom-cross-talk"). The goal of his research is to use *Ulva mutabilis* as a model organism to study bacteria-induced morphogenesis and adaptation processes of the holobiont in stressful and changing situations. To better understand the underlying principles of eco-physiological processes, the research group employs a variety of combined methodologies in chemical ecology, analytical chemistry (metabolomics), microbiology and molecular biology (genetic tool kit) resulting in more than 90 scientific publications. Wichard has received research funding from organizations such as the German Research Foundation DFG-SFB 1127 [ChemBioSys] and DFG-SPP 1158 [Antarctic Research], as well as the Marie Skodowska-Curie Innovative Training Network [ALFF]. He works as an Associate Editor for the journal Botanica Marina.

ULVA AND ITS ASSOCIATED AMINO ACIDS: CHEMOSENSORY RESPONSE OF A VALUABLE SEA URCHIN SPECIES

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In the present study, we characterized the biochemical composition (proximate, fatty acids, and amino acids) of wild *Ulva sp.* collected in a coastal lagoon of south Sardinia where seasonal blooms of such biomass supply the feed for the experimental farming of the valuable sea urchin *Paracentrotus lividus*. Successively we tested the effect of *Ulva sp.* as chemostimulating compounds for sea urchins using three different doses of the supernatant of *Ulva sp.* Sea urchins' chemical sensitivity was then estimated by the movements of spines, pedicellariae, and tube feet, which were captured by video recordings followed by computer analysis of the movements from frame to frame and producing an "urchinogram", estimated by the changes in the mean squared difference in light intensity between successive frames (5 frames/s). Once obtained the behavioral response from sea urchins, a set of single amino acids characterizing *Ulva's* biochemical composition (alanine, glycine, glutamine, serine and valine) was used to test the detailed chemical sensitivity of sea urchins by "urchinograms" analysis. Our results showed that the presence of the *Ulva's* supernatant at the highest dose led to a behavioral response of *P. lividus*. Moreover, the animals displayed differential sensitivity to the different Ulva's amino acids, where alanine resulted the most stimulating compound, even if only at the highest concentration, while no sensitivity to serine and valine was observed regardless of the dose tested. These results provide a first indication of the role of *Ulva sp.* and its most representative amino acids as chemostimulant compounds for such herbivore species. From an applied point of view, the presence of potential food-related compounds in *Ulva sp.*, acting as chemoattractants (to reduce food searching time) and/or feeding stimulants (to stimulate ingestion), would improve the several applications of these seaweeds in the formulation of the feeds for aquaculture.

LIFE CYCLE ASSESSMENT OF SEAWEED CULTIVATION: OPPORTUNITIES AND CHALLENGES

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Seaweed of the genus *Ulva* is a promising source of biomass for the future. Biomass of *Ulva* can be used for wide range of products starting from food and feed to valuable compounds like ulvan to and bioenergy. Different cultivation techniques can be used for *Ulva* cultivation and manipulative experiments and different aquaculture approaches can be applied to achieve direct effects on the biochemical composition of the algae biomass.

Life cycle assessment (LCA) can be performed to assess environmental impact of *Ulva* seaweed cultivation process. LCA is a holistic methodology to identify the environmental impact of a product or production system. The results of an LCA are used to identify stages of the cultivation process which can be improved to minimize the impacts and optimize the production.

LCA is composed of four phases: (1) goal and scope definition, (2) life cycle inventory analysis, (3) life cycle impact assessment, and (4) interpretation. LCA is performed according to international standard ISO 14040-44 (ISO 2006).

Performing an LCA at the initial stage of *Ulva* production allows to determine which of the cultivation stages has the greatest environmental impact. LCA allows to work with different scenarios to see how the impact can be minimized. The main challenge is to provide LCA study that would cover environmental impact of *Ulva* cultivation in particular region. Many factors have to be taken into an account since the details can be significantly important. On-shore and of-shore cultivation have completely different production lines, therefore the environmental impact factors, that highlights particular environmental problems, are completely different. LCA is highly affected by data quality and *Ulva* cultivation and conversion are still at its infancy, and consequently data are often based on pilot scale trials or laboratory experiments. On one hand, this makes LCA results more uncertain, but on the other hand, this allows LCA to provide recommendation at the development phase where changes can be applied without significant economic investments.

STATE OF KNOWLEDGE REGARDING THE POTENTIAL OF MACROALGAE CULTIVATION IN PROVIDING CLIMATE-RELATED AND OTHER ECOSYSTEM SERVICES

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Seaweed aquaculture can potentially provide many ecosystem services, including climate change mitigation. Nevertheless, there are still many constraints and knowledge gaps that need to be overcome, as well as potential negative impacts or scale-dependent effects that need to be considered, before macroalgae cultivation in Europe can be scaled up successfully and sustainably. To investigate these uncertainties, a multiple expert consultation with Delphi process in combination with a Quick Scoping Review (QSR) was performed. While the results of each method differed in many ways, both methods identified the following top six ecosystem services provided by seaweed cultivation: i) provisioning food and feed, ii) provisioning hydrocolloids, iii) regulating water quality, iv) provisioning habitats, v) provisioning of nurseries and vi) regulating climate. Diverse technological knowledge gaps precluding the scaling up of a sustainable seaweed aquaculture in Europe were identified by both methods at all scales of the macroalgae cultivation process, followed by economic and environmental knowledge gaps depending on the method used. The most commonly identified potential negative impacts of macroalgae cultivation both Delphi and QSR were unknown environmental impacts, e.g. to deep sea, benthic and pelagic ecosystems. One of the main hurdles recognised by the EWG was the understanding of ES themselves by the different stakeholders, as well as the issue of scale. Studies providing clear evidence of ES provided by seaweed cultivation and/or valorisation of these services were lacking in the literature, and some aspects, like cultural impact etc. were missing in the responses to the questionnaires during the Delphi process. The issue of scale and scaling-up was omnipresent both in assessing the ES provided and in identifying knowledge gaps, constraints and potential negative impacts. For example, the ES provided will depend on the scale of cultivation, the main technological knowledge gaps were often related to scale of cultivation. Likewise at a large scale of operations, there could be multiple associated potential side effects, which need to be further investigated. Based on the outcomes of this investigation, we provide an outlook with open questions that need to be answered to support the sustainable scaling-up of seaweed cultivation in Europe.

TOWARDS A COMPREHENSIVE OVERVIEW OF *ULVA* SPECIES DIVERSITY IN EUROPE

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The green seaweed *Ulva* is one of the most important macroalgal genera from both an ecological and an economic perspective. Unfortunately, reliable identification of *Ulva* species remains a challenge, resulting in major shortcomings with respect to understanding the diversity and distribution of *Ulva* species in Europe, which in turn negatively affect ecological and applied research. The use of DNA sequence data as barcoding markers to identify *Ulva* species (e.g. ITS rDNA, rbcL, and tufA) has only partially resolved this knowledge gap. Major outstanding hurdles include, 1) the unreliability of names associated with DNA sequences in public databases, 2) the inconsistent use of barcoding markers, and 3) the legacy of over 200 years of systematics which resulted in the description of several hundred species of uncertain identity. Recent initiatives to sequence type specimens resulted in several clarifications of species name usage, but the majority of historic names remains uncharacterised using modern DNA-based methodologies. In this presentation we outline perspectives for *Ulva* taxonomy including the consistent use of multiple DNA-barcode markers assisted by species delimitation methods and potentially applications of genomic data. To be able to deliver a stable nomenclature we outline the benefits and shortcomings of adhering to the rules and practices of the International Code of Nomenclature for algae, fungi, and plants, for example by sequencing name-bearing types, and discuss alternative approaches.

ULVA MICROBIAL CONTRASTS BETWEEN LAGOON AND OPEN COAST HABITATS IN SOUTHERN PORTUGAL

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Seaweed associated bacterial communities (microbiomes) have been recognized to influence essential processes within the *Ulva* holobiont. How the microbiome composition is determined remains a controversial debate and different theories exist to explain the process (i.e. lottery, host-specificity, "functional host-specificity", and environmental factors). In this study we aimed to assess the importance of *Ulva* identity and habitat or region for the determination of the microbiome, protein content and fatty acid composition across southern Portugal comparing
open coast and lagoon specimen. Specimen were barcoding by sequencing the chloroplast elongation factor (tufA) gene. Microbiomes were analysed using partial 16S rRNA gene profiling. Some shotgun data was generated to obtain more holistic microbiome insights in taxonomy and function. Fatty acid profiles were analyzed by gas chromatography - mass spectrometry and total protein content through thermal combustion. Six different *Ulva* species (*U. rigida, U. compressa*, *U. californica/flexuosa*, *U. australis*, *Ulva sp.*1, *Ulva sp.*2) were identified, among them the nonindigenous species (NIS) *U. australis* was recorded for the first time in Portugal, and two unknown entities. Total protein content, in *U. rigida* sampled in coastal locations was higher than in the lagoon locations, but did not differ among species within the lagoon. Fatty acid profiles, did not differ across coastal and Ria Formosa locations nor among species within the Ria Formosa.
However, within the lagoon *U. compressa* had a significantly higher PUFA content than *U. rigida* and *U. fasciata.* Host-specificity emerged as primary factor for microbiome composition with signs of secondary habitat/region-specificity on a within-host level, as well as clear differentiation between morphotypes of *U. compressa*. Our study lifted probably only a fraction of the richness and differences of *Ulvas* in southern Portugal, but clearly demonstrated that a rich potential in the region for *Ulva* research.

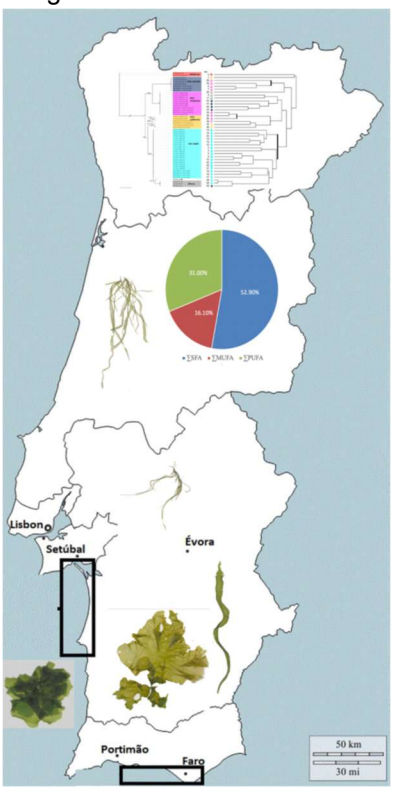


Figure 4 Ulva microbial contrasts between lagoon and open coast habitats in southern Portugal

EFFECT OF DIETARY NON-PROCESSED ALGAE MEALS ON NUTRIENT DIGESTIBILITY AND INFLAMMATORY RESPONSE OF EUROPEAN SEABASS (DICENTRARCHUS LABRAX)

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In the present study, we characterized the biochemical composition (proximate, fatty acids, and amino acids) of wild *Ulva sp.* collected in a coastal lagoon of south Sardinia where seasonal blooms of such biomass supply the feed for the experimental farming of the valuable sea urchin Paracentrotus lividus. Successively we tested the effect of *Ulva sp.* as chemostimulating compounds for sea urchins using three different doses of the supernatant of *Ulva sp.* Sea urchins' chemical sensitivity was then estimated by the movements of spines, pedicellariae, and tube feet, which were captured by video recordings followed by computer analysis of the movements from frame to frame and producing an "urchinogram", estimated by the changes in the mean squared difference in light intensity between successive frames (5 frames/s). Once obtained the behavioral response from sea urchins, a set of single amino acids characterizing Ulva's biochemical composition (alanine, glycine, glutamine, serine and valine) was used to test the detailed chemical sensitivity of sea urchins by "urchinograms" analysis. Our results showed that the presence of the Ulva's supernatant at the highest dose led to a behavioral response of P. lividus. Moreover, the animals displayed differential sensitivity to the different Ulva's amino acids, where alanine resulted the most stimulating compound, even if only at the highest concentration, while no sensitivity to serine and valine was observed regardless of the dose tested. These results provide a first indication of the role of *Ulva sp.* and its most representative amino acids as chemostimulant compounds for such herbivore species. From an applied point of view, the presence of potential food-related compounds in *Ulva sp.*, acting as chemoattractants (to reduce food searching time) and/or feeding stimulants (to stimulate ingestion), would improve the several applications of these seaweeds in the formulation of the feeds for aquaculture.

MACROALGAE AS BIOINDICATOR OF PHARMACEUTICAL´S ENVIRONMENTAL CONTAMINATION

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The increasing use of macroalgae in animal feed, including in the aquaculture industry, has several advantages, one of them their high nutritional properties. Another interesting benefit is related with their ability to function as biofilters, with the capacity to bioaccumulate environmental contaminants. Their presence in the ecosystems is of global concern, with the constant release of veterinary and human drugs to the environment through wastewater treatment plants, runoffs from intensive animal production, and the consequent contamination of the aquatic ecosystems with what are currently considered as emerging pollutants. *Ulva* species, considered the wheat of the sea, is commonly reared in aquaculture systems and could be used as bioindicator of the presence of such contaminants. A multi-detection and multi-class method, based on ultra-high-performance liquid chromatography coupled with high resolution mass spectrometry detector, time-of-flight (UHPLC-ToF-MS), was developed and validated to access the presence and quantification of more than 60 compounds from diverse family drugs, including antibiotics, anti-inflammatory, psychiatric, antidepressants and anticonvulsants drugs. Parameters of validation included: LoD, LoQ, precision, recovery, linearity, selectivity and specificity.
The important featured of the presented method is the ability to reevaluate the results in the future, to search untargeted compounds at the present time.

ULVA AS MODEL ORGANISM IN A NEW APPROACH OF URBAN FARMING

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Facing the rising interest to diversify the local food supply using alternative food sources, to provide food security and promoting a healthy diet, environmentally controlled and sustainable production of alternative foods (e.g. macroalgae, halophytes) and breeds (e.g. insects, jellyfishes) comes in the interest of the urban farming. To meet the current challenge of integrating cultivation systems within existing urban infrastructure, transdisciplinary approaches are required. The joint project food4future brings together novel technologies, such as lightweight materials and new LED irradiation concepts, for co-cultivation of marine and terrestrial organisms in a saline environment. Different edible species of the genus *Ulva* are applied to test different cultivation conditions. In addition to development, growth and biomass production, strong emphasis is given to the metabolic composition and nutritional value of the cultivated species. Combining different technologies and expertise, the genus *Ulva* is used to test the feasibility of macroalgae becoming an integrative part of the urban agriculture.

THREE BECOME ONE IN A TRIPARTITE COMMUNITY: REASSESSING THE COLLABORATIVE ADAPTATION OF *ULVA* AND ITS MICROBIOME TO COLD TEMPERATURES IN ANTARCTICA

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Chemical communication using infochemical-mediated strategies plays an essential role in ecological interactions in marine ecosystem. These chemical signals in algal–bacterial interaction are not still completely known but given that bacteria release algal growth- and morphogenesis promoting factors (AGMPFs) required for (green) macroalgae growth and development, adaptive responses to environmental stressors must be considered within the community structure.

To evaluate the contribution of macroalgae and its microbiome to various stress factors particularly temperature, the reductionistic model system of the tripartite community formed by *U. mutabilis* and its two essential bacteria, Roseovarius sp. strain MS2 and Maribacter sp. Strain MS6, which release all essential AGMPFs, was investigated. This analysis will let us to determine the stress response of each algal and bacterial symbionts within this cross-kingdom interactions and will help to understand the ecological success of Ulva.

I will present the morphogenetic effect of recently isolated bacteria from Potter Cove, King George Island (Isla 25 de Mayo) in Antarctica, on the model system *U. mutabilis* Føyn starting with axenic gametes. The results indicate that cold-adapted bacteria release sufficient amounts of AGMPFs, inducing cell differentiation, and cell division in axenic cultures. In particular, metabolite profiling of polar low molecular weight compounds revealed insights into the species-dependent cold stress response of the green seaweed holobiont *Ulva* (Chlorophyta). Integrating the chemical ecology to aquatic-microbiome investigations will allow us to further explore underlying adaptation and acclimation mechanisms in macroalgae to stress situations.

FABRICATION AND CHARACTERIZATION OF BIODEGRADABLE FILM FROM *ULVA* SPP.

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Biodegradable packaging materials can be developed from macroalgae, which are affordable, abundant, renewable, and readily available. Biodegradable films were manufactured with *Ulva* spp. The residual from the acid-base procedure has been used to produce a film-forming solution and *Ulva* biopolymer film was prepared via solvent casting technique. All the films have been characterized using a scanning electron microscope (SEM), Fourier transform infrared spectroscopy (FTIR), physicochemical, and mechanical and barrier properties. Total phenolic compounds and DPPH radical scavenging effect assay indicated that Ulva-based biopolymer film showed higher antioxidant properties than control film. It demonstrated that has been improved not only in the physicochemical properties but also in mechanical properties for the Ulva-based biopolymer film. The results of this investigation show that Ulva-based biopolymer films created, could be employed in packaging applications, such as food preservation films.

RISK ASSESSMENT OF COST PROJECT: PRINCIPLES AND METHOD

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Life presents us with unexpected occurrences that may contradict our aims. This is especially true for innovative projects – let alone multi-national ones. In order to reduce the probability of undesirable events, and to mitigate the consequences of such events once they occurred, we at COST employ a rigorous system of Risk Management.

The risks we are addressing include biological risks (such as diseases), technical risk (failure of systems and equipment), managerial risks, marketing risks, financial risks, transportation risk, and occupational safety risks (accidents and ill health). The target populations (i.e., who may be hurt) include the COST teams as well as the public who is exposed to *Ulva* products. Anything that may compromise our Project milestones can be regarded as a risk as well as anything that can harm people, environments, facilities, and reputation.

Our Risk Assessment (RA) is based on the following principles

- a single body who directs and oversees the RA activities of every team (DAS Ltd.)
- each team is responsible to carry out its own RA - every team has a local RA contact
- RA is based on Hazard identification, Risk Assessment and Risk Control (mitigation)
- COST Project will help with forms and Checklists to facilitate RA
- generating Hazard Log for every team in order to follow-up timely countermeasures
- avoidance of dangerous activities
- reducing risks As Low As Reasonably Practicable

A BACTERIAL-BASED PROCESS FOR DECOMPOSITION OF *ULVA* POLYSACCHARIDES TOWARD IMPROVING OF BIOMASS DIGESTIBILITY AND GAINED ENERGY

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Algal biorefinery processes need halotolerant enzymes for the hydrolysis of poly- and oligo- saccharides in low water. Efficient breakdown of ulvan which is the primary polysaccharide in *Ulva* (up to 55% of dry biomass) release trapped amino acids and increase biomass digestibility and the gained green energy from downstream fermentation. We hypothesized that the gut of the algivorous sea urchin *Tripneustes gratilla elatensis* contains novel enzymes for decomposition of ulvan and other algal polysaccharides. This is since this animal can gain energy from diet of a single algal species which may lead to the development of a unique microbial assembly that aid in polysaccharides decomposition. We compared the gut microbial assembly of the sea urchin when fed a mono-specific algal diet of either *Ulva fasciata* or *Gracilaria conferta*, or an algal-free diet. Dietary *Ulva* facilitated unique microbes and associations in the gut resulting in a denser and highly connected network. *Ulva*-unique microbes of *Bacteroidetes* were rich in genes for carbohydrate active enzymes which may aid in decomposition of the specific polysaccharides in this diet. A further effort resulted in the isolation of a novel bacterial strain of *Alkalihalobacillus sp.* which contained in its genome carbohydrate active enzymes as GH1 (glucto- and galactosidases), 13 (α-amylases), and 16 (agarases, laminarinases, galactanases), for decomposition of various algal polysaccharides. In vitro trials with isolated *Alkalihalobacillus sp.*

PACKAGING SOLUTIONS FROM LAND-BASED MACROALGAE AQUACULTURE

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Figure 5 Packaging solutions from land-based macroalgae aquaculture

The use of single-use packaging materials has increased dramatically in recent decades in parallel with increasing trends in convenience and fast-food. Most of these packaging materials are made of non-biodegradable, petroleum-based polymers that have degradative impacts on the environment and contribute to the global plastic pollution crisis. Finding alternative packaging materials is an important step towards building a bio-based circular economy. Sustainable landbased macroalgae cultivation can provide a solution, as it eliminates land-use pressure on coastal areas, doesn't interfere with recreational activities or agriculture, reduces seasonal limitations, allows for complete control over product quality, and ensures consistent quality and traceability. Here, we present the success story of land-based macroalgae packaging solutions in the food industry via the Mak-Pak and Mak-Pak Scale-Up projects.

EVALUATING *ULVA SP.* CULTIVATION FOR CLIMATE CHANGE MITIGATION: GROWTH, CARBON UPTAKE AND LONG-TERM STORAGE OF INORGANIC CARBON

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A key factor behind on-going climate changes on planet Earth is the high levels of anthropogenic CO2 released into the atmosphere. There are several options on hand to meet the needs of carbon reduction on a global basis. One such options refers to the sequestration of CO2 via photosynthesis of marine macroalgae, which are largely responsible for the normal functioning of marine ecosystems. Seaweeds such as *Ulva* species can also deliver important additional benefits and services including food, proteins and minerals, or active natural molecules, therefore alleviating human needs in the near future. Specifically, in both nature and during cultivation, *Ulva* species can keep fast growth during most seasons associated with high nutrient uptake capacity, hence accumulating significant amounts of dissolve inorganic carbon in their tissues. This presentation elaborates on the global potential use of seaweeds for such objective and focus on *Ulva* species as a unique tool to sequester excessive atmospheric CO2. Growth capacity and carbon uptake traits during semi-controlled, land-based cultivation will be presented, focusing on total carbon budgets and footprints for *Ulva sp.* from the Israeli Mediterranean Sea. This talk will address the seasonal aspect of biomass production in *Ulva sp.*, and discuss on the base of quantitative data of CO2 uptake during biomass production, the future potential of using *Ulva sp.* as a valuable tool to alleviate climate change.

TRANSITION OF AQUAPONICS TOWARDS SALTWATER: MODEL BASED DESIGN OF A MULTI-LOOP MARAPONICS SYSTEM

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Figure 6 Transition of aquaponics towards saltwater: Modelbased Design of a Multi-Loop Maraponics System

The Green Deal encourages European farmers to contribute to climate goals. For fish farmers sustainability means that the input, process and output for production are less harmful to farmed fish, people and the environment. There are various technical solutions for this. Aquaponics is such a solution. In aquaponics nutrient, water and energy cycles in the production process of freshwater fish and plants are closed as much as possible. However, agriculture, climate change and overpopulation contribute to freshwater scarcity while there is a surplus of saltwater. Hence, this raises the question: Can't food production be done smarter through Maraponics that uses saltwater instead of freshwater?

The objective of this work was to investigate and further develop potential multi-loop maraponics systems in terms of economic revenue, use of biogas, nutrient use efficiency of nitrogen and phosphorous, and water use efficiency.

To reach the objective, a model-based multi-loop maraponics system has been designed. The model helped to analyse the characteristics of a potential multi-loop maraponics system. In this study, European locations where aquaponic practices are already in place were investigated first, followed by a research on the types of marine fish and crops, a.o. Ulva, in a multi-loop maraponics system such that profitable and sustainable yields can be achieved.

The Azores islands appears to be the most suitable location, because the temperatures are warm but moderate. These weather and climate conditions give the best results regarding nutrient, water and energy use efficiency and economic revenue. *Ulva lactuca* is the highest-potential crop species due to the high growth rate, salinity tolerance and the market price when *Ulva* is used as input for the pharmaceutical industry. The fish species with the highest potential is turbot, due to its flat body, allowing a relatively high fish density with limited use of nutrients, water and energy. Furthermore, turbot has a profitable market price.

The research results may support the European Agriculture Policy and the European farmers by reaching the Green Deal. The research especificially supports fish farmers with their farm close to saltwater sources and who are interested in sustainable farming approaches for people, profit and planet.

STATE OF THE ART OF APPLICATIONS OF *ULVA* AND ITS EXTRACTS IN FISH AND INVERTEBRATE AQUACULTURE

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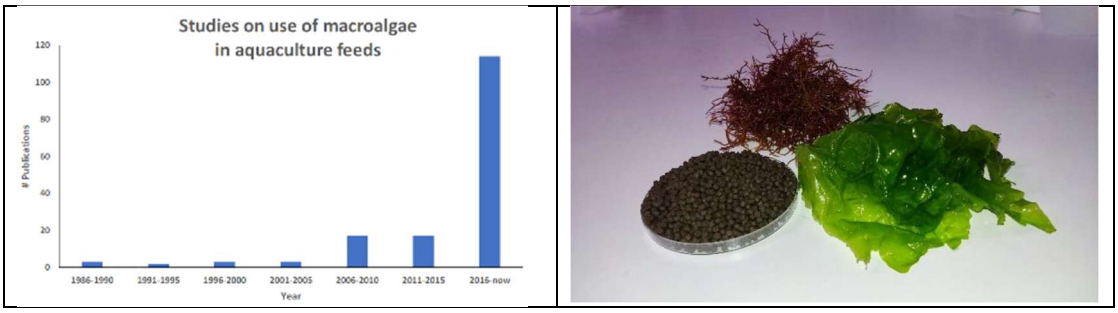


Figure 7 State of the art of Applications of Ulva and its

To be able to feed the ever-growing world human population, animal and plant marine aquaculture is considered as one of the most promising strategies. As it does not rely on fresh water and can be carried out at sea or in transitional areas on saline soils, it does not compete with agriculture for space and water. However, the production of animal protein from the sea with the current practice still has various sustainability issues, in particular in the case of finfish and crustacean aquaculture. These cultures largely rely on fish meal and other animal components in the feeds that are collected by trawl fishing, increasing the strain on already overexploited natural populations. Furthermore, soy and wheat are important compounds, for which they compete directly with human and farm animal feeds. Therefore, there is a need for more sustainable feed and feed compounds that not only does have excellent nutritional capacities but also increase the wellbeing of the animals produced (so-called functional feeds).

Macroalgae could form an important source of more sustainable feed ingredients of aquaculture feeds, as their production does not depend on fresh water. Moreover, they can be produced in IMTA-systems growing on the nutrients released by intensive aquaculture systems, thereby mitigating some of the negative effects of aquaculture.

The use of macroalgae as nutritional and/or functional compound is however still in its infancy. Although the first publications evaluating the nutritional potential of macroalgae in seaweeds stem from 1986, only in the last few years the number of publications on macroalgae as aquaculture feeds has grown exponentially.

This paper pretends to give an overview of the current state of the art of the use of macroalgae in aquaculture feeds, based on a literature study of over 150 peer-reviewed papers. Among other things, the different macroalgal species used will be discussed as well as the most common forms of use and the animal cultures for which they are used. The focus will be on the genus *Ulva*, as this is the genus most widely used in these studies (in over 2/3 of all papers). Apart from the use of whole algae, I will also go deeper into the use of specific macroalgae extracts (in particular polysaccharides as ulvan), including some recent results of our own studies. To conclude, some of the barriers (be it technical and/or socio-economical) and knowledge gaps affecting the potential of *Ulva* to contribute to a more sustainable aquaculture feed will be discussed.

CAROTENOIDS AS VERSATILE COMPOUNDS FOR THE SUSTAINABLE PRODUCTION OF HEALTH-PROMOTING PRODUCTS

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Carotenoids are isoprenoids compounds of grat versatility. They are biosynthesized by photosynthetic organisms as well as by some bacteria, fungi and arthropods. Most animals can incorporate them through the diet. They play key roles in photosynthesis (photoprotection, accessory light harvesting, assembly of protein-pigment complexes, etc.) the engine of life on planet Earth. They are also important from an ecological point of view for pollination and seed dispersal. Altogether, it is clear that they are key for food security. Besides, they can be metabolized into many compounds including phytohormones such as abscisic acid and strigolactones, that play important roles in plants, some of them of great importance for their subsistence and therefore also for food security. Their interest in foods is owed not only to their roles as pigments and vitamin A precursors, but also as lipophilic antioxidants and healthpromoting compounds. Recently there is a renewed interest in their use with cosmetic purposes, concomitant with the growing interest in nutricosmetics. *Ulva* is a good source of carotenoids, including lutein and β-carotene, which are among the main dietary carotenoids and the most widely studied in relation to their applications as colorants and ingredient of health-promoting compounds (Meléndez-Martínez et al., 2021). In this contribution, the importance of carotenoids in agro-food and health in the context of food security, sustainability and health promotion is summarized.

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PHOTOSYNTHETIC AND GROWTH STRATEGIES OF *ULVA* LACINULATA TO SURVIVE THE SEASONAL FLUCTUATING CONDITIONS IN A EUTROPHIC MEDITERRANEAN SEA BAY

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Ulva lacinulata (Kützing) Wittrock is a marine species with distribution in the Mediterranean, Tropical-Subtropical Western Atlantic Ocean, and New Zealand coasts, which inhabits rocky and shallow subtidal zones. This species was sampled nine (9) times from the eutrophic Thessaloniki Gulf, Greece, between June 2019 and March 2021, where key environmental parameters, e.g., temperature (mean value = 20.22°C ± 7.2SD), salinity (36.74±0.89), suspended solids (17.65±10.32mg/l), total dissolved inorganic nitrogen ($N\text{-}NO_3$=9.15±6.45 µmol/l), and dissolved reactive phosphorus (0.53±0.21 µmol/l) were measured. These data were analysed for the factor “time” by using one-way Anova and were correlated with subcellular (Rapid Light Curve, JIP-test, Photochemical Quenching analysis, and pigment content) and organismal (Relative Growth Rate-RGR) species parameters that were investigated under field conditions simulated in the laboratory by using multivariate analysis (RDA). The aim was to gain knowledge on photosynthetic and growth responses (a) to explain species’ seasonal acclimation strategies and (b) to support species’ sea or land-based mass cultivation. The results showed a statistically significant variation ($p<0.05$) for time of all (26) tested physiological parameters. RDA analysis showed temperature as the main factor ($p<0.001$) affecting the species' photosynthetic performance (qP, ΦPSII, rETRmax, Ek), higher during the warm period and associated with high irradiance levels, and lowest during the cold period. However, increased Chlorophyll/Carotenoids ratios at 27-28°C in July and September 2019, except in July 2020 (after Covid lockdown), indicate stress or damage to the photosynthetic apparatus. A heavy summer rainfall on July 2919 that lowered the salinity (34.4) and accumulated nitrogen (11.28 µmol/l) and suspended sediment (41.11 mg/l) reduced the photosynthetic efficiency (alpha) and the density of active Reactive Centres per Photosystem II (RC/ABS) and resulted in partial inactivity of RCs. This result indicates that some active RCs were converted into heat sinks, enhancing the energy dissipation, as also indicated by high DI0/RC (total dissipation of untrapped excitation energy from all RCs concerning the number of active RCs) values. Maximum RGR values that were estimated during March 2021 coincided with maximum biomass in the field. Based on the species' potential monthly growth yield (>70% of the maximum), the farming at the collection site could be successful from October to June.

Acknowledgements: This study was supported by the BIOALGAFOOD project (code: T1ΕΔΚ-00232) cofinanced by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH—CREATE—INNOVATE.

PAST, PRESENT AND FUTURE DEVELOPMENTS IN *ULVA* SPECIES CULTIVATION AND BIOMASS APPLICATIONS IN THE CANARY ISLANDS

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During the last 30 years, native *Ulva* species were the main assayed organism for the development of intensive seaweed aquaculture in the Canary Islands. In particular, *Ulva rigida* C. Agardh biomass have been produced year-round from laboratory experimental setups to tubular photobioreactors, tanks and raceways at pilot plant scale in our facilities at Taliarte (Gran Canaria). As far as successful outdoor productivities, reaching seasonal values higher that 100 gDW m-2 d-1, and biomass qualities were obtained during the first experimental trials, integrated multi-trophic aquaculture methodologies were evaluated and established as sustainable and environmental-friendly principles for the semi-industrial development of the sector in the region.

Physiological and biochemical studies have been regularly carried out, in order to evaluate how the different established culture conditions affected growth characteristics and biomass composition. So far, *Ulva* protoplast isolation, their photosynthetic characteristics and capabilities as seeds to regenerate new plants were evaluated in the beginning. Pathologies associated to endophytes growth or the effects of heavy metals (i.e. cadmium) were studied from the physiological point of view as causal agents for biomass losses in cultures. Also the availability of inorganic nitrogen and dynamics on tank-cultivated biomass, grown under enriched and depleted conditions, deeply affected growth, photosynthetic characteristics and polysaccharides and dietary fibre, pigments, proteins or lipids and fatty acid composition among other metabolites.

More recently, intensive tank produced *Ulva* biomass have been evaluated for multiple applications related to sectors as food and feed or those related with the use of high value metabolites as the cosmeceutical or nutraceutical. It is particularly relevant the characterization of biomass with different biochemical profiles, obtained under specific growth controlled conditions, as feed for molluscs such as the abalone or as novel dietary ingredients for aquafeeds improving the immune response in aquaculture. All these knowledge and expertise will be reviewed and considered in the near future as the basis for a new project, “RuBisCO-Algarden”, with the aim to evaluate the carbon sequestration and footprint reduction related to port area activities in a scenario of global change, by assessing a pilot open-sea cultivation unit of the native seaweed *Ulva rigida* and its biomass valorization for biofuels and agriculture applications.

SHEDDING LIGHT ON THE *ULVA* HOLOBIONT: THE ROLE OF LIGHT IN MICROBIAL INTERACTIONS WITH IMPLICATION IN IMTA-RAS

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The effect of light on the population behaviour of *Ulva* species, and on bacteria-seaweed interactions, has in general been studied very little. Natural populations of *Ulva* seem well adapted to conditions of light heterogeneity, and this adaptation could suggest the involvement of quorum sensing mechanisms and microbial interactions.

Since the bacterial communities associated with *Ulva sp.* play an important role both in the morphogenesis and in the reproduction of Ulva, it is posible that bacteria also play a role in *Ulva sp.* adaptation to light. Moreover, *Ulva* laminar thallus provides an important niche for biofilmforming bacteria, such as those from the Phaeobacter genus, that are antagonistic towards pathogens (e.g. Vibrio anguillarum). The probiotic effect of U. ohnoi experimentally colonized with a Phaeobacter strain, previously isolated from *Ulva sp.*, was demonstrated in experimental infections with V. anguillarum. Those results paved the path to the possible manipulation of Ulvaassociated bacterial communities as strategy for Vibrio control in fish-*Ulva* IMTA-RAS cultures.

The UlvaQuo project investigated the existence of biochemical communication in U. ohnoi in response to light intensity and heterogeneity, the identification of the chemical signals which could mediate that communication, and the effect of light on the Ulva-epiphytic microbiota.

Microbial communities and the exo-metabolome of *Ulva* surfaces were analysed to elucidate the underpinning mechanisms in response to light variables that could improve U. ohnoi cultivation in IMTA-RAS and the maintenance of Phaeobacter. A negative influence of light intensity on the maintenance of Phaeobacter bacteria on U. ohnoi was observed. We observed that adapting *Ulva* cultivation within the IMTA-RAS systems to include a light and dark phase, could ensure Phaeobacter was retained. Light management strategies to maximize U. ohnoi productivity in IMTA systems and also to promote probiotic effect induced by Phaeobacter colonization were developped in a pilot-scale system with effluents from sole (S. senegalensis) cultures.

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BIO-ECONOMIC MODEL OF *ULVA* CULTIVATION UNDER CC

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By the end of the current century atmospheric CO2 concentration may reach 1000 ppm, more than twice the present level set at ca. 400 ppm. Marine macroalgae (seaweeds) contribute to
global primary production and by taking up CO2 they may ameliorate and regulate global climate change. Seaweeds also have direct and indirect economic importance by providing food and bioactive compounds for human benefit. Nonetheless, all these benefits could be jeopardized by the ongoing pressures, both local and global, on marine environments. In this study we examine the effects of dissolved CO2 and seasonal seawater temperature on the growth rates (measured weekly changes in biomass and expressed on a daily basis) of two model species, *Ulva rigida* (Chlorophyta) and Gracilaria conferta (Rhodophyta), which are common in the intertidal zone of the Israeli Mediterranean Sea, and cultivated by the local seaweed industry. The seaweeds were grown in land-based 40 L fiberglass tanks fertilized with sufficient N and P, supplied with running seawater and continuous air bubbling to keep equal exposure of the seaweeds to nutrients and light. The tanks were also provided with aeration with regular air (ambient CO2, ~ 400 ppm) or CO2-enriched air (~780 ppm). Seaweeds exposed to CO2–enriched seawater grew faster, 32.5 and 8.5% growth per day for U. rigida and G. conferta, respectively. Following calculations of productivity rates, market price, and input cost, we estimate production and show a quadratic production function with respect to temperature for each CO2 concentration. Thus, there is an optimal temperature that maximizes seaweed output. Based on the production function estimates and using market prices, maximal short-run profits were obtained at ca. 22.5 °C and 27.5 °C for U. rigida and G. conferta, respectively. These results may provide useful information for seaweed growers on what and where to grow seasonally, and how farming activities should adapt to external changes in temperature and CO2 concentration.

LARGE-SCALE OFF-SHORE CULTIVATION OF ULVA: SEASONALITY AFFECTS THE BIOMASS YIELD, PERFORMANCE AND BIOCHEMICAL PROFILE OF THE BIOMASS

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Compared to Asia, seaweed aquaculture is in its infancy in Western Europe. However, due to the multi-applicable usage of this renewable resource there is a rapidly growing interest in efficient production technologies to foster a large-scale industrial production of seaweed biomass. Especially the sustainable oceanic cultivation of seaweeds is attractive as it does not compete with terrestrial crops for space and freshwater, and as extractive organisms seaweeds do not afford fertilization whilst providing high biomass yields.

This study monitored the effect of seasonality on the overall biomass performance (growth, biomass yield), chemical composition (fatty acid, protein, carbohydrate, pigment, phenolic, biochar, ash, and element composition), fertility (total amount of fertile thallus tissue), and biofouling (total coverage) of Swedish off-shore cultivated *Ulva* fenestrata, in order to find suitable harvest times for biorefinery purposes. Specimens of U. fenestrata were cultivated in an off-shore seafarm in the Kosterfjord, Sweden from October to five different harvesting points in April, May and June. Statistical analyses confirmed that there was a significant difference in overall biomass performance and biochemical composition among the time points. Our study confirmed the large scale off-shore cultivation potential of northern hemisphere U. fenestrata and underpins suitable harvest time points to facilitate industrial valorization processes of the off-shore cultivated biomass. Together, these results indicate that seasonality and the selection of harvest periods are crucial factors to consider in order to facilitate high yields, respective quality as well as desired biochemical traits in future oceanic *Ulva* farms.

THE SEAWEED *ULVA LACTUCA* AS A NUTRITIONAL 'TOOL' TO PREVENT CHRONIC DEGENERATIVE WELFARE DISEASES (CDDS) IN THE HUMAN POPULATION.

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For the ≈10 billion people who will inhabit our planet around AD 2050 Bluegreentechnologies.nl set for quality-of-life MG-1: "The right for healthy food" at the first place. We studied the efficacy of seaweeds as a source for long chain polyunsaturated fatty acids (LC-PUFAs), which are associated with the prevention most obesity related CDDs such as type-2 diabetes, insulin resistance, cardiovascular diseases≈ on a global scale death-cause no.1, NAFDL, atherosclerosis, mental disorders, and some cancers.

The most striking observations on the lipid profile of *Ulva lactuca* are the following: Conversion of Palmitic acid (C16:0, SFA) to Palmitoleic acid (C16:1, ω9) via the enzyme Δ-9 desaturase or SCD-1 in a process termed 'palmitoylation' is low. SCD-1 activity is associated with most obesity related harmful CDDs. The two essential fatty acids (EFAs) Linoleic acid (C18:2, ω-6; LA) and α-Linolenic acid (C18:3, ω-3; ALA) are high, while the strongly pro-inflammatory Arachidonic acid (C20:4, ω6), operating via eicosanoids, is relatively low. An important observation is that of all in this study by GCMS measured seaweeds (green-, brown- & red-) Docosapentaenoic acid (C22:5) -an important ω3 'fish-oil' in human brain- is only detected in *Ulva lactuca*. DPA has recently been attributed the function of buffering between the two important ω3 'fish oil' LC-PUFAs EPA(C20:5) & DHA(C22:6) also operating via eicosanoids in an anti-inflammatoryway. An important observation is that the two rate-limitingenzymesystems of Δ-6 desaturase: ω6: from LA=> γ-Linolenicacid (C18:3) & ω3: ALA=> Stearidonic acid (C18:4) areboth present.Striking, the rare Δ-17 desaturase, catalysingconversion of ω6 ARA(C20:4) to ω3 'fishoil' EPA(C20:5) is present. ω3 'fish oil' LC-PUFAs EPA(C20:5) & DHA(C22:6) also operating via eicosanoids in an antiinflammatory way. An important observation is that the two rate-limiting enzyme systems of Δ-6 desaturase: ω6: from LA=> γ-Linolenic acid (C18:3) & ω3: ALA=> Stearidonic acid (C18:4) are both present. Striking, the rare Δ-17 desaturase, catalysing conversion of ω6 ARA(C20:4) to ω3 'fish-oil'

EPA(C20:5) is present.

Ulva lactuca is a good and virtually inexhaustible source for PUFAs with an ω6/ω3 ratio of ≈1.0=>presently≈25-50! in our Western diet causing many CDDs. A biotechnological challenge is to transfer the genetic code for DHA from Sargassum natans to a new *Ulva lactuca* strain to fight the medically expensive CDDs.

ULVA AND SEAGRASSES, A TALE OF LOVE AND COLDNESS.

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It is well known that eutrophication causes negative effects on shallow coastal ecosystems. Often these effects trigger massive proliferations of ephemorohytes such as *Ulva* (green tides), which can harm seagrass meadows due to shading by dense overgrowing *Ulva* mats. In addition, seagrass development can be undermined by direct ammonium toxicity, since some seagrass species do not control their uptake efficiently. However, the story of the relationship between *Ulva* and seagrasses is far from being simple. For more than 25 years, our research group has studied the interactions between the development of *Ulva* blooms and seagrass meadows. While we have documented evidence that a massive development of *Ulva* causes the disappearance of seagrass meadows (i.e., Palmones River estuary in the 90´s), over the years we have got evidences that a moderate proliferation of *Ulva* can be beneficial for seagrasses, based on the higher nutrient uptake rates of the *Ulva* mats, which reduces the toxic effect of ammonium on seagrasses. Similarly, beneficial effects of dense *Ulva* mats on seagrasses have been shown to involve a direct transfer of dissolved organic carbon from the algal mats to the seagrasses.

SUSTAINED GROWTH IN CULTURE OF BARCODED *ULVA SP.* GERMLINGS FOR BIOPROCESSING

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Figure 8 Sustained growth in culture of barcoded Ulva sp. germlings for bioprocessing

Ulva species have cryptic morphologies with few distinctive features, as well as significant intraspecific variation. This often renders them difficult to identify. This study reports the sustained growth of *Ulva sp.* germlings in culture and their identification via morphological characters and DNA barcoding.

Culturing of unialgal germlings involved sectioning thallus fragments a few millimeters in size. These were used to initiate new cultures and their sustained growth was achieved in enriched sea water. *Ulva sp.* were grown at a twelve hour photoperiod using cool white fluorescent light, together with aeration. Biomass is being produced for further downstream bioprocessing.

Sequencing of the chloroplast RUBISCO LSU (rbcL) gene, the elongation factor Tu (tufA) and the nuclear internal transcribed spacer (ITS) was undertaken. Of the various *Ulva sp.* that are being grown in culture, U. compressa, U. torta and U. californica have been successfully barcoded so far.



From Ulva aquaculture to food and feed production: state-of-the-art, bottlenecks, risks and gaps

Cadiz, Spain

POSTER PRESENTATIONS

Ulva

The Future Wheat of the Sea

ENRICHING *ULVA SP.* WITH NUTRITIONAL COMPOUNDS BY EXPLOITING IMTA

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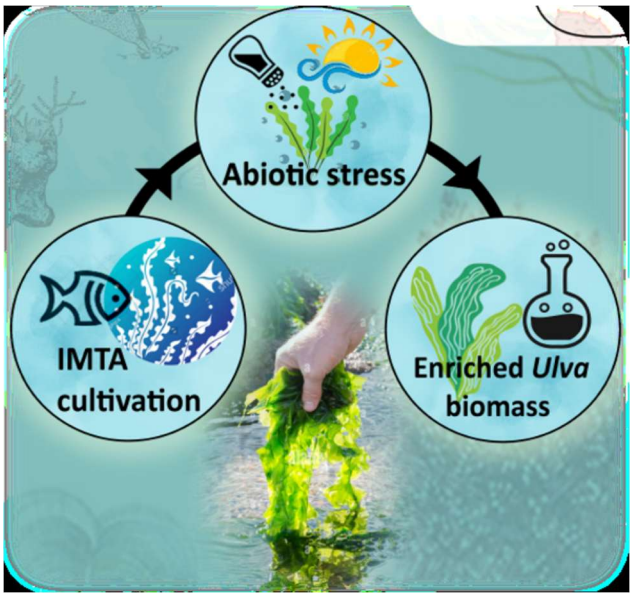


Figure 9 Enriching Ulva sp. with nutritional compounds by exploiting IMTA

Shifting from agriculture to mari-culture is viewed as an essential step towards food security in an era of global climate change, habitat destruction, rapid soil erosion and severe shortage of freshwater. Shifting from agriculture to mari-culture is viewed as an essential step towards food security in an era of global climate change, habitat destruction, rapid soil erosion and severe shortage of freshwater. Cultivation of the green seaweed *Ulva sp.* is of a particular interest thanks to its multifunctional attributes: elevated bioremediation capabilities, no freshwater use, high growth rates, and potentially rich with essential elements for human nutrition. Following the rising public awareness of alternative, healthy food sources, there is a global increase in demand for seaweed biomass. However, there are yet knowledge gaps regarding the accurate control of specific metabolites during the cultivation of seaweeds. Our study explores an innovative, two stage integrated aquaculture designed to control and stimulate the content of nutritional compounds in Ulva. We integrated a finfish (*Sparus aurata*) culture, the effluents of which are diverted into a series of mixed *Ulva rigida* and *Ulva* ohnoi cultivation tanks.

The seaweeds were then exposed to short-term abiotic stressors (i.e., high irradiance, nutrient starvation, and high salinity) to stimulate synthesis of desired compounds in their tissues. The seaweeds were then exposed to short-term abiotic stressors (i.e., high irradiance, nutrient starvation, and high salinity) to stimulate synthesis of desired compounds in their tissues. Our methodology enabled high growth rates (25% biomass increase per day), with significant enhancements in the amount of protein, starch, and minerals. Moreover, the seaweeds removed up to 90% of the Ammonia, NO3 and PO4. Our Ulva based experimental approach suggests a promising direction towards production of functional seaweeds, enriched with valued compounds for the emerging food and health industries.

INFLUENCE OF ABIOTIC FACTORS ON VALUABLE COMPOUNDS IN NATURAL AND CULTIVATED BIOMASS OF *ULVA SP.* INFLUENCE OF ABIOTIC FACTORS ON VALUABLE COMPOUNDS IN NATURAL AND CULTIVATEDBIOMASS OF *ULVA SP.*

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The genus *Ulva* is globally highly abundant and widely distributed within coastal marine macroalgal communities. In addition to its ecological significance as an important primary producer, its commercial value is increasingly recognised. As the world's population continues to rise, it is imperative to focus on the sustainable production of new food sources. *Ulva sp.* (Chlorophyceae) contain a unique fatty acid profile with high amounts of omega-3 with precursors synthesisable to increase its levels in human tissue, as well as chlorophyll and carotenoids that function as free-radical scavengers. Inthis
In this sense, it becomes increasingly vital to determine how the chemical variability of *Ulva* can be affected by abiotic factors such as light, temperature, salinity, water movement, and nutrient availability.The genus *Ulva* is globally highly abundant and widely distributed within coastal marine macroalgal communities. In addition to its ecological significance as an important primary producer, its commercial value is increasingly recognised. As the world's population continues to rise, it is imperative to focus on the sustainable production of new food sources. *Ulva sp.* (Chlorophyceae) contain a unique fatty acid profile with high amounts of omega-3 with precursors synthesisable to increase its levels in human tissue, as well as chlorophyll and carotenoids that function as free-radical scavengers. In this sense, it becomes increasingly vital to determine how the chemical variability of *Ulva* can be affected by abiotic factors such as light, temperature, salinity, watermovement, and nutrient availability.
One of the aims of this large-scale project investigating seaweed-microbe interactions across commercially valuable macroalgae (red, green, brown) was to characterize biochemical spatial-temporal changes in the internal compounds of Irish *Ulva sp.*, as well as the pathogens of public health concern presents in their natural environment. Multivariate analyses were employed to evaluate spatial-temporal profiles established across four seasons and four locations in western Ireland. In Ulva, significant variation was observed in pigments and fatty acids across seasons and locations, with higher pigment levels observed, in general, in winter months, coinciding with higher monounsaturated and omega-3 fatty acids levels. Positive bacterial responses to CASE medium (Salmonella spp., Enterobacter spp. & Klebsiella spp., E. coli, Shigella spp.) were strongest in coldest months at all coastal locations.
Using experimental cultures, fluctuations in biochemical compounds of commercial interest were induced by interactions of temperature and irradiance applied as natural stressors. Higher chlorophyll concentrations were observed at lower light and temperature, coinciding with higher algal photosynthetic fitness. Higher monounsaturated and omega-3 values were associated with high light, and high or low temperatures, respectively. In this sense, bioaccumulation of valuable compounds responded to interacting stressors; results reflect the physiological capacity of *Ulva* to adjust to temporal and spatial fluctuations in its natural habitat and highlight its suitability for optimized valuable biomass production through cultivation.

SALINITY AS A KEY FACTOR FOR STRAIN SELECTION AND OPTIMIZATION OF *ULVA SP.* FOR LAND-BASED CULTIVATION

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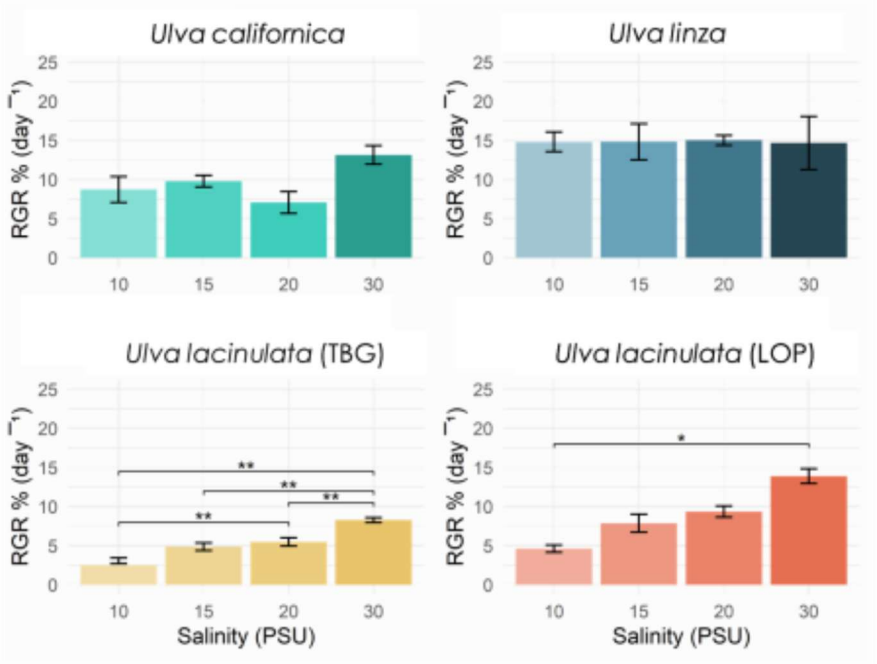


Figure 10 Relative Growth Rate (in % day -1) of Ulva's germlings after 3 weeks under different salinity conditions and statistical comparison between treatments (pvalue ≤ 0.05)

The green seaweed *Ulva sp.* has been vastly explored for its functional properties in diverse industries including cosmetics, pharmaceuticals, food, feed, and even the ecosystem services it provides, such as bioremediation. More recently, its potential as a biological alternative to plastic packaging has been investigated. The mission of the Mak- Pak Scale-Up project (2021-present) is to develop a seaweed-based food packaging material material and to sustainably scale-up the production of seaweed, including *Ulva sp.*, in a recirculating land-based facility supplemented with artificial seawater. Such a system requires a selection and optimization of the seaweed of interest. The *Ulva* genus has previously shown high plasticity and capacity to acclimate and develop under broad ranges of environmental conditions, suggesting this may be a promising candidate for production in a recirculating aquaculture system with low salinity artificial seawater. In this case, selecting an *Ulva* strain well adapted to low salinity conditions will help reduce the costs of salt and, therefore, optimize production. The aim of this study was to determine how one abiotic factor (salinity) can be used and adapted throughout the entire *Ulva* production to define several key points in the production chain: 1) strain selection 2) nursery and seeding 3) optimized production in adult *Ulva* and 4) increasing functionality (e.g. high antioxidant activity). Based on the results of three different experiments, it was possible to conclude how salinity impacts different species and strains of Ulva, during three key moments of their production chain and with that, conclude which species would be more suited to be scaled up within the scope of landbased production. At the same time, the data presented serve as a baseline for further work with *Ulva* under different cultivation conditions.

ULVA MUTABILIS REMOVES THE ENDOCRINE DISRUPTOR BISPHENOL A WITHOUT BACTERIAL SUPPORT

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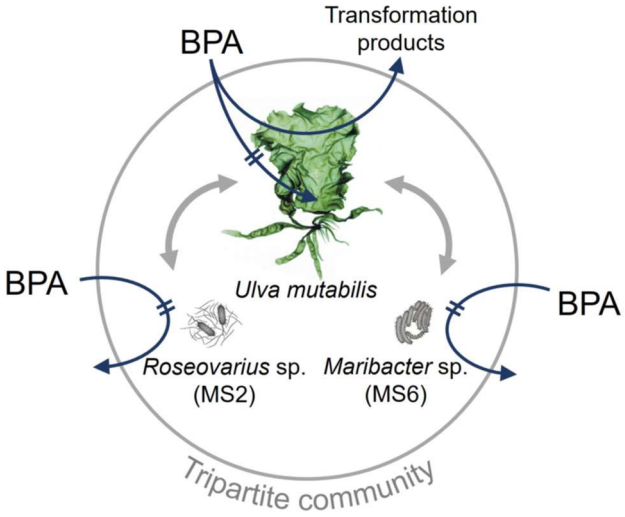


Figure 11 Ulva mutabilis removes the endocrine disruptor bisphenol A without bacterial support

Emerging pollutants are environmental hazards due to their ecological and human health impacts. We aimed to uncover the removal mechanisms of the endocrine disruptor bisphenol A (BPA) and its substitutes by a model system composed of *Ulva mutabilis* and two symbiotic bacterial strains, Roseovarius sp. (MS2) and Maribacter sp. (MS6). Cultivation experiments were performed with the tripartite community and compared to the individual species; axenic *Ulva* was cultivated both as deformed callus and with complete morphogenesis using the bacterial growth factors. Medium and algal tissue samples were taken after 14 days of incubation, and spiked with isotopically labelled BPA as internal standard prior to analysis. Liquid samples were analyzed using ultra high performance liquid chromatography (UHPLC) coupled with high resolution mass spectrometry (HRMS). Tissue samples were analyzed using gas-chromatography (GC) coupled with HRMS after extraction and subsequent silylation.

The tripartite community provided exceptionally effective removal of high concentrations of BPA (13.2 mg L-1) from the medium with a removal efficiency of over 99 % after two weeks. When looking at the individual species, the two bacterial strains failed to change the BPA concentration significantly in both complex and minimal media. However, axenic Ulva, both with and without complete morphogenesis, displayed similar removal capabilities compared to the tripartite community. Since tissue extraction showed no BPA uptake or sorption, biological transformation by *Ulva* seems to be the most probable removal mechanism. Moreover, through BPA detoxification, *Ulva* supports the growth of associated bacteria, which are sensitive to high BPA concentrations. This ensures the production of growth factors important for algal development and morphogenesis by the bacteria. It is another example of cooperative behavior between associated organisms. According to our results, *Ulva mutabilis* shows excellent capability in the removal of BPA and is a promising candidate for bioremediation.

ULVA SP. BASED-ALGAE BLENDS: UNVEILING THE LIPID FRACTION POTENTIAL FOR FOOD AND NUTRACEUTICAL APPLICATIONS

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Rising health consciousness, the need for alternative and sustainable nutrients sources, and the increasing knowledge on health-promoting effects of algae consumption are boosting the demand and market for edible algae, including macroalgae and microalgae, in Western countries. However, each algae species has unique nutritional and functional characteristics. Some species are richer in certain compounds than others, and there are some compounds that are species specific. The preparation of blends, with mixing of several species, allows to obtain in a single product distinct characteristics, impossible to obtain with a single species.

Regarding macroalgae, *Ulva* species are among the most abundant and can be found in coastal benthic habitats all around the world. It's one of the most versatile macroalgae, with diverse applications, such as in food, nutraceutical and pharmaceutical, and it has been used as the main component of algae blends.

In this work, we aimed to develop and characterize five algae blends (four of macroalgae and one of macro and microalgae) containing *Ulva rigida* produced under organic conditions in Europe. The blends were evaluated for their composition in ashes, proteins, lipids and total carbohydrates, and the fatty acid (FA) profile analysis was performed by gas chromatography coupled with mass spectrometry (GC-MS). Considering the FA profiles, the lipid quality indices of atherogenicity (IA), thrombogenicity (IT) and unsaturation (IU) were calculated.

The results showed that all blends contained considerable amounts of plant-based protein (15.40±3.17 - 28.68±0.15%), low lipid contents (0.48±0.11 - 3.67±0.18 g/100g), but a significant content of polyunsaturated FA in the lipid pool (35.77±2.02 - 49.73±7.24% of total FA). Each blend was characterized by its own FA profile, although all contained essential and n-3 FA, and had healthy lipid indices (IA: 0.41±0.01 - 0.72±0.04; IT: 0.23±0.09 - 0.45±0.04; IU: 153.03±1.49 - 245.11±35.32). The macroalgae blends differed in most parameters evaluated only from the blend that added macro and microalgae.

As nutritional and environmental attractive food products, the consumption of the studied *Ulva* containing blends can contribute to a healthy lifestyle, and to sustainable diets. The lipid fractions revealed a great potential to contribute to the prevention and handling of cardiovascular and inflammatory diseases.

Keywords: Algae blends; Chemical composition; Fatty acid profile; Lipidomics

Acknowledge: Thanks are due to the COST Action 20106 SEAWHEAT and CESAM, LAQV-REQUIMTE, UA and ALGAplus, Ltd.

ULVA OHNOI – SOLE IMTA-RAS SYSTEM: LIGHT MANAGEMENT STRATEGIES TO MAXIMIZE *ULVA* PRODUCTIVITY AND TO PROMOTE PROBIOTIC EFFECTS INDUCED BY MICROBIOTA

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One of the bottlenecks in multitrophic recirculation culture systems (IMTA-RAS) of fish and macroalgae is to increase the productivity and reduce the land surface required for seaweed production. Increasing of productivity has to be studied involving management of densities, incident photon flux density and photoperiod. The UlvaPro project investigated the spatial distribution of the photon flux density and spatial distribution of the seaweed fronds in tanks, to obtain the best macroalgae production, both from the quantitative and qualitative point of view.

The project has been carried out in an IMTA-RAS system (Sole-U. ohnoi) equipped with biological and mechanical filter and water temperature control. U. ohnoi were cultivated in indoor tanks (90 and 180 L) with bottom aeration and illuminated by LED light sources (Fig. 1)

In the project three main points were studied (Fig 2):

- The distribution of light in seaweed tanks in function on irradiance, stocking densities and chlorophyll content of the Ulva: Light measurements were made in tanks with stocking densities from 0.3 to 3 kg m^{-2}, and incident PAR in the tank water surface from 150 to 1000 µmol m^{-2} s^{-1}

-The effects of light distribution on growth and photoinhibition processes in Ulva: Specific growth rate and productivity, chlorophyll fluorescence and chlorophyll content were determined under different combinations of stocking densities and incident PAR in the tank water surface -The effects of light distribution on nutritional quality and food safety of the Ulva.: heavy metals, and ash, protein, carbon and dietary fiber content were determined in seaweed fronds cultured under different combinations of stocking densities and incident PAR in the tank water surface

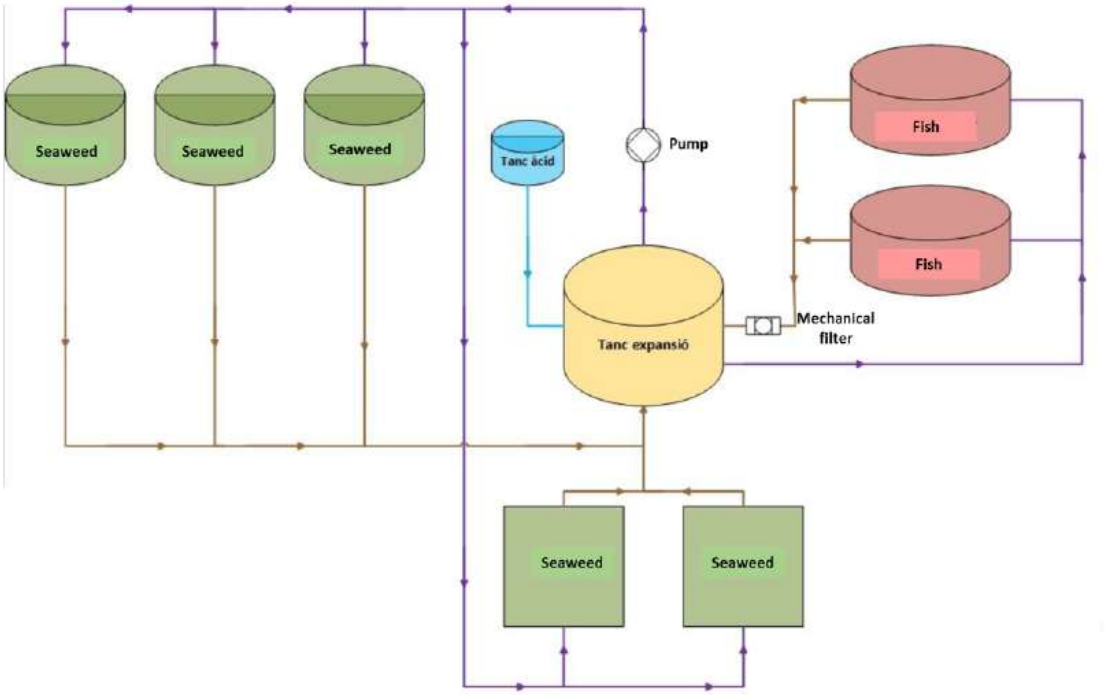


Figure 12 Fig 1. IMTA-RAS system (Sole-U. ohnoi)

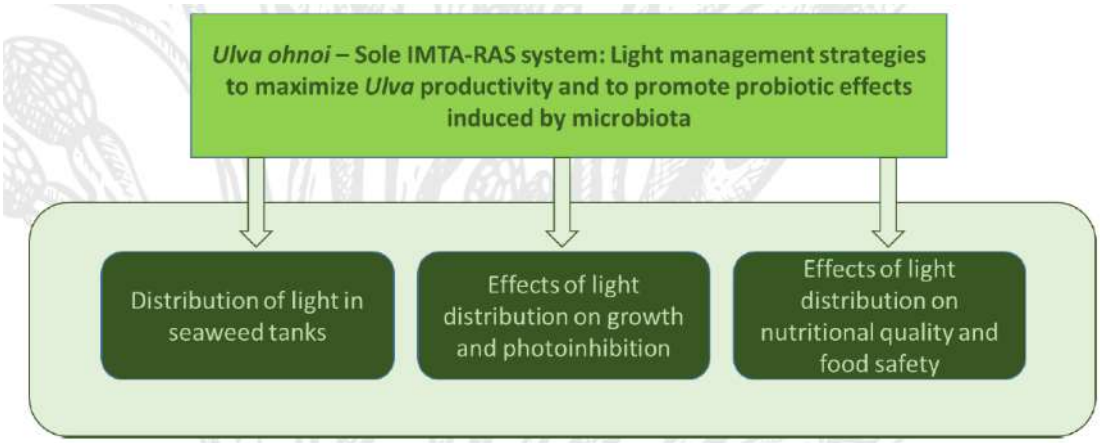


Figure 13 Fig. 2: Main points of the UlvaPro project

Acknowledgments

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ULVA AND SEA URCHINS IN A RECIRCULATING AQUACULTURE SYSTEM: A PILOT STUDY TO MAXIMISE WASTE RECYCLING

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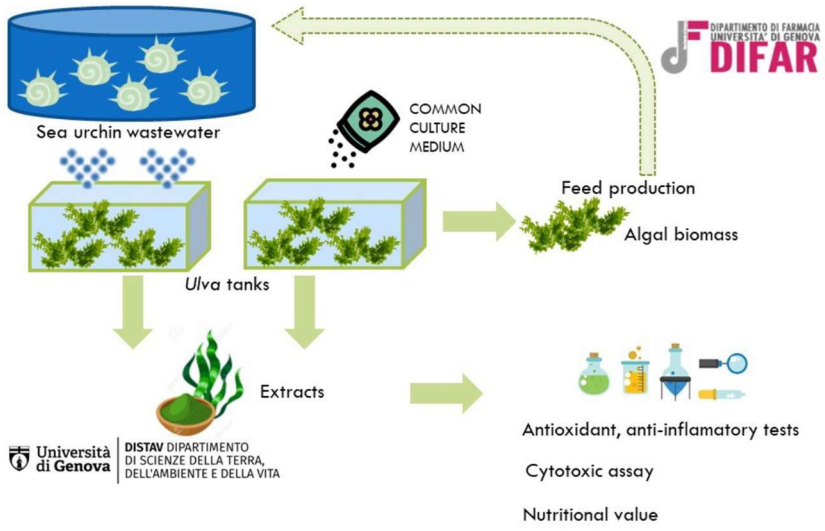
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Waste recycling and valorisation are presently the main challenges for the society and two of the pillars of the circular economy approach. A waste of a production process could be reused with the aim of producing resources, with a strong impact on our daily life and on the health and general well-being of the environment. The waste from aquaculture farms can be used in an eco-efficient practice for the cultivation of macroalgae, minimizing the environmental impact and contributing to the production of precious resources such as food, feed and biomaterials.

In this context, the aim of our trial is to develop a recirculating cultivation system using wastewater from sea urchin aquaculture, i.e. Paracentrotus lividus, a species common in the Mediterranean Sea with considerable commercial interest, as a source of nourishment for *Ulva lactuca*, a marine macroalga with huge potential as food, feed and for the extraction of bioactive compounds. This integrated aquaculture system of algae and animals would respond to the need of i) green wastewater treatment for aquaculture systems and ii) producing new marine derived valuable compounds in a sustainable process, non-impacting environmental resources.

The main objective of this study is the development of an innovative integrated cultivation system of marine macroalgae, which will be used both as wastewater biodepurators and as a source of substances of commercial interest in a circular economy perspective.

This experimentation complements a project that aims to reuse sea urchin waste from the food industry (BRITEs project, https://www.riccicliamo.it/brites/) to produce highvaluable materials, as marine collagen (from sea urchin peristome) and bioactive calcium-rich flour (grounding sea urchin test). This flour is used in the production of sea urchin feed together with *Ulva* biomass produced in the recirculating system, potentially developing an optimal feed for sea urchins that will allow to completely close the “waste circle” while promoting sea urchin aquaculture and reducing the impacts on natural stocks.



Macroalgal-bacteria interactions: screening for siderophores and their ecophysiological role in the

Macroalgal-bacteria interactions: screening for siderophores and their ecophysiological role in the chemosphere of the green tide forming macroalga.

Figure 14 Ulva and sea urchins in a recirculating aquaculture system: a pilot study to maximise waste recycling

CHEMOSPHERE O F THE GREEN TIDE FORMING MACROALGA *ULVA* (CHLOROPHYTA)

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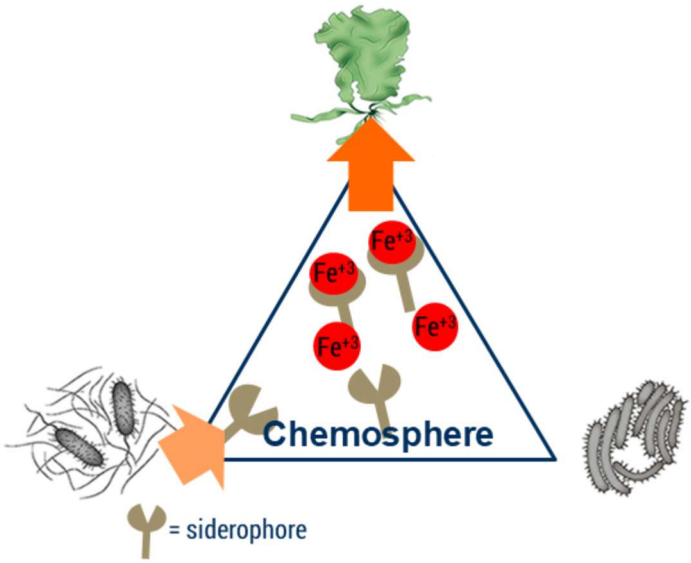


Figure 15 Macroalgal-bacteria interactions: screening for siderophores and their ecophysiological role in the chemosphere o f the green tide forming macroalga Ulva (Chlorophyta)

Ulva sp. life is tightly dependent on its microbiome. Bacteria produce algal growth and morphogenesis promoting factors (AGMPF) and contribute to *Ulva's* growth and development. Recently, in chemical ecology, *Ulva mutabilis* has been considered a good and established model organism. *U. mutabilis* and its two essential bacteria establish a tripartite community where Halomonas sp. MS1 induces cell division and Maribacter sp. MS6 promotes rhizoid and cell wall formation.

Ulva and its associated bacteria require iron to manage essential biological processes like most organisms. However, due to low iron bioavailability in marine ecosystems, organisms have developed several recruitment strategies. For example, bacteria release siderophores to acquire iron from the environment. As bacteria can support algal growth, we hypothesize that bacterial siderophores contribute to their algal host's iron homeostasis. Thus, the "carbon for iron" hypothesis suggests the iron exchange against carbon between bacteria and algae within their chemosphere.

A screening of the chemosphere of *Ulva* using metal isotope-coded profiling (MICP) identified several bacterial siderophore released by Halomonas sp. MS1. Subsequently, one siderophore was isolated and preliminarily characterized from the culture supernatant of Halomonas sp. MS1. Structure elucidation of the isolated siderophore refers to an amphiphilic siderophore with an unusual moiety. Fe(III)-siderophore complex was used in short-term uptake as iron sources, showing that Halomonas sp. does not utilize its own siderophore; however, *Ulva* gametes utilize the bacterial siderophores to uptake iron. We conclude that our study supports the "carbon for iron" hypothesis, which indicates that bacteria and *Ulva* collaborate within their cross-kingdom interactions and benefit from each other by "public goods". The study contributes to a better understanding of the crosskingdom interactions potentially applied in the maintenance of algal aquacultures.

DEVELOPING A PROTOCOL FOR THE EFFECTIVE APLICATION OF PHAEOBACTER IN *ULVA* CULTURES FOR PATHOGEN CONTROL IN IMTA-RAS

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The co-culture of Solea senegalensis and *Ulva* ohnoi in an integrated multi-trophic aquaculture recirculation systems (IMTA-RAS), is one of the most promising possibilities for the development of a more sustainable aquaculture. Moreover, *Ulva* not only improves water quality by removing nutrientexcess and oxygenating the water, it also provides a niche for biofilm-forming bacteria, like Phaeobacter sp. with the ability to antagonize fish pathogens such as Vibrio anguillarum, through the production of tropodithietic acid (TDA). The colonization of Phaeobacter over *Ulva* can be used as a pathogen control strategy in IMTA-RAS, however, this colonization can be influence by environmental factors such as light intensity. In previous experiments, it was determined that light affected negatively the maintenance of Phaeobacter biofilms in *Ulva* and a period of darkness was necessary to maintain an adequate concentration of the probiotic on the algae surface. Considering that, the purpose of this research was to design an IMTA-RAS system based on the tripartite Sole-Ulva-Phaeobacter and check the biological control capacity of Phaeobacter against Vibrio anguillarum outbreaks. The prototype system consisted of three 3L-tanks connected in cascade, one exposed to light with algae actively growing, other in darkness, with algae inoculated with Phaeobacter connected to a third tank where nutrients were added (simulating the presence of fishes) as well as Vibrio anguillarum (simulating an infection in the system). During the experiment, the presence of Phaeobacter and Vibrio, as well as bacterial communities, were monitored by colony counting, qPCR and 16S rRNA gene sequencing, both in water and algal surface. The results indicated that Phaeobacter colonised the entire system satisfactorily and did not interfere with algal growth. The presence of Phaeobacter reduced Vibrio concentration in water, being undetectable at the end of the experiment, compared to the control. Considering this, a basis can be laid for designing the system on a larger scale for an industrial purpose.

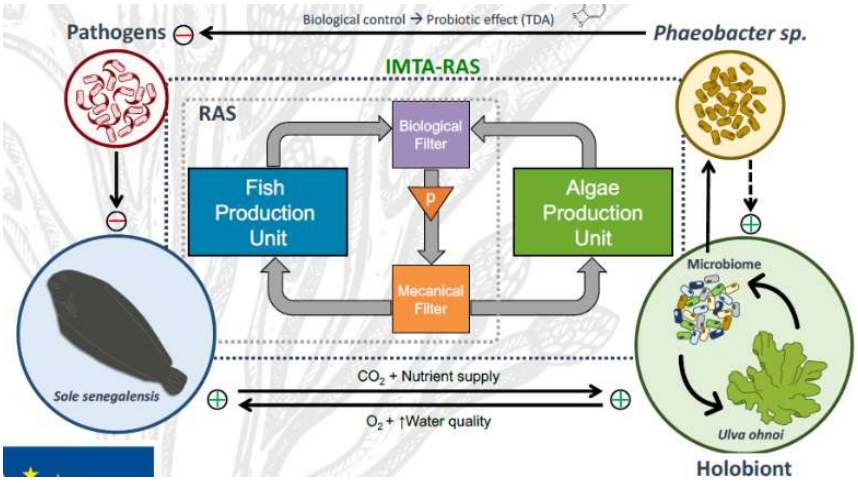


Figure 16 Developing a protocol for the effective aplication of Phaeobacter in Ulva cultures for pathogen control in IMTA-RAS

HOW DOES LIGHT AFFECTS ANTIBACTERIAL ACTIVITY OF PHAEOBACTER IN THE *ULVA* OHNOI HOLOBIONT?

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Studying seaweeds as holobionts has proven to be fundamental to understand their physiology. Among seaweeds, *Ulva sp.* is an economical relevant genus with severalStudying seaweeds as holobionts has proven to be fundamental to understand their physiology. Among seaweeds, *Ulva sp.* is an economical relevant genus with several applications in the industry, including aquaculture. Thanks to *Ulva sp.* high growth rate and physiological plasticity, *Ulva sp.* has a high potential in aquaculture, in which it can be integrated in closed multitrophic cultivation systems, playing a role as a biofilter, and promoting a favorable pH and O2 concentration in the seawater. Furthermore, *Ulva sp.* provides an important niche for biofilm-forming bacteria, including species belonging to the genus Phaeobacter, producing antibiotic compounds such as TDA (Tropodithietic acid) with antagonistic activity towards fish pathogens such as Vibrio anguillarum. It is thus, interesting from an economic point of view to understand the environmental conditions that promote the growth of Phaeobacter on *Ulva sp.* surface, and its concomitant production of TDA.

In this context, algal-bacterial interactions within the seaweed holobiont may be affected by environmental parameters (e.g. light intensity) and by changes in the physico-chemical parameters at the seaweed diffusive boundary layer (DBL) caused by the algal metabolism (pH, O2 concentration and ROS). This work intends to address the effect of light on the production and activity of TDA by P. galliciensis on Ulva's biofilm. In this study we investigate the effect of light and darkness on the inhibitory effect of P. galliciensis growing on living and dead U. ohnoi surfaces against V. anguillarum. According to our results, when growing on inert surfaces (dead U. ohnoi), the inhibitory effect of P. galliciensis on V. anguillarum is not affected by light. However, light affected the maintenance of Phaeobacter in growing U. ohnoi under light conditions and its influence on concentration and inhibitory capacity of TDA produced by P. galliciensis is currently being studied.

FEASIBILITY OF PRODUCTION OF *ULVA SP.* IN RACEWAY REACTORS, AN APPROACH TO SUSTAINABILITY IN-LAND AQUACULTURE

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The *Ulva* species are postulated as one of the main macroalgae for aquaculture production due to high acclimation to a broad range of enviromental conditions as solar radiation, salinity, pH and temperature. *Ulva* presents high growth rate based in the high inorganic nitrogen consumption. *Ulva* biomass has great interest for food, feed and cosmeceutic industries among others. Our goal is to demonstrate efficient growth of *Ulva* in raceway reactors, normally used in microalgal aquaculture , for the production of biomass to be used in the above mentioned sectors. Growth rate and photosynthetic capacity by using in vivo chlorophyll a fluorescenece of photosystem II were monitored during several weeks in late Spring-beginning of Summer in Malaga, Southern Iberian Peninsula. The culture was conducted without injection of CO2 . The source of inorganic nitrogen was NH4NO3 (200 µM) and phosphate was of KH2PO4 (24µM) applied 3 times per week. The water temperature during the experimental time oscillated between 20-35 oC. Effective quantum yield range form 0.6 to 0.1 and electron transport rate (ETR) as estimator of photosynthetic capacity remained high during the experimental period with slight decrease as noon as consequence of dynamic photoinhibition.The stock conditions in tanks ranged from 50 to 80 g L-1 under high radiation at densities of 10 g L-1 in raceway (3000L and 10cm depth). The results obtained during 1 month ofcultivation under natural conditions, with chemical fertilizers and without the additionof CO2 , showed promise for obtaining high algal biomass. The growth rate was 27-33%per week of algae, with daily pH variations between 8-11 demonstrating that itsphotosynthesis is not inhibited by changes in pH (due to the change of carbon forms)and reducinge the risk of the microalgae growth in the *Ulva* ponds.

EARTHEN POND TO FORK: THE JOURNEY OF THE SEA LETTUCE FROM THE BAY OF CÁDIZ TO THE CONSUMER´S PLATE

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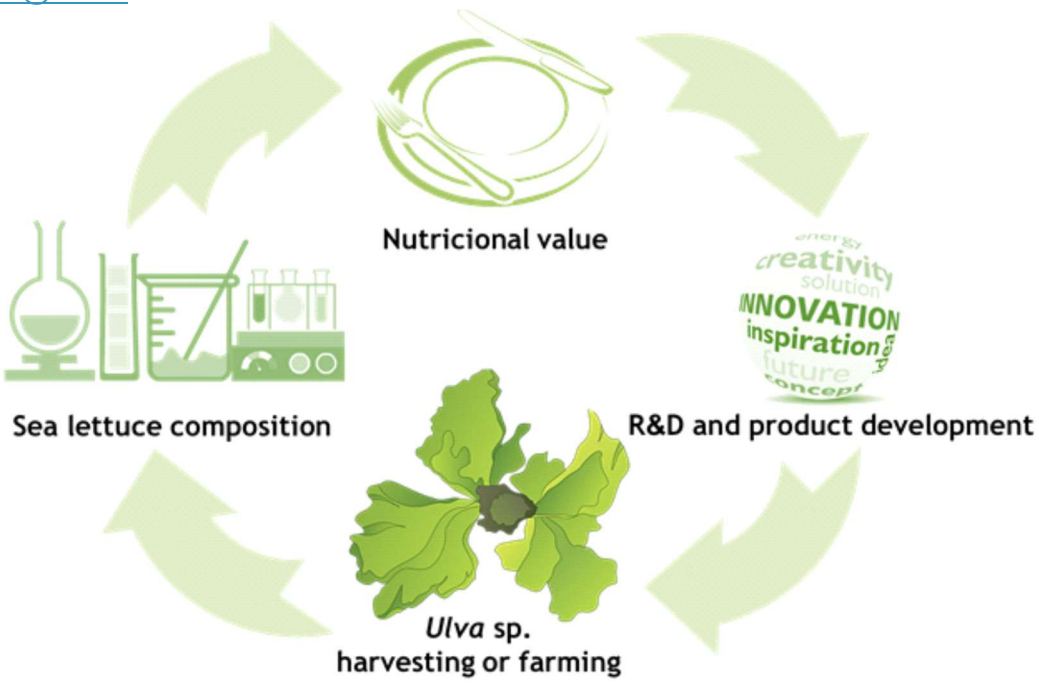


Figure 17 Earthen pond to fork: the journey of the sea lettuce from the bay of Cádiz to the consumer´s plate

Marine algae offer great nutritional richness and are considered a key product in the search for new nutritional sources for human consumption. In addition, the growing demand for healthy, natural and sustainable foods has promoted culinary interest in marine algae in recent years, and even a new trend known as "phycogastronomy" has recently emerged. In Spain, however, despite its rich coastline, seaweed is not a regular part of our diet and, therefore, not of our gastronomy. However, in recent years, interest in these marine products for direct consumption or as an ingredient in the development of new products has increased. In Andalusia, and specifically in the Bay of Cádiz, macroalgae such as *Ulva sp.* are being developed in places such as earthen ponds, areas belonging to former salt mines and, therefore, with particular environmental characteristics conditioned by the salt production system.

This work proposes the harvesting and possible cultivation of sea lettuce (*Ulva sp.*) for human consumption, and for this purpose, the nutritional properties of this species in the area throughout the year have been evaluated. In addition, the possible applications of this seaweed in the formulation and development of new products have been developed.

The results show how *Ulva* from earthen ponds is a rich source of proteins, lipids, fatty acids, amino acids and minerals, allowing its use both for direct consumption and for the manufacturing of products based on algae. These products (seaweed mousse, sherry vinegar dressing flavored with seaweed, snacks, pesto, savory biscuits and vegan hamburgers) have been widely accepted by different groups of consumers, proving good marketing possibilities. So, *Ulva sp.* from earthen ponds is a viable raw material for human consumption, both for direct use and in the development of new nutritious and healthy products based on algae. Besides, the harvesting of this species and also on its possible cultivation would serve as a basis for promoting the development of a sustainable industry in the Bay of Cádiz.

THE APPLICATION OF ULVAN FOR THE PROTEOLYTIC STABILIZATION OF NISIN

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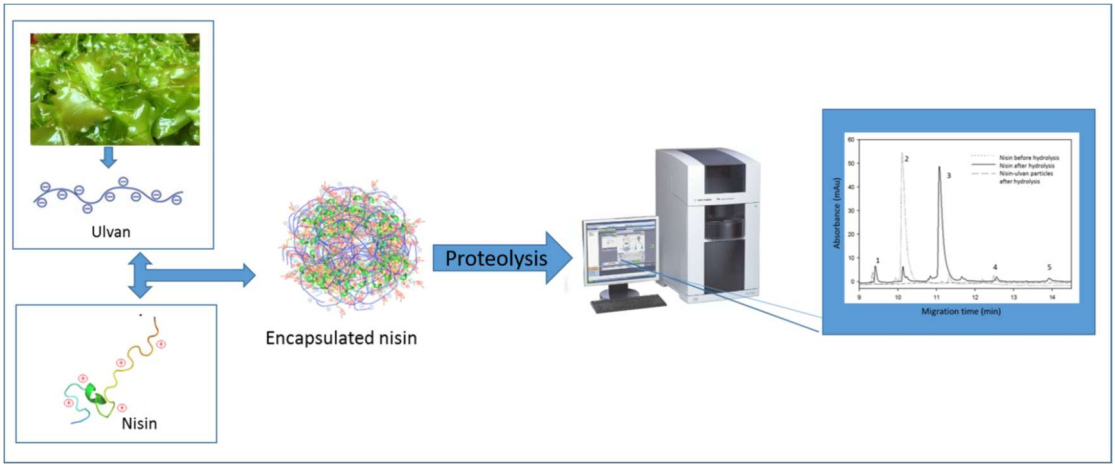


Figure 18 The application of ulvan for the proteolytic stabilization of nisin

Ulvan is a water-soluble sulfated polysaccharide. The carbohydrate composition of ulvan is complex and variable and depends on the particular green algal species, growth conditions, as well as extraction and purification methods. Ulvan is a cell wall polysaccharide with a backbone composed of α- and β-(1,4)-linked monosaccharides, namely rhamnose, xylose, glucuronic acid and iduronic acid. The sulfation sites are localized mainly on rhamnose.

Nisin is a small cationic peptide composed of 34 amino acid residues and produced by Lactococcus lactis subsp. lactis. Nisin is generally recognized as safe (GRAS) and approved by the US Food and Drug Administration for food applications. It is also recog- nized as a food additive in the EU and assigned the number E234. Nisin is highly active against Gram-positive bacteria, including Listeria monocytogenes and Clostridium botulinum. However, nisin being a peptide can easily lose its activity due to proteolytic degradation. If pure nisin is directly added to food, its long-term stability is debatable. To overcome the drawback, various encapsulation technologies were developed.

The aim of this study was to prepare nisin-loaded ulvan particles using the complexation method and compare the proteolytic stability of encapsulated nisin with free nisin.

The particles were prepared in the pH range of 4.0-7.0 and at the final ulvan and nisin concentrations of 0.4 and 0.2-0.5 mg/mL, respectively. For the determination of proteolytic stability, two types of proteases with different pH optimum and cleavage specificity were used, i.e. the protease Type XIII from Aspergillus saitoi and trypsin from porcine pancreas. For the first and second protease, the reaction was carried out at pH 5.0 and 7.0, respectively, and 37 °C for 24 h. The products of proteolytic degradation were analysed by the capillary zone electrophoresis method. The profile of the electropherogram of encapsulated and free nisin is the same. However, the area of peaks is different compared with free nisin due to the suppression of proteolytic degradation. The experiments show that ulvan could be successfully used for the stabilization of antimicrobial peptide nisin.

Acknowledgement: The authors thank prof. Vassilios Roussis from National and Kapodistrian University of Athens for providing us ulvan from green alga *Ulva rigida*.

A RAPID METHODOLOGY FOR THE SELECTION OF *ULVA* ELITE STRAINS TAILORED TO SPECIFIC GROWTH CONDITIONS

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Sea lettuce (*Ulva sp.*) is recognised for its potential in food, pharmaceutical, nutraceutical, biorefinery and bioremediation industries and is increasingly being cultivated in aquaculture. The demands of industrial applications vary widely in terms of biomass composition and cultivation requirements. To screen a large diversity of wildisolates, we tested the suitability of garden experiments, i.e. co-cultivation of many strains under specific growth conditions. We have characterised species composition over time among foliose strains cultivated in seawater and brackish waters, the latter being suited for bioremediation of land-based wastewaters. Our findings reflect the competitive advantage of strains displaying fastest growth in both environments. Interestingly, growth rates after a month were very similar, suggesting that selected strains cope equally well in either media. Further, we found significant variation in the composition of biomass produced in both conditions, in particular protein and carbohydrate content. We have now applied this protocol to the identification of filamentous *Ulva* strains with improved tolerance to long-term low salinity and are establishing similar trials for selecting locally-sourced strains adapted to aquaculture and industrial waste-water treatment. The established bulk-selection protocol provides a distinct advantage in efficiently screening large numbers of strains for their suitability to a target application.

QUANTIFICATION OF THE MORPHOGEN THALLUSIN IN MARINE BACTERIA INDUCING GROWTH AND MORPHOGENESIS OF THE GREEN MACROALGA *ULVA* (CHLOROPHYTA): APPLICATION IN AQUACULTURE MONITORING

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Growth and development of the sea lettuce *Ulva sp.* (Chlorophyta) depend on growth- and morphogenesis-promoting factors released by associated bacteria. Hereby, the morphogen thallusin induces the rhizoid and cell wall formation in *Ulva* at a very low concentration of 11 pmol L-1. It is mainly produced by various species of Maribacter spp. and Zobellia spp. (Bacteriodites). This study aims to quantify thallusin in bacterial and algal cultures and determine ecologically relevant concentrations.

The high biological activity of thallusin requires an ultra-trace analysis, considering that thallusin also complexes with iron and makes chromatographic separation difficult. We thus developed a mass spectrometric method for quantifying thallusin upon solid phase extraction (C18) of sterilefiltered growth media.

The concentration of thallusin was measured using ultra-high-performance liquid chromatography (UHPLC) coupled with electrospray ionization high-resolution orbitrap mass spectrometry (ESI-HRMS). Before the analysis, a derivatization step of the carboxylic acid groups was crucial to avoid ironcomplexing, which improved peak shape and calibration. Using this approach, the limit of quantification for 1 L of culture was 22.2 pmol L-1, and the detection limit was 7.6 pmol L-1.

The bacterial thallusin concentration was measured at selected growth phases with values of 0.16 ± 0.01 amol cell-1 (turning point of the exponential growth), of 0.27 ± 0.05 amol cell-1 (end of exponential growth) and of 0.86 ± 0.13 amol cell-1 (late stationary phase) for Maribacter sp. The concentration of 22 ± 4 pmol L-1 determined in an *Ulva* aquaculture (20 g L-1 fresh weight), grown under laboratory conditions, was sufficient to induce the morphogenesis. We only need 1 L of *Ulva* aquaculture supernatant to quantify thallusin using our newly developed method. This method will thus be an important tool for monitoring thallusin concentrations in aquaculture and natural habitats, and it may contribute to the maintenance of aquaculture.



ULVA: Tomorrows' Seawheat and the green wave of innovation

Bremerhaven, Germany

September 2-4, 2025

Ulva

The Future Wheat of the Sea

SEAWHEAT COST ACTION CA20106 SEAWHEAT BEYOND 2025: BUILDING A SUSTAINABLE *ULVA* FUTURE

Shpigel, Muki [1,2,3]

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The COST Action "SeaWheat" (CA20106, 2021–2025) aimed to promote a pan-European, interdisciplinary approach to the sustainable cultivation and application of *Ulva* spp. (sea lettuce). *Ulva* is a promising green macroalga with potential uses in aquaculture, food systems, bioactive products, and environmental services.

SeaWheat addressed this challenge by establishing a strong, coordinated network of scientists, industry stakeholders, and policymakers. The final conference, held at the Alfred Wegener Institute in Bremerhaven showcased 4 years of work and collaboration by our members.

SeaWheat brought together 448 participants from 53 countries, including 17 Inclusiveness Target Countries and 17 Small and Medium-sized Enterprises (SMEs). Structured around seven Working Groups, spanning biology and aquaculture to food, bioactive compounds, ecosystem services and regulatory frameworks, SeaWheat delivered significant advances along the *Ulva* value chain.

The initiative supported 24 Short-Term Scientific Missions (STSMs), five training schools, and four workshops, promoting hands-on collaboration and training for early-career researchers. Its scientific output included over 30 peer-reviewed publications and four major review articles. In addition, a database of more than 200 *Ulva*-related publications authored by network members was compiled.

SeaWheat COST Action initiated the launch of E*ULVA*, a collaborative initiative focused on the taxonomy and standardization of *Ulva* cultivation.

Public outreach was a key strength of the Action. SeaWheat produced three animated films (in 14 languages), a multilingual *Ulva* recipe book, and 16 widely attended "Lunch with *Ulva*" webinars. These initiatives helped raise awareness among the public and policymakers about the value and potential of *Ulva*.

A notable achievement of SeaWheat was the empowerment of early-career researchers and the promotion of inclusive participation across Europe. The results of the Action have been integrated into academic programs, industrial R&D, and policy discussions, setting the stage for a thriving *Ulva*-based bioeconomy.

By effectively uniting research, innovation, and outreach, SeaWheat advanced the science and applications of *Ulva*. Its legacy will continue through ongoing collaborations, the E*ULVA* initiative, and the vibrant community it has fostered.

There is no doubt that the success of the SeaWheat COST Action is due to the dedicated collaboration of the Working Group leaders and Core Group members, who voluntarily led and organized all our activities over the past four years.

CONSIDERATION OF THE NUTRITIONAL AND TOXICOLOGICAL ASPECTS OF *ULVA* SPP. FOR ALIMENTARY AND ZOOTECHNICAL APPLICATIONS

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The genus *Ulva* (Ulvophyceae, Chlorophyta), also known as sea lettuce, is the most common, widely distributed green alga [1]. These algae can adapt to a variety of habitats and environmental factors (e.g. light, nutrients, temperatures, etc.) and reproduce in various ways, including parthenocarpy and asexual reproduction via biflagellate zoospores [2]. There are many recognised commercial applications of *Ulva* spp., including use as a fertiliser, nutraceutical, biomaterial, biofilter, human food (e.g. "aonori") and animal feed (e.g. abalone, shrimp and sea urchin). This makes them a valuable seaweed. [3, 4]. Researchers have extensively analysed the biochemical composition of *Ulva* spp., showing that it is rich in proteins, dietary fibres, minerals, vitamins and bioactive secondary metabolites. This indicates that it is suitable for human consumption and has applications in the feed industry [1]. In addition, sea lettuce is regarded as a potent attractant and feeding stimulant, capable of augmenting the feeding intake and promoting the conversion rate of marine herbivores [5, 6].

Although researchers are familiar with the nutritional potential of *Ulva* spp., they have not thoroughly investigated its toxicological aspects or potential risks to human and animal health.

Depending on the environmental conditions, chemical and microbiological contamination can occur on wild and cultivated seaweed.

It is known the capacity of seaweeds to uptake and concentrate environmental pollutants, such as those belonging to the family of persistent organic pollutants (POPs), heavy metals and metalloids [7]. Several studies have been carried out on the bacterial diversity in brown (Phaeophyceae), green (Chlorophyta), and red (Rhodophyta) macroalgae (henceforward: seaweed). Seaweed is home to a variety of bacteria, including Proteobacteria, Actinobacteria, Bacteroidetes (CFB group), Cyanobacteria, Firmicutes, Planctomycetes, Verrumicrobia, Chloroflexi, Deinococcus-Thermus, Fusobacteria, and Tenericutes. Among these, Gammaproteobacteria are the most prevalent bacterial clade [8]. However, only a few studies have clarified the prevalence of human pathogens in edible seaweed, and further research is needed [9].

This in-depth study aims to show the two sides of the coin so as not to create false myths and unwarranted alarmism.

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Acknowledgments

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Short Biography of the speaker

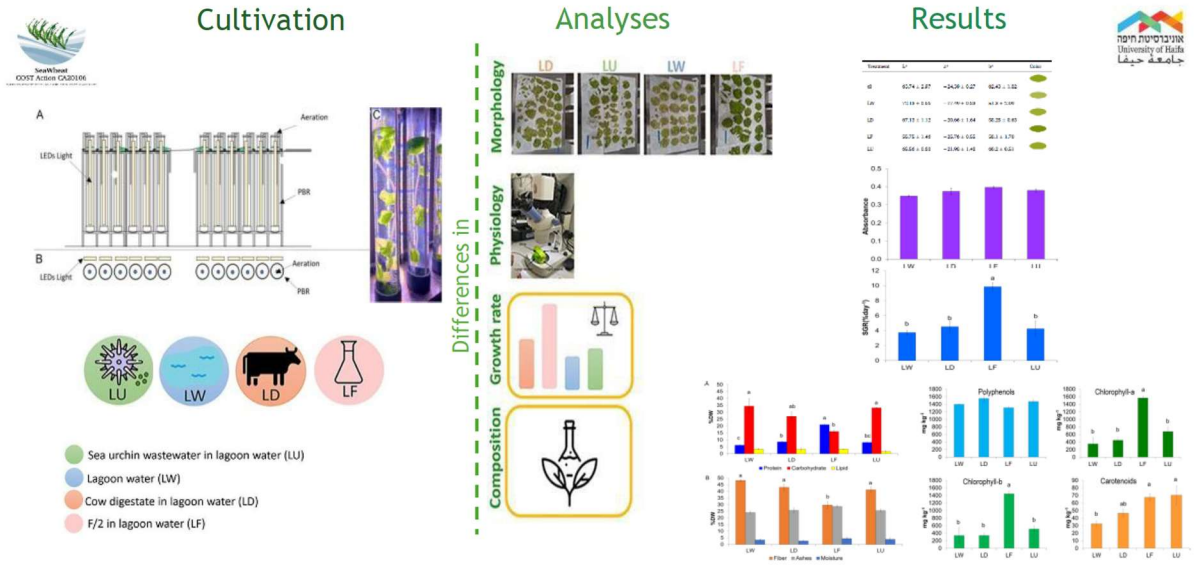
Prof. Alberto Angioni is a full professor of food chemistry at the University of Cagliari, teaching food chemistry, technology, and analysis. He is responsible for the Laboratory of Chemical Analysis of Food, which is included in the list of regional laboratories. President of the Italian Group of Plant Protection Products and the Environment (GRIFA), Past-president of the Italian Group of Food Chemistry (ItaChemFood), member of the National Committee for Food Safety (CNSA) of the Ministry of Health, and reference person for the University of Cagliari for the evaluation of Dossiers on plant protection products for the Ministry of Health. Prof. Angioni is a member of the Technical Scientific Committee of the "Phytopathological Days" and of the Technical Scientific Committee of the Montiferru Olive Oil Award.

EFFECTS OF DIFFERENT CULTURE MEDIA ON GROWTH, COMPOSITION AND QUALITY OF THE GREEN ALGAE ULVA SP. CULTIVATED IN CYLINDRICAL PHOTOBIOREACTORS

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Ulva spp. have been identified as an effective and sustainable biomass source. They are macroalgae with high growth capacity and high nutritional content, which have gained considerable importance in the food and feed sector, as well as in ecosystem services and green technologies [1, 2].

The growth rate, morphology, physiology and biochemical characteristics of *Ulva* sp. produced by an indoor vertical cylindrical photobioreactor (PBR) system were evaluated using four different culture media such as lagoon water, lagoon water enriched with f/2 synthetic media, sea urchin wastewater and bovine digestate [3].

It was found that the media influenced all the parameters examined, the lagoon water enriched with f/2 synthetic media biomass weight was double compared to the other treatments and showed a slightly higher absorbance. Colorimetric analyses reported a significant darker color in *Ulva* sp. grown under enriched media. *Ulva* sp. showed higher nutrient removal potential in lagoon water enriched with f/2 synthetic media. The lipid content did not vary (2–3 % dry weight, DW), while the protein content ranged from 21 % in f/2 synthetic media to 6–9 % in the other treatments. Carbohydrates and fiber content were significantly lower in f/2 synthetic media (16 % and 30 %) compared to the other treatments, 27–34 %, and 41–48 %, respectively. Pigment content significantly varied, being higher in biomass grown in lagoon water enriched with f/2 synthetic media and sea urchin wastewater. This study shows how different nutrient sources affect the biochemical composition, growth and quality of *Ulva* sp. The PBR system tested here proved to be efficient in ensuring significant biomass growth in a short time frame, ensuring controlled growing conditions, gas and nutrient exchange and adequate amounts of light. Moreover, it was highlighted how the different media used determines important variations regarding growth rate, morphology and biochemical composition of the biomass.

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Acknowledgments

We acknowledge the support of the COST Action CA20106 "Tomorrow's wheat of the sea': *Ulva*, a model for an innovative mariculture", supported by COST (European Cooperation in Science and Technology, www.cost.eu).

Short Biography of the speaker

Nicola Arru is a PhD student at the Department of Life, Environmental and Pharmaceutical Sciences of the University of Cagliari. His doctoral project "BIO-Bank: catalog of behavioural, biochemical and physiological responses of marine organisms conditioned in mesocosms", has as its objective the evaluation of the chemical and biochemical characteristics of lagoonal and marine model species of the Sardinian coast.

The study of these species will also be able to provide useful information regarding the diffusion of pollutants in the marine environment and in transition waters and, furthermore, be able to identify the sources of pollution. He obtained a master's degree in Chemical Sciences at the University of Cagliari. His studies concern food chemistry and nutrition.

ULVA, AN EMERGING GREEN SEAWEED MODEL FOR MOLECULAR BIOLOGY

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Green seaweeds exhibit a wide range of morphologies and occupy various ecological niches, spanning from freshwater to marine and terrestrial habitats. These organisms, which predominantly belong to the class Ulvophyceae, showcase a remarkable instance of parallel evolution toward complex multicellularity and macroscopic thalli in the Viridiplantae lineage. Within the green seaweeds, several *Ulva* species ("sea lettuce") are model organisms for studying carbon assimilation, interactions with bacteria, life cycle progression, and morphogenesis. *Ulva* species are also notorious for their fast growth and capacity to dominate nutrient-rich, anthropogenically disturbed coastal ecosystems during "green tide" blooms. From an economic perspective, *Ulva* has garnered increasing attention as a promising feedstock for the production of food, feed, and biobased products, also as a means of removing excess nutrients from the environment.

We propose that *Ulva* is poised to further develop as a model in green seaweed research. In this talk, I will focus on *Ulva mutabilis/compressa* as a model species and highlight the molecular data and tools that are currently available or in development. I will focus on our efforts in improving *Ulva* genomic data. The use of our molecular tools will be demonstrated with a case study on the carbon concentrating mechanisms of *Ulva*. Here, we combined large-scale gene-tagging with targeted characterisation of proteins in vivo, in vitro and at the structural level. Our spatial model for inorganic carbon capture in *Ulva* reveals unique Rubisco condensation mechanisms in the pyrenoid.

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Short Biography of the speaker

Jonas Blomme is a molecular biologist. My PhD focused on understanding land plant growth and development using mutant studies and systems-biology approaches. Then, from my postdoc onwards, I have focused on -omics research and molecular tool development in the green seaweed *Ulva* (vectors available at https://vectorvault.vib.be/*Ulva*-mutabilis). I generated the first stable transgenic seaweed lines with tagged transgenes and one of my core activities is to further improve these molecular tools to allow a better understanding of seaweed developmental biology.

For example, in an ongoing FWO ERC starting grant runner up project, I am determined to enable CRISPR screens in *Ulva*. My ambition is to develop and use system-biology approaches to understand *Ulva* growth and development. In an FWO-funded research project we use automated phenotyping, generation of mapping populations and ecological genomics to understand the growth potential of *Ulva*. Doing research at the intersection of (population) ecology, genomics and molecular biology in an ecological and economical relevant organism holds promise to further translate academic results into industrial applications. In total, I have (co-)authored 23 publications (h-index 13; 706 citations), eight during my PhD and fifteen during my postdoc. Since September 2024 I have obtained an associate professorship position at Ghent University.

LAB SCALE FEASIBILITY STUDY INVESTIGATING THE POTENTIAL USE OF THE SHELLFISH TOWER DESIGN FOR OPEN OCEAN CULTIVATION OF ULVA COMPRESSA IN DIFFERENT SALINITY ENVIRONMENTS

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Abstract

Low trophic aquatic food provides the potential to reduce food and nutrition insecurity and tackle malnutrition, while simultaneously posing little stress on the climate and environment, thereby delivering essential ecosystem services and enabling the achievement of the UN SDGs. The Shellfish Tower is an Innovative system design for open ocean aquaculture of shellfish, a low trophic aquatic food. In the current study, we examined the feasibility of using the Shellfish Tower for cultivation of *Ulva compressa*. *Ulva compressa* is widespread in the Baltic and North Seas and can exhibit two different morphologies: a tubular form, most common in the Baltic, and a foliose form, most common in the North Sea. Therefore, we tested the hypotheses that the Shellfish Tower could be used to cultivate *Ulva compressa* in different salinity environments, and that the morphology, physiology and biochemical properties of *U. compressa* would differ in the Baltic and North Sea salinity scenarios. Nets were seeded with *U. compressa* gametes after fertility induction and incubated for one month at 15°C, 30 PSU and 100 µmol photons m-2 s-1 light intensity. Once the *U. compressa* reached 2-3 cm in size, the nets were wrapped around the outer frame of 20 miniature Shellfish Towers, which were constructed based on the original frame design. The frames with seeded *U. compressa* were distributed to four different salinities: 10, 15, 30 and 35 PSU. Growth rates, photosynthetic efficiency and cell size were monitored weekly for 5 weeks. Biochemical properties (protein, carbohydrate, fiber, ash and chlorophyll and carotenoid content) were measured at the end of the experiment. *Ulva compressa* successfully grew on the miniature Shellfish Towers at a lab scale (Figure 21). Significant differences in growth rates and photosynthetic efficiency were observed between the low and high salinities (Figure 20). Differences in cell size and biochemical properties will be discussed, but we did not observe the foliose morphology of *U. compressa* in any salinity treatment. This study shows that the Shellfish Tower can be used to cultivate *U. compressa* in different salinity environments, but the success in an open ocean environment must still be evaluated, as well as the impact of the vertical distribution of light and its impact on seaweed growth.

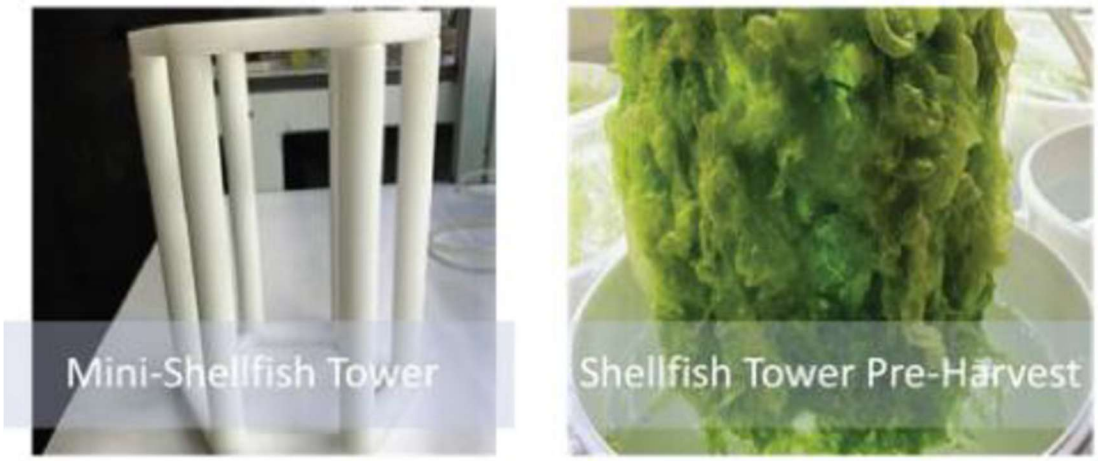


Figure 19 Miniature Shellfish Tower before seeding and directly prior to harvest

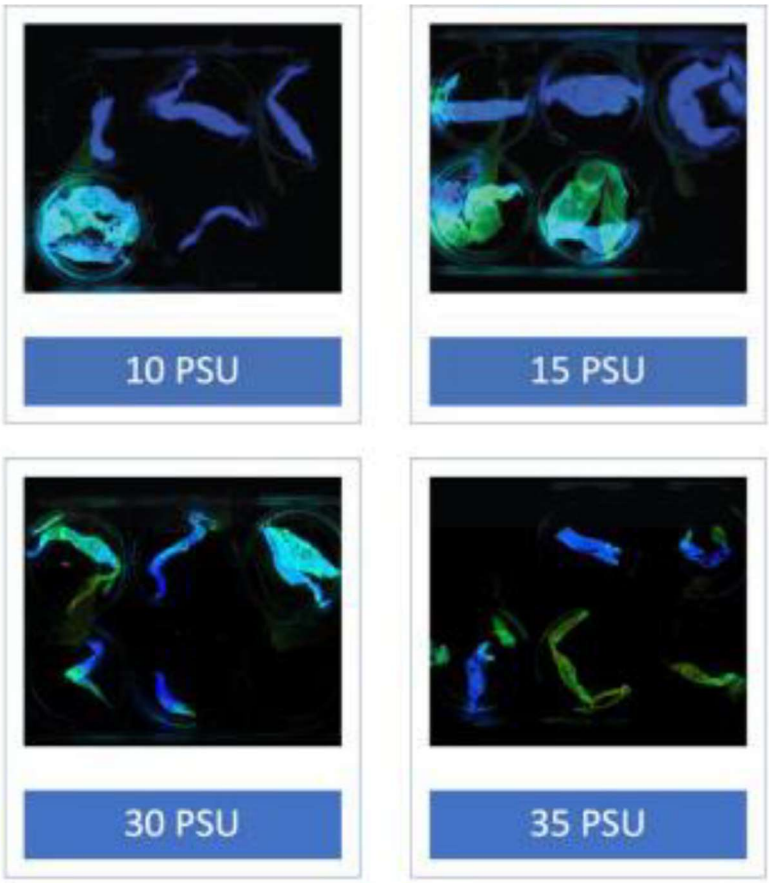


Figure 20 Visualization of maximum photosynthetic efficiency (Fv/Fm) of U. compressan exposed to different salinities

Short Biography of the speaker

Clarissa Böhme is a Master's student in the Biotechnology program at Bremerhaven University of Applied Sciences. Her research focuses on microbiology and aquatic biotechnology, with a focus on sustainable aquaculture and the cultivation of macroalgae. She presented the results of her bachelor's thesis at the international AQUA 2024 conference in Copenhagen. She is currently deepening her knowledge of marine biotechnology and is aiming for a scientific career in the field of sustainable aquatic systems.

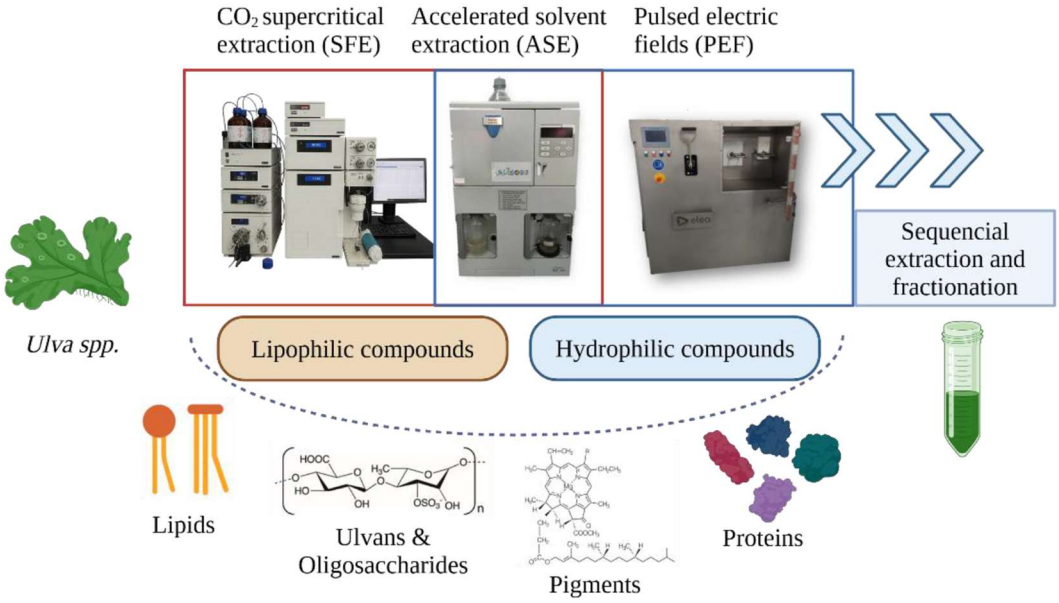
NOVEL EXTRACTION OF BIOACTIVES FROM ALGAE AND PLANTS: A SEQUENTIAL APPROACH WITH PEF, ASE AND SFE

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Sustainable and innovative extraction technologies such as pulsed electric fields (PEF), accelerated solvent extraction (ASE), and supercritical fluid extraction (SFE) have significantly advanced the recovery and preservation of bioactive compounds from complex food matrices like *Ulva spp*. Nevertheless, sequential extraction approaches offer a powerful strategy for fractionating and concentrating target compounds based on their chemical affinities, thereby maximizing the valorization potential of marine biomass. In this study, sequential extractions combining SFE, ASE, and PEF were applied to obtain both hydrophilic and lipophilic fractions from *Ulva* spp., followed by comprehensive chemical characterization. Carbohydrates were analyzed by GC-FID and colorimetry, proteins via elemental nitrogen analysis, fatty acids by GC-MS, and total pigments (chlorophylls and carotenoids) were quantified spectrophotometrically. Results revealed that aqueous ASE extracts were particularly enriched in sugars (25–51%) and proteins (~10%), with a notable presence of uronic acids and rhamnose associated with *Ulva*ns. In contrast, ethanolic extracts from ASE and SFE showed low sugar content but were rich in fatty acids. PEF-treated samples exhibited the highest antioxidant capacity and pigment concentration compared to ASE. Overall, the integration of these three technologies in a sequential manner enhanced both the yield and selectivity of bioactive compound recovery, offering a viable and scalable approach for the sustainable exploitation of *Ulva* spp. and similar marine-derived matrices.

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Short Biography of the speaker

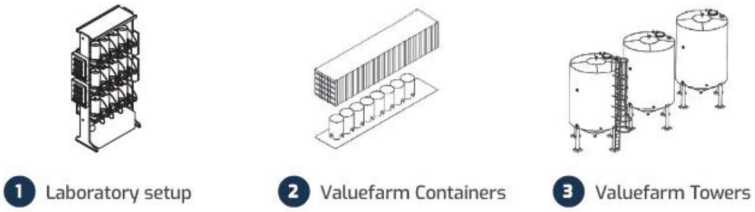
Mara Calleja is a PhD candidate in Food Science at the University of Valencia, affiliated with the Department of Preventive Medicine and Food Science. As a member of the research group Innovative Technologies for Sustainable Food (ALISOST), her work focuses on promoting sustainable food production through the application of advanced preservation strategies and the valorization of agri-food by-products. In this context, her research integrates a range of emerging technologies such as pulsed electric fields (PEF), supercritical fluid extraction (SFE), accelerated solvent extraction (ASE), and ultrasound (USN), with the aim of improving the recovery bioactive compounds while maintaining the nutritional and sensory integrity. Additionally, her work explores the *in vitro* therapeutic potential of the recovered compounds and the evaluation of contaminants and associated risks in both food and soil matrices.

SCALING UP EMISSION CAPTURE AND UTILIZATION WITH *ULVA*: A THREE-PHASE APPROACH

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The potential for macroalgae to play a transformative role in carbon and nutrient capture is significant, yet largely untapped. *Ulva* species, in particular, offer fast growth, high biomass yields, and valuable compounds for food, feed, and biotech applications. This presentation outlines Pure Algae's three-phase approach to industrializing *Ulva* cultivation with a focus on emissions capture and circular resource use [1].

Phase 1 begins with laboratory-verified productivity of *Ulva* in controlled environments, where precision cultivation enables reliable data on growth performance, nutrient uptake, and strain selection. With the use of Pure Algae's Laboratory Setup, early-stage feasibility studies help determine optimal species and conditions, supporting data-driven decisions for larger-scale installations.

Phase 2 focuses on medium-scale production in VALUEFARM Container systems. These plug-and-play cultivation units are CE-certified and deliver stable yields of 5–13 kg fresh weight per PBR per week. Integrated with recirculating aquaculture systems (RAS), they utilize CO_2 and dissolved nutrients from fish production, creating a low-impact IMRAS (Integrated Multitrophic Recirculating Aquaculture System) model that converts emissions into high-value biomass.

Phase 3 targets full industrial scalability through the VALUEFARM Tower platform. Each modular tower holds 5,000 to 10,000 liters and is optimized for automation, nutrient cycling, and real-time monitoring. These units enable emission-intensive industries to integrate carbon and nitrogen utilization directly into their infrastructure – reducing emissions while generating new bio-based value streams.

Results from ongoing trials demonstrate that *Ulva* cultivation in these systems can outperform state-of-the-art land-based models, with productivity reaching up to 15.6 kg fresh weight/m³/week at 10% dry weight. The systems are designed to scale modularly, adapt to site-specific parameters, and produce consistent outputs for both local and export markets.

This phased approach allows for rapid deployment, learning, and scale-up, facilitating climate-positive growth in aquaculture, biotech, and land-based emissions-heavy sectors. It combines science, engineering, and entrepreneurship to turn a global emissions challenge into a green economic opportunity.

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Short Biography of the speaker

Esben R. Christiansen is the founder and CEO of Pure Algae, a Danish company pioneering land-based seaweed cultivation. With a background in biotechnology and bioremediation, he has developed scalable systems that convert emissions from aquaculture and industry into high-value biomass. Esben has played a central role in advancing EU-supported innovations in aquaculture, turning Pure Algae into a trusted provider of emission-utilizing cultivation solutions

SOME LESSONS LEARNED FROM ONLAND CULTIVATION OF SELECTED SEAWEEDS INCLUDING MANAGEMENT TECHNIQUES

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The history of cultivation of a few, select seaweeds, at scale, is but a few centuries old. More recently, advanced and applied techniques have evolved to reach an equivalency to terrestrial farming that has been called "phyconomy". Some open water farmed seaweeds cannot be cultivated without at least a seeding/seedling stage on land, in a nursery (e.g., laver, kelps). The commercial volumes and economic values of seaweeds produced are regularly documented by the Food and Agriculture Organisation of the United Nations, the data from which were queried (see Buschmann et al. for an early warning call) and were also re-evaluated recently by the World Bank as investment opportunities. It is of great concern that the production of some cultivated seaweeds is falling, particularly in some surprising traditional, key areas. A number of issues arising will be presented.

An even smaller group of selected, and bespoke seaweeds (i.e., strains and variants) are currently farmed entirely on land for (hopefully) high-value applications and this has been coined as "precision phyconomy". These practices can be traced from using earthen ponds with co-cultivation of seaweeds and fish (e.g., agarophytes and milk fish), but generally the practice of on land (unialgal) seaweed cultivation for high-value production of biomass, is only a few decades old.

Sadly, all is not well in the seaweed garden and there are pressing issues facing the future of economically successful phyconomic activies - either in open water, or on land. Not least the very small number of currently (reliably) cultivatable seaweeds and their cultivars, plus increasing pest and diseases. Some of these issues are made worse by rapid climate change, especially is shallow waters. These are red flag warnings as to a clear and present danger to the future of the industry. The status quo is no longer an option. Resiliency of applied techniques and types of farmed seaweeds have to be established by urgent current research. There is little room for delay. Management of seaweeds in cultivation is a combination of science and evolving art and practice. Keep on keeping on, growing the "same old, same old" and responses of: "we have always done it that way" will no longer "work". This presentation will highlight some selected issues which it is hoped will evoke discussion and urgently needed directions for research.

Short Biography of the speaker

Dr Alan T. Critchley (https://orcid.org/0000-0003-1704-458X) is an adaptive phyconomist with 20 years' experience as an academic in southern Africa focused on seaweeds. He moved to sustainable and industrial-scale applications of commercial seaweeds in early 2001. He has helped co-edit two books on Tropical Phyconomy and currently two volumes on Temperate Phyconomy are in preparation for Springer.

He is Research Fellow at the Verschuren Centre, Canada and a consultant working on aspects of industrial-scale production of selected seaweeds, their biomass conversion and value-added applications.

MANIPULATING FISH GUT BACTERIA FOR IMPROVING *ULVA* NUTRITIONAL VALUE IN AQUAFEEDS

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The incorporation of *Ulva* sp., a nutrient-rich green macroalga, into aquafeeds offers a sustainable approach to reducing the aquaculture industry's dependence on fishmeal [1]. Still, the high presence of indigestible fibers, such as non-starch sulfated polysaccharides, limits *Ulva's* digestibility and nutritional value in aquafeeds for finfish species [2]. To address this challenge, the present study investigates the feasibility of manipulating the gut microbiota of gilthead sea bream (*Sparus aurata)* through probiotic supplementation as a strategy to enhance the utilization of *Ulva*-rich diets. For this aim, a preliminary feeding trial was performed over 12 weeks in which fish were fed commercial aquafeed with different inoculation rates of the probiotic bacterium of 1x108 , 1x101 0, and 1x101 2 CFU/Kg compared to a probiotic-free diet control. Fish growth rate, digestibility, immune response, and microbial community composition were measured throughout the culture period. Sequentially, trials to examine feed digestibility and fish resilience when challenged with pathogenic *Streptococcus agalactiae* were conducted using identical diets. Results indicated no effect of probiotic treatment on fish growth performances, but protein and lipid digestion were improved when the probiotic was supplemented at 1x101 0 CFU/Kg, proposing an improved absorption of these macronutrients. Such a level of the probiotic supplement also induced antiprotease activity and led to a more stable gut microbial community in terms of its diversity. Last but not least, the probiotic treatment improved fish survival after challenging them with *S. agalactiae*, with highest survival rate of 70% in the probiotic supplementation level of 1x101 2 CFU/Kg. Overall, the probiotic bacterium appears to be efficient in culture of *Sparus aurata* by improving fish resilience and enhancing its nutrition via improved protein and lipid uptake and digestibility.

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Short Biography of the speaker

Ajit Dake working as a Ph.D. student in Prof. Lior Guttman's lab at the IOLR- National Center for Mariculture in Eilat, Israel, with a keen interest in microbiology, molecular biology, and aquaculture. His current project focuses on the use of novel probiotics to enhance growth, health, and feed digestibility in gilthead seabream. His academic journey reflects a commitment to research excellence and innovation. He actively engages in both national and international conferences, contributing to the dissemination and exchange of knowledge within his field.

YEAR-ROUND CULTIVATION OF *ULVA* SP. ASSOCIATED WITH A RAS FISH FARM: BIOMASS PRODUCTIVITY AND ITS VALORISATION POTENTIAL

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Recirculating aquaculture systems (RAS) can reduce impacts on the environment, however the nutrient-rich water produced by RAS can still affect both local wildlife and the water quality which the industry depends on [1]. Integrating a seaweed cultivation close-by to these systems could be an effective approach to reduce nutrient levels and produce biomass for further use, supporting sustainability and the circular economy [2,4]. *Ulva* spp. are a fast-growing green macroalgae, rich in proteins, pigments, polysaccharides and other. These traits make *Ulva* species an ideal organism not only to bioremediate fish aquacultures but also as a source of food, feed, and bioproducts [3].

The work presented here demonstrates the feability of cultivating *Ulva* sp. in association to a land-based intensive aquaculture of *Solea senegalensis* operating in RAS (Aquacria Piscícolas S.A., from the Sea8 group), showing its biomass productivity, its biochemical characterization and its nutrient removal potential over a year-round experiment.

The cultivation conditions of *Ulva* sp. were optimized by testing different stock densities (1, 2, and 3 kg·m²) and water flows (LF – 45 L·h-1 and HF – 180 L·h-1) during a year-round. Physico-chemical parameters (salinity, temperature and pH) and growth performance (growth rate and productivity) were monitored weekly, whereas bioremediation performance (carbon and nitrogen) and biochemical characterization (pigments, proteins, ashes, polysaccharides, phenols and antioxidant capacity) was assessed monthly. The nutrient concentration of the RAS water (inlet water) was followed every 15 days.

In a nutshell, environmental conditions in the HF tanks were closer to those in the fish farm water, showing more stable temperatures and lower pH compared to the LF tanks. Salinity was not influenced by the cultivation conditions. Growth performance and nutrient removal increased steadily for all conditions, peaking in summer. The highest growth rate (25%d-1), productivity (4.5 kg FW m-2 wk-1) and carbon/nitrogen removal (210 g C/ 29 g N g DW m-2 wk-1) was achieved at 1kg m-2 and 180 L·h-1. A variation was observed, for both cultivation conditions over the year, for total pigments (4.8-11.1 mg /g DW), proteins (17.2-25.8%), ashes (12.9-25.8 %), polysaccharides (9.6-41.4 %), phenols (0.6-2.6 mg /g DW) and antioxidant capacity (14-86 mg TE/ g DW).

This presentation will explore the influence of the cultivation conditions and the seasonal effect on the growth, bioremediation performance and biochemical composition of the produced biomass, assessing its potential valorization.

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Short Biography of the speaker

Aires M. Duarte holds a bachelor's degree in Biotechnology from the University of Évora (2013) and a master's degree in Functional Biology and Biotechnology of Plants from the University of Porto (2017). He is currently a PhD candidate in Marine Biotechnology and Aquaculture at the Interdisciplinary Centre of Marine and Environmental Research (CIIMAR) and the Faculty of Sciences, University of Porto (FCUP). His research focuses on macroalgae cultivation, particularly *Ulva* species, and their integration into aquaculture systems to improve water quality, as well as the valorisation of biomass from a circular economy and biorefinery perspective.

BIODEGRADATION AND TRANSFORMATION OF BISPHENOLS IN *ULVA*

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Anthropogenic xenoestrogens represent a significant threat to both human health and the environment due to their endocrine-disrupting effects [1]. The green macroalga *Ulva* (Chlorophyta), known for its ability to thrive across a wide range of salinities and to form large green tides in polluted and eutrophic waters, shows strong potential for applications in wastewater treatment and bioremediation.

In this study, we examined the removal of various bisphenols and ethinylestradiol, focusing on the underlying mechanisms involved, using the tripartite community of *Ulva mutabilis* - *Roseovarius* sp. (MS2) - *Maribacter* sp. (MS6) [2]. The model organism *Ulva* demonstrated over 99% removal efficiency for bisphenols A, B, E, F, P, and Z, with partial removal observed for bisphenol S. Notably, complete removal occurred even under axenic conditions, indicating that associated bacteria *Roseovarius* sp. (MS2) and *Maribacter* sp. (MS6) played no significant role. A concentration of 6.6 mg L^{-1} of bisphenol A was entirely removed within two days, with a half-life of 1.85 hours [3].

Stable isotope labeling combined with high-resolution mass spectrometry revealed that bisphenol A underwent a range of transformation reactions including bromination, sulfation, iodination, and radical cleavage. In total, 20 transformation products were identified, with radical cleavage being the dominant removal mechanism. Bioaccumulation was minimal, suggesting that chemical transformation, rather than storage, drives pollutant removal [4].

The results demonstrate that *Ulva* is not only effective at removing xenoestrogens but also degrades them into less harmful byproducts. These findings highlight the potential of *Ulva* as a scalable and sustainable tool for bioremediation, particularly in wastewater treatment systems. Future applications could integrate *Ulva* into aquaculture or coastal treatment facilities, offering a nature-based solution to persistent organic pollutants.

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Short biography of the speaker

Maximilian Knips is a PhD candidate in chemistry at the Friedrich Schiller University of Jena in Germany. He holds a bachelor's degree in Chemistry and a master's degree in "Chemistry – Energy – Environment". He is investigating the ability of *Ulva* to biodegrade anthropogenic micropollutants and its application in wastewater treatment plants. His research is funded by the Deutschen Bundesstiftung Umwelt (German Federal Environmental Foundation).

DOES MORPHOLOGICAL INSTABILITY IN THE CROP *ULVA FENESTRATA* RESULTS FROM POLYPLOIDY?

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Ulva fenestrata is gaining attention as a promising future crop for aquaculture in the Northern Hemisphere, sparking interest in methods to improve its growth and maintain morphological consistency. One potential strategy involves selecting fast-growing "giant" individuals. However, it remains unclear whether this distinctive trait —enabling them to outcompete others in growth— emerges randomly or is associated with polyploidy. Here, we sampled 30 individuals of *Ulva fenestrata* along the Swedish west coast and further included 5 long-term cultivated crop strains. All algae were incubated to induce fertility, swarmers were isolated and cultured to generate an F1 generation. This generation was screened for individuals displaying the giant phenotype. Selected F1 gametophytic giants will be parthenogenetically propagated and the inheritance of the trait will be assessed while in parallel, we aim to determine ploidy levels in giant individuals.

Our findings may inform strain selection in future *Ulva* aquaculture by clarifying the role of polyploidy in morphological stability and genetic diversity. This knowledge is essential for identifying robust traits in cultivated strains and advancing our understanding of *Ulva* biology at both molecular and physiological levels.

Acknowledgments

We would like to thank Elena Schroffner-Brunner from University of Gothenburg, Department of Marine Sciences for her help with *Ulva* sampling and setting up of experiments, as well as Annelous Oerbekke from Nordic SeaFarm for providing cultivation strains. We acknowledge invaluable support from the Blue Bio Boost Project (grant number: SBEP2023-476), as well as the European Cooperation in Science and Technology.

Short Biography of the speaker

Inga is a postdoctoral researcher at the Steinhagen lab at University of Gothenburg, Tjärnö Marine Laboratory, working on the cultivation and selection of green algae *Ulva* for aquaculture purposes. Prior she obtained her PhD in marine glycobiochemistry at MARUM Center for Environmental Research at University of Bremen and Max-Planck Institute for Marine Microbiology, Bremen, focusing on the potential of brown algae as a carbon sink.

MORPHOGEN-INDUCED METABOLIC SHIFTS IN *ULVA*

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The green macroalga *Ulva mutabilis* (conspecific with *Ulva compressa*) serves as a valuable model for the investigation of algal development, particularly due to its reliance on the symbiotic marine bacteria *Roseovarius* sp. MS2 and *Maribacter* sp. MS6 [1]. The bacteria excrete algal-growth and morphogenesis-promoting factors (AGMPFs) essential for the normal development of the alga [2, 3]. *Roseovarius* sp. promotes cell division, while *Maribacter* sp. induces morphogenesis and proper cell wall formation in axenic gametes. The AGMPF from *Maribacter* sp. has been identified as (−)-thallusin [4] and successfully synthesised [5, 6], whereas the corresponding factor from *Roseovarius* sp. is still unidentified.

We demonstrated the axenic callus derived from *Ulva* gametes is a suitable model for investigating the metabolic effects of AGMPFs [7]. After two weeks of exposure to AGMPFs, treated calli showed visible signs of regeneration and significant metabolic shifts compared to untreated controls using metabolomics. Targeted fatty acid profiling also revealed pronounced alterations in the fatty acid profiles compared to untreated controls. Particularly ω3 and ω6 fatty acids were affected [7]. In addition, we analysed the monosaccharide profiles of *Ulva* treated with bacteria or AGMPFs for three months to compare their carbohydrate composition to that of the axenic calli control cultures. Notably, treatments with *Maribacter* sp. or (−)-thallusin revealed clear differences compared to control conditions. Overall, our poster illustrates how omics approaches can elucidate the mechanisms of morphogen-induced signal transduction. The findings underscore the crucial role of bacterial-derived compounds in regulating metabolic and developmental processes in *Ulva*, highlighting their potential utility in interfering with algal–microbe interactions for aquaculture applications.

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Acknowledgments

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Short biography of the presenter

Hermann Holbl is a PhD candidate in chemistry and a member of the Collaborative Research Centre 1127 Chembiosys at the Friedrich Schiller University Jena in Germany. He holds a bachelor's degree in chemistry and a master's degree in "Chemistry – Energy – Environment". He studies the symbiotic relationships between *Ulva* and its associated bacteria, *Roseovarius* sp. and *Maribacter* sp., focusing on the algal growth- and morphogenesis-promoting factor thallusin released by *Maribacter* sp.

SEAWEED CULTIVATION AND COMMERCIAL EFFORTS IN DENMARK

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Over the past two decades, Denmark has witnessed the emergence of a seaweed sector transitioning from historical hydrocolloid processing based on imported or wild-harvested biomass, to a small but growing ecosystem of seaweed cultivation, processing, and product innovation. While Denmark lacks extensive intertidal zones and large native seaweed forests, pioneering efforts since 2007 have driven both sea-based and land-based cultivation of species such as *Saccharina latissima*, *Ulva* spp., and *Palmaria palmata*.

This presentation outlines the development of the Danish seaweed industry with a focus on cultivated species, commercial applications, and the evolving network of small- and medium-sized enterprises. We present results from three national SWOT analyses (2012, 2021, 2023) capturing stakeholder perspectives, and summarize trends in funding, peer-reviewed research output, and industry consolidation. A particular emphasis is placed on the role of formalized science-industry networks—such as Tangnetværket, AlgaeCenter Denmark, and the Danish Seaweed Organization—in supporting knowledge exchange and enabling collaboration. Despite positive developments, challenges remain related to funding structures, regulatory clarity, and the shift from enthusiastic entrepreneurship to economically sustainable business models. The Danish case offers valuable insight into how coordinated national efforts can support the emergence of a blue bioeconomy sector in a European context.

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Short Biography of the speaker

Susan Løvstad Holdt, Associate Professor at DTU Food is expert in research within seaweed composition, bioactives and technologies for extraction and processing. Furthermore, she is involved in research on seaweed as a food, legislation, standardization, retaining the qualities of seaweed, but also having focus on reducing the contaminant found in seaweed. SLH is Vice President of the International Seaweed Association and chair of the Seaweed Network in Denmark. SLH's list of publications incl. FAO reports and book chapter, and with more than 40 peer-reviewed publications counting the highly cited (>1350) review on Bioactive compounds in seaweed: functional food applications and legislation (Holdt and Kraan, 2011).

A SALINE WATER SOURCE FOR *ULVA* LAND-BASED CULTIVATION: FROM SPA TO BIOMASS FOR *ULVA*N AND BIOPLASTIC PRODUCTION

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Ulva biomass is typically produced in land-based settings using seawater and attempts to establish sea-based cultivation strategies are under way [1,2,3]. Further, only a handful studies using saline water sources to explore seaweed culture have been reported, and they include *Ulva* [4,5,6]. This talk presents a compilation of a 1-y experimental trials in which the growth rates of *Ulva* (likely a combination of the morphologically similar *U. rigida* and *U. ohnoi*) were measured in 1,000 L PVC tanks using a saline (i.e. thermo-mineral water) source chemically resembling natural seawater, with temperatures ranging between 25-28 °C. The experiments run from August 2024 through July 2025. During an initial period of several weeks the thermo-mineral water source tested was highly nuisance for algal growth, apparently related to high Fe levels and alkalinity, resulting in calcite deposition, very low or negligible growth, triggering several sporulation events. This may reflect a stress response to acclimation to a new cultivation environment. A set of filters down to 5 µ were of significant benefit and towards the coming fall and winter seasons growth rates ranged between 50-150% per week. Altogether, the biomass quality was arbitrarily ranged as "low" or average" compared to similar cultures in seawater, yet *Ulva*n estimates were c.a. 10% on a DW basis. This study provides an encouraging option for land-based *Ulva* cultivation in saline environments, other than seawater, specially when looking at on-going climate changes and alarming anthropogenic global pollution occurring in the marine environment.

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Short Biography of the speaker

Álvaro Israel is a senior scientist at Israel Oceanographic & Limnological Research, Ltd. The National Institute of Oceanography, Haifa, Israel. During his career he engaged in studying photosynthesis, carbon fixation and ecology of marine macroalgae, acquiring a vast experience in seaweed aquaculture and strain selection intended for the production of food and valuable molecules. Seaweed taxonomy using molecular tools, seaweed invasions and issues related to global change are also under current investigation in his lab.

FROM LAB TO SEA: EXPLORING *ULVA* SPORULATION AND GROWTH DYNAMICS FOR OPEN-WATER AQUACULTURE ON GRAN CANARIA

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Seaweed cultivation in coastal areas is often limited by spatial constraints and environmental variability. To support the expansion of seaweed farming, it is necessary to explore alternative regions with suitable environmental parameters. Gran Canaria is particularly promising due to its abundant sunlight and stable, warm water temperatures.

This study presents the first trials of open water cultivation of *Ulva* in nearshore waters off Gran Canaria, aiming to assess whether the local environmental conditions support its growth. The experiments were conducted over a period of approximately one month between March and May. Two cultivation approaches were employed: cage and rope-based systems. A biomass of exactly 50 g (wet weight) of young *Ulva* specimen was placed in each cage (n=3) at two different depths: the surface and 1.5 meters. Adult *Ulva* was attached to ropes fiddling the specimen into the twine (n=4 + one control without any biomass) and grown under three treatments. Attached to a frame without a net, with a net and submerged at 1 m with a net.

Cage cultivation achieved growth rates of up to 12.5% wet weight (WW) per day during the first week. Growth at 1.5 m depth led to significant higher RGR ($p = 0.014$, Post hoc tukey test) then the cages kept floating at the surface, potentially due to reduced biofouling and lower irradiance or photoinhibition effects at depth. Despite increasing biotic stressors, including grazing and biofouling, the biomass doubled twice during the experimental period. Further in contrast, the rope-based system faced significant challenges due to heavy biofouling, which hindered the algae's development and resulted in total decay of the biomass. Additionally, the age of the used biomass might have impacted the growth potential. Adjustments to this system are planned for future trials to mitigate fouling and grazing impacts.

Additionally, sporulation experiments using a local *Ulva* species offered preliminary insights into reproductive timing and potential regulatory factors, setting the stage for future reproductive studies. These findings underline the importance of refining cultivation techniques and understanding biotic interactions for sustainable *Ulva* aquaculture in nearshore environments.

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Acknowledgments

I acknowledge the Alfred Wegener Institute (AWI) for funding this research, the Spanish Bank of Algae for their support and provision of laboratory infrastructure, and Macrocarbon for their financial contributions, which not only supported this study but also enabled access to their boat and barrier system, making the fieldwork possible.

Short Biography of the speaker

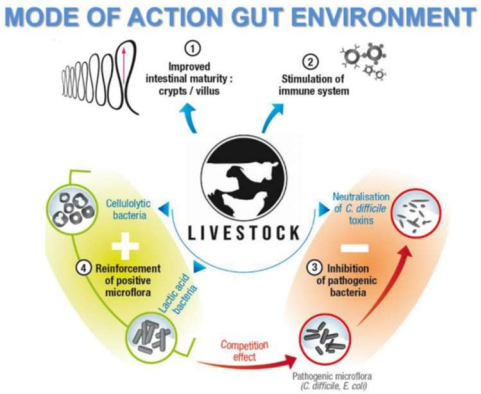
I began my academic journey with a Bachelor's degree in Biology at Leibniz University Hannover, specializing in plant genetics and completing a thesis on Arabidopsis thaliana mutants under abiotic stress. I then earned a Master's in Marine Environmental Sciences from the University of Oldenburg, conducting my thesis research at Aarhus University on the responses of sub-Arctic benthic macrophytes to warming. In early 2023, I worked at the Department of Ecoscience in Aarhus and later completed a research stay at the Greenland Institute of Natural Resources in Nuuk. Since March 2024, I have been a research assistant at Macrocarbon, designing and running experiments to optimize *Ulva* cultivation and managing aquaculture systems such as photobioreactors. In January 2025, I began my PhD at the Alfred-Wegener Institute as part of the INNOva project, which focuses on sustainable open-water *Ulva* farming and its climate and social benefits. These experiences have given me a strong foundation in marine plant research and applied aquaculture. I'm especially interested in the role of seaweed in climate change mitigation and sustainable resource use. My work is driven by a commitment to bridging marine science with practical environmental solutions.

USE OF SEAWEEDS, EXTRACTS AND INGREDIENTS FROM SEAWEEDS TO IMPROVE HEALTH AND WELLBEING IN LIVESTOCK.

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There are about 1200 chlorophytes or green algal, 1750 phaeophytes or brown algal and 6000 rhodophytes or red algal species. These macroalgal species or more popular called seaweeds, contain a plethora of secondary metabolites that play a role in a variety of metabolic processes in plants, animals and humans. Seaweeds possess several bioactive molecules that are studied for their prebiotic, anti-microbial, antioxidant, anti-inflammatory and immunomodulatory effects.

Seaweed benefits are related to their content of sulfated polysaccharides, phlorotannins, diterpenes, omega-3 polyunsaturated fatty acids, minerals and vitamins. Modern agricultural and farming practices depend heavily on chemicals to control pests such as a variety of parasitic organism either internal or external in animal farming and crop production. Unique biochemical compounds from algae have been applied as potential biocides against these parasitic organisms. This presentation as part for the final COST action "*Ulva* Wheat of the Sea" will place specific emphasis on *Ulva* as feed ingredient for livestock in agriculture.

Over the last decades several chemical biocides have become less effective and many chemicals currently used have a negative environmental impact (e.g., Zinc Oxide, bromoform) and have possible side effects to humans and other non-target organisms. Marine algae have a demonstrated potential to act as environmentally friendly biocides with no negative side effects and could fulfill the need of alternative and safe supplements to increase health and welfare in livestock. This presentation reviews the effects of seaweeds (*Ulva* in particular) and seaweed extracts in farmed animals as prebiotics, anti-microbial, antioxidant, anti-inflammatory and immune-modulatory effects, promoting intestinal well-being and improving digestibility. Special focus will be on the green seaweed *Ulva.*

Short Biography of the speaker

Obtained his PhD on phylogenetics and aquaculture of edible seaweeds at the National University of Ireland, Galway in 2001, while in parallel managed of the Irish Seaweed Industry Organisation and established the Irish Seaweed Centre in 2001, a dedicated R&D centre for seaweed-based research and development at the University of Galway Ireland. Resigned from University life and established Ocean Harvest Technology Ltd to pursue and develop some commercial ideas using seaweeds for functional food ingredients for fish farming and livestock. After 8 years he resigned and co-founded The Seaweed Company in 2018. A company with complete horizontal integration developing the whole seaweed value chain from cultivation to biostimulant feed, food, and biomaterials with as highlight SeaMeat™, a processed meat replacement ingredient. Stepped out of the Seaweed Company in December 2024. Currently he is developing seaweed cultivation in India and Indonesia, producing agarose, biostimulant and works on biopaper and bioplastics. In his position for Blaaker AS, Norway is also involved in developing and applying biodegradable rope and materials for seaweed cultivation and aquaculture in general. He was President of the International Seaweed Association (2016- 2019) and is currently President for the International Society for Applied Phycology (2024- 2027). He was business coach of 14 Indonesian seaweed companies under the Dutch Ministry of Economics and Climate Policy's development program (CBI/RVO) to help diversify and increase export to Europe. Main fields of expertise are aquaculture of seaweeds, sustainable development of algal resources, industrial applications of seaweeds and usage of seaweeds in animal feed, aquaculture, biotechnology and biomedicine

HOW COLLABORATIONS WITHIN THE SEAWHEAT NETWORK ALLOWED FOR AN HOLISTIC APPROACH OF TEMPERATURE EFFECTS ON *ULVA* BIOMASS QUALITY AND YIELD.

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The wonders of *Ulva* biology are being researched in all corners of the world with every research group having special expertise and niches. The SeaWheat COST Action has provided incredible opportunities for early-career researchers to learn from several research groups through training schools, workshops, and short-term-scientific-missions (STSM). All these opportunities allow young researchers to gain a more holistic and nuanced approached to their own research.

Several questions arise, as the research and development of *Ulva* cultivation systems is being investigated in Denmark: "Which *Ulva* species are we working with?", "How does it grow?", "What can it be used for?". To answer these questions, the expertise of several *Ulva* research groups is needed, as the dynamics of *Ulva* biology often vary among *Ulva* strains and cultivation conditions.

In this study, the effect of temperature on a single German *Ulva compressa* strain was investigated across a temperature gradient (10 – 31 ℃) in a steady-state, high-nutrient pilot setup. The examined parameters included: growth rates, oxygen release, nitrogen uptake, microbiome composition, and biochemical composition. To complete this study an early-career researcher travelled between several SeaWheat member institutes to learn methods of molecular biology, seaweed cultivation, and biochemistry.

Temperature affected growth rates, with high growth rates being observed (19 ± 2% FW day-1) up to 19 ℃, and gradually reduced growth above 22 ℃ (0.2 – 12% FW day-1). *Ulva*n content was highest at 10 – 13 ℃ (148.5 ± 34.2 mg g-1 DW), while amino acid content was higher at the temperature extremes (10, 28, and 31 ℃). Nitrogen uptake potential was highest at 10 - 19 ℃ and accounted for 60 - 80% of added inorganic nitrogen. The results document effects of cultivation temperature on the biomass yield and quality of a single *U. compressa* strain at pilot scale. Furthermore, these results present a prime example of how international cooperation within the COST Action framework have promoted learning, networking, and increased the quality of research caried out by young scientists.

Acknowledgments

The authors would like to acknowledge the technical and academic staff of Aarhus University, Ghent University, and the University of Galway who were essential to this work. Additionally, the authors would like to thank everyone involved in hosting and organising the SeaWheat COST Action training schools, workshops, and STSMs for their dedication and inspirational discussions.

Short Biography of the speaker

PhD student, Department of Ecoscience, Aarhus University, Denmark Originally from the West coast of Denmark, Kristoffer grew up with a curiosity for marine life and coastal ecosystems. Kristoffer is passionate about the mitigation of anthropogenic effects on natural ecosystems and educated as an artic and marine ecologist he aims to quantify the ecosystem services and disservices of *Ulva* cultivation in Danish waters.

ULVA IN LAND-BASED INTEGRATED AQUACULTURE WITH ABALONE IN SOUTH AFRICA: AN UPDATE AFTER 25 YEARS OF COMMERCIAL CULTIVATION

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The land-based abalone farming industry in South Africa developed rapidly in the 1990s. By the early 2000s the first commercial IMTA systems were initiated, using *Ulva* grown in abalone effluent to produce feed for cultivated abalone. All the farms feed formulated feed to the abalone, with some providing additions of freshly harvested kelp (*Ecklonia maxima*) and/or cultivated *Ulva*. In 2006 the first integrated recirculating system was developed using *Ulva* to bioremediate the effluent enabling partial recirculation of water. Bolton et al. (2009) carried out a SWOT analysis of this initial system. Since then national abalone production has increased from 1100t to 2600t yr-1 , and *Ulva* production, predominantly on 4 large farms, has increased from 1000t to ca. 2400t yr-1 . Two of these farms were built a little over a decade ago and designed to operate fully on 50% recirculation: Bufflejags Abalone and Diamond Coast Abalone. The ASTRAL project (2021-4: https://www.astral-project.eu/) 'IMTAlab South Africa' carried out detailed research on the recirculating IMTA system at Buffeljags Abalone Farm. The farm (Figure 22) is designed in 'clusters' consisting of 42 ca. 5m-long abalone tanks linked to a D-ended ca. 30m long paddle raceway producing *Ulva lacinulata*. The latter propagates vegetatively in the system, as demonstrated by continual production without thallus deterioration and the lack of attached foliose *Ulva* sporelings on the raceways.

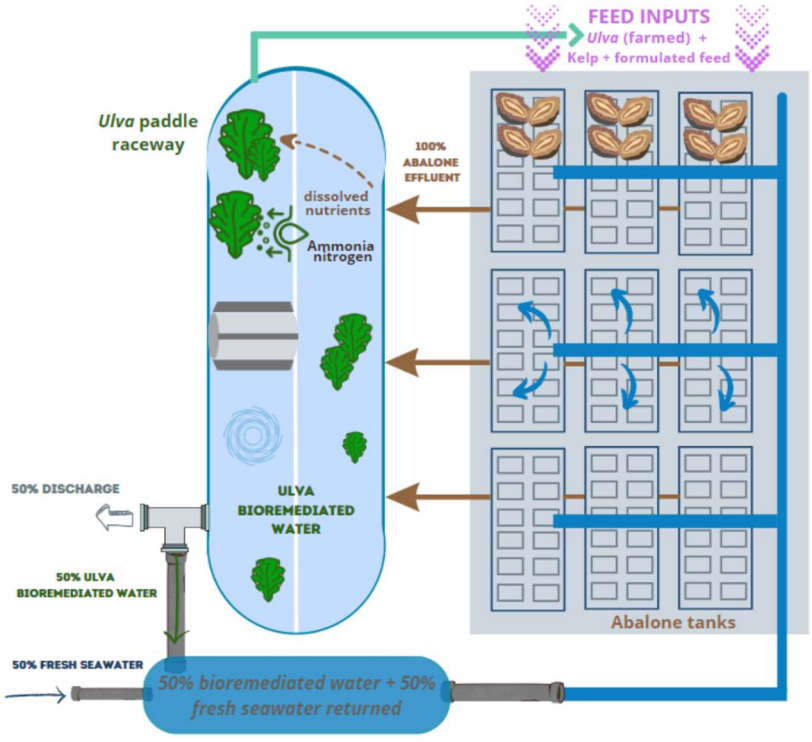


Figure 21 Single cultivation cluster at Buffeljags abalone farm. From Macey et al. (2024)

Ulva has a remarkable list of benefits apart from additional feed provision and enabling partial recirculation. These include: providing feed when formulated feed causes difficulties at high summer temperatures/ improved abalone health through the addition of fresh feed/ feeding stimulation leading to increased consumption reducing use of protein-rich formulated feed/ significant reduction in nutrient release to the environment/ potential to increase recirculation rates to 75% with no apparent adverse effects/ potential to run on 100% recirculation for

a few days - a benefit in the event of a short-term Harmful Algal Bloom/ and modulation of microbiome in the system including reduction in potential opportunistic pathogens. Despite the demonstrated benefits, only ca. 35% of South African abalone farms grow seaweeds. As previously highlighted (Bolton et al. 2009), main barriers to IMTA development in South Africa are: biosecurity concerns with refeeding seaweed grown in effluent/ use of costly land-space for seaweed rather than abalone cultivation/ concerns over potential for reduced growth or health of abalone with increasing recirculation/ and the extra input necessary to grow a second aquaculture species. An additional threat is the recent price reduction for the high-value abalone, sold predominantly to Asia.

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Acknowledgments

This study received funding from the EU Horizon 2020 Research & Innovation Programme ASTRAL Project under Grant Agreement No. 863034.

Short biography of the speaker

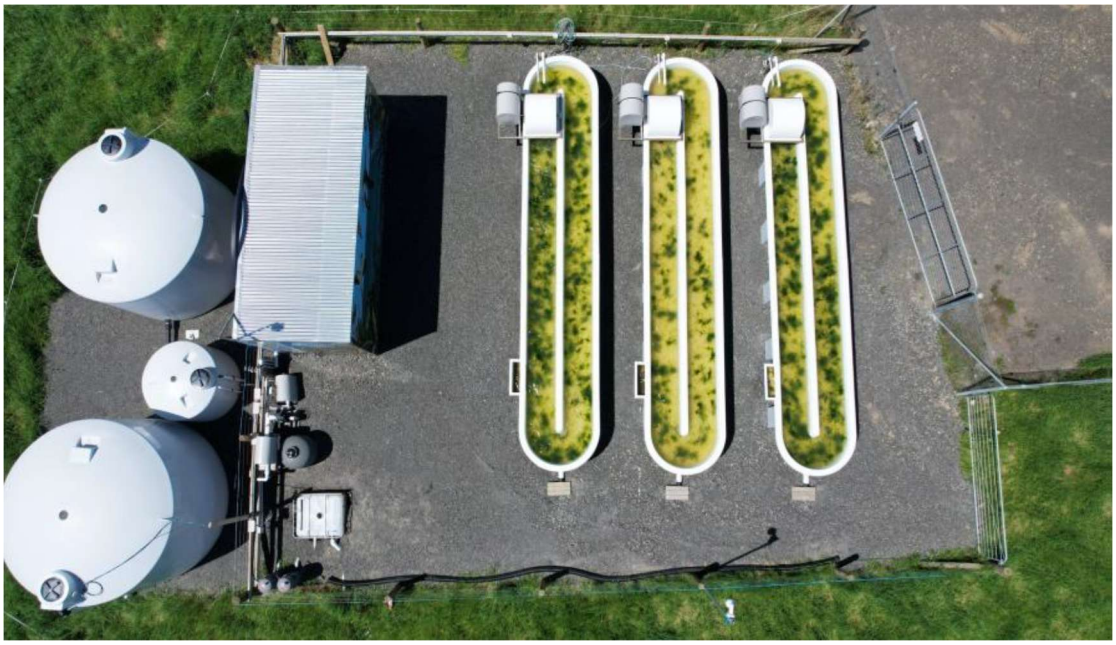
Dr Brett M Macey is a specialist scientist in the South African Department of Forestry, Fisheries and the Environment (DFFE). He conducts inter- and transdisciplinary research to tackle nutritional and sustainability challenges of marine aquaculture, focusing specifically on advancing the health and sustainability of farming systems, the development of disease prevention strategies and fostering a better understanding of the role microorganisms play in the health of aquatic animals and farming practices. Current research encompasses the creation of probiotics/immuno-stimulants, establishment of innovative molecular methods for diagnosing new and emerging pathogens, and the formulation of functional feeds—such as probiotic and seaweed-supplemented diets—with emphasis on culture technologies aimed at enhancing the health and sustainability of aquaculture systems and nutritional content of farmed species, particularly through the integration of low trophic species and seaweeds.

PILOT SCALE *ULVA* BIOREMEDIATION OF DIFFUSE SOURCE NUTRIENTS IN A NEW ZEALAND ESTUARY

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Terrestrial run-off has resulted in nutrient (e.g. nitrogen and phosphorus) enrichment of rivers and adjacent marine ecosystems in New Zealand, with risk of significant environmental harm. However, an opportunity exists to recover and re-use some of these nutrients using a land-based algal bioremediation strategy. Seaweed based bioremediation is used commercially for point-source discharges elsewhere, e.g. from land-based aquaculture, but there is limited evidence of the efficacy of this approach for remediating diffuse source nutrients in a warm-temperate climate.

To test this, we designed and commissioned a pilot-scale aquaculture pond system suitable for the land-based cultivation of *Ulva* seaweed (sea lettuce) for the bioremediation of diffuse source nutrients from the Waihou River estuary on the North Island of New Zealand. We isolated and scaled up a locally present species of *Ulva*, *Ulva australis*, at our University farm, where we maintained broodstock and scaled up biomass for inoculating the three 10m raceway ponds at the test site. We then quantified seasonal performance of biomass productivity and bioremediation capacity (nutrient removal) over seasonal growth periods during 2022-2023. The ponds were managed with weekly harvests at stocking densities of 0.5, 1.0, and 1.5 g fresh weight *Ulva* L-1, and water flow rates of 2.4, 3.6, and 4.8 pond volumes day-1 using a 2-level 2-factor blocked design with replicated centre points. On average per seasonal block, seaweed ponds removed 70% to 86% of the dissolved nitrogen (NOx-N) and up to 70% of dissolved phosphorous (as PO_4-P) from the in-flow water taken from the Waihou River. Productivities (seaweed growth) were excellent during the warmer months, producing over 100kg of seaweed from this relatively small pilot system per month, with somewhat lower growth in the cooler months, as expected. Overall, the project demonstrated the potential for year-round land-based seaweed production and continuous nutrient removal in conditions of ambient sunlight, water temperatures, and availability of nutrients for growth in a rural setting.

Acknowledgments

This project was a collaboration between the University of Waikato and AgriSea Ltd. who also provided funding, supported by the Ministry for Primary Industries Sustainable Food and Fibre Futures fund (MPI SFFF), the Agricultural and Marketing Research and Development Trust (AGMARDT), and the Hauraki & Thames-Coromandel District Council, Te Waka, Veolia, and PWC, with the blessing of local Iwi.

Short Biography of the speaker

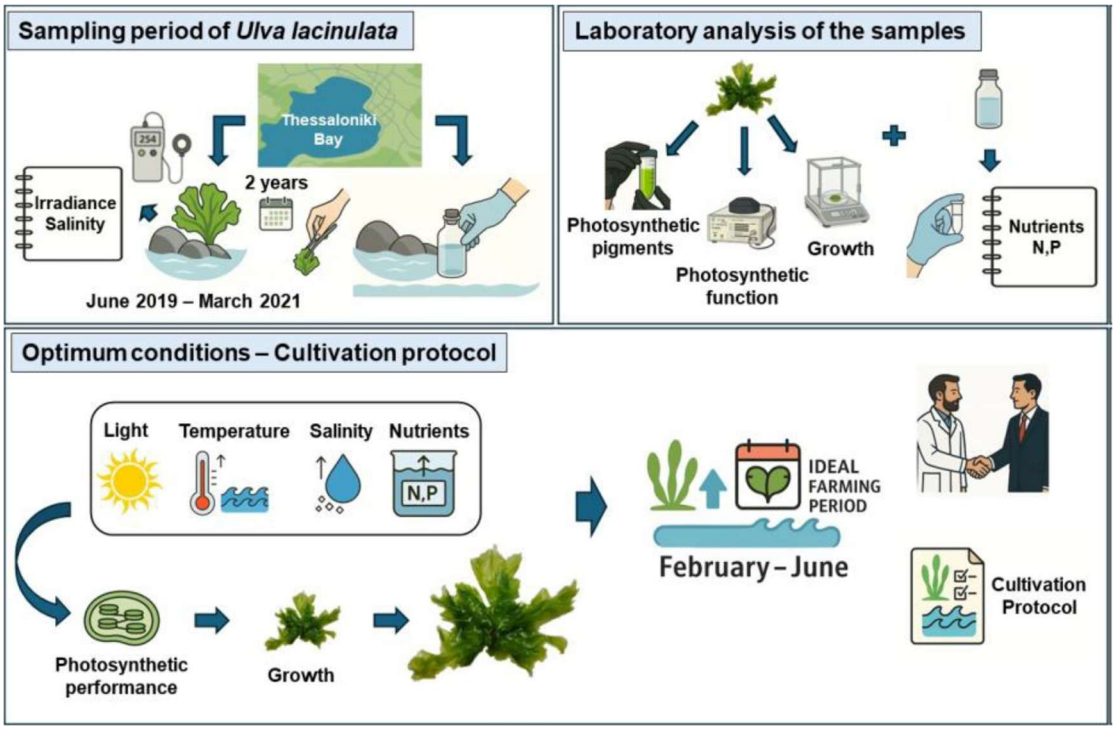
I am a phycologist and lead a multidisciplinary research group at the University of Waikato's Tauranga campus, delivering fundamental and applied science underpinning NZ's emergent seaweed aquaculture sector. I have a passion for using science and seaweed to develop practical and sustainable solutions for communities and diverse environmental and primary industry sectors. I led the commissioning of our University pilot scale recirculating algal aquaculture farm licensed for commercial transfers of seaweed stock to support the research and its technology transfer. My team and I have developed and established seaweed hatcheries, farming methods for local species, and a culture collection of key seaweed aquaculture targets for NZ. Current work focusses on seaweed microbiomes, and the application of seaweed sulfated polysaccharides as elicitors of plant immunity to develop horticultural crop protection products.

PHOTOSYNTETIC AND GROWTH ACCLIMATION OF *ULVA LACINULATA* TO EUTROPHIC THESSALONIKI BAY (GREECE): AN INTEGRATED, MULTILEVER ANALYSIS

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Seaweeds acclimate their photosynthesis and growth to environmental factors to optimize their performance under predictable, seasonal, and unpredictable extreme weather variations [1]. Heat waves and heavy storms will likely become more frequent and intense under the human-induced climate crisis, changing nutrient loadings in coastal habitats [2] and further testing seaweed acclimation potential. Among the seaweeds, fast-growing *Ulva* species can thrive in shallow eutrophic conditions worldwide, with their utility in bioremediation and as a source of valuable products [3]. Understanding the underlying biological mechanisms controlling *Ulva*'s life cycles in nature is fundamental to developing cultivation protocols for these economically important species [4].

We studied *Ulva lacinulata* (Kützing) Wittrock in the eutrophic Thessaloniki Bay, Greece, between June 2019 and March 2021, to study photosynthetic and growth responses to understand species' acclimation strategies at different integrated biological levels, to develop species' sea or land-based mass cultivation protocols. At each sampling effort, while temperature and salinity were recorded by a WTW LF330 portable conductivity meter, 2 l of seawater was collected and transferred by a portable refrigerator in plastic containers to analyze its nutrient content. From each sampled material 18 pieces of *U. lacinulata* thalli were carefully cleaned of epiphytes and selected as replicates (n = 6) for the experiments in the simulation laboratory. While temperature and salinity have been adjusted to field measurement, nutrients (6 µmol/L N-NO3- and 0.06 µmol/L P-PO4-) and irradiance (100 µmol photons m-2 s-1; 12 h light per 24 hours) were kept constant.

Critical seawater variables were analyzed by using one-way ANOVA and were correlated with biochemical (photo-pigment content), physiological (fast-term JIP-test and slow-term Rapid Light Curve photosynthesis protocols), and organismal (Relative Growth Rate-RGR) species variables by using multivariate analysis (RDA).

In order, nitrates, temperature, and phosphates were the abiotic factors most significantly affected *Ulva*'s photosynthesis and growth, in agreement with other studies [5]. While extreme temperatures during September

2029 triggered photosynthetic changes in PSII structure (photon absorption and trapping), function (electron transport from QA^- to QB), and photopigments composition (chl/carotenoids ratio), heavy rainfall during June 2019 reduced salinity and increased nitrates, lowering *Ulva lacinulata*'s photosynthetic efficiency (alpha), quantum yield (ΦP0), and active PSII centers (RC/ABS) and increasing energy dissipation (DI0/RC). Variations in winter-summer temperature and nitrogen-to-phosphate ratio affected photosynthetic performance (rETRmax, Ek) and productivity (ETRmax/NPQmax). As expected [1], seasonal changes and extreme events significantly drove *U. lacinulata*'s physiology. However, they operated on different timescales (fast vs. short photo-acclimation), eliciting different responses.

Unimodal is the typical pattern of *Ulva* spp. [6] and *Ulva lacinulata* [7] growth in temperate coastal waters. It involves a period of biomass accumulation starting in winter or early spring as water temperatures rise and light availability increases, leading to a peak in biomass, usually occurring in late spring or early summer. Late winter and early spring conditions supported the highest relative growth rates, primarily due to increased nitrogen availability despite moderate irradiance and low temperatures.

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Acknowledgments

This study was supported by the BIOALGAFOOD project (code: T1EΔK-00232) co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH—CREATE—INNOVATE.

Short Biography of the speaker

Dr. Sotiris Orfanidis, Research Director, is a Marine Botanist with expertise in seaweed ecophysiology and cultivation. His expertise includes the ecology and ecophysiology of macroalgae for their usage as bioindicators of water quality of coastal and transitional waters, the cultivation of macroalgae independently or within multi-trophic aquaculture systems (IMTA), and marine forest restoration. Participated in more than 40 national and European projects and has more than 150 publications, 79 of them in peer-reviewed international journals, one in Nature Communications, as listed in Google Scholar database (h-index = 31, i-10 = 61). He is also a reviewer for international journals and conferences and a member of the editorial board of Mediterranean Marine Science, Water and Frontiers in Marine Science.

REGULATION OF *ULVA* SPORULATION AS PART OF A CULTIVATION STRATEGY: WHAT DO WE KNOW?

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One of the principal conclusions of the Seawheat workshop "From *Ulva* aquaculture to food and feed production: state-of-the-art, bottlenecks, risks and gaps" (Lisbon, 23-24 May 2023) was that one of the main challenges preventing the development of *Ulva* cultivation in Europe is its financial cost. This relates to all parts of the production phase and all types of production systems (e.g., monoculture, integrated) at sea or on land. Sporulation is one of the key parts of the production cycle.

In land-based cultivation systems using vegetative propagation (currently the dominant commercial cultivation model), spontaneous sporulation, fragmentation or protoplast formation might result in important loss of biomass, depending on the species or strain. For sea-based cultivation on longlines, sporulation is essential in developing a labour extensive cultivation process. In addition, sporulation is important in the process of strain selection and to maintain genetic diversity in vegetatively growing material. Hence, achieving control of the sporulation process, or the production of other initial phases of individuals, such as biomass is of great importance for upscaling commercial *Ulva* cultivation.

The life-cycle of *Ulva* is typical for many green algae (alternance of gametophytes and sporophytes), however, many short-cuts and alternative routes have been observed in laboratory situations. The plasticity in the reproduction mode is species or even strain specific and the triggers are largely unknown. In this presentation, the state of the art of knowledge for reproduction control of *Ulva* will be summarised and discussed. Main focus will be on the role of sporulation inhibiting factors, their chemical identification, and how they regulate the reproduction process.

Based on this knowledge, developed in the last two decades, a general protocol for sporulation has been developed and some specific methods have been identified for individual species or strains, which is part of the objective for task 2.2 of WP2 "*Ulva* in Aquaculture" of this COST action. In addition, potential alternatives to sporulation, such as protoplast development (incl. spontaneous events of protoplast development) will be discussed.

Acknowledgments

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Short Biography of the speaker

Erik-jan Malta PhD, is Chief Scientific Officer and researcher seaweed cultivation and sustainable

aquaculture at the Aquaculture Technology Centre (CTAQUA), Spain. A biologist by training, Erik has more than 25 years of international research experience in marine ecosystem and seaweed ecology, seaweed cultivation and applications and sustainable aquaculture systems as Integrated Multitrophic Aquaculture (IMTA). He has been PI of several research projects in Spain and Portugal and is coordinator of the Interreg Atlantic Area project AQUAFISH0.0 and collaborator and in various other past and present EU funded projects. He has published >25 papers in peer-reviewed journals (H-index = 19) as well as contributions to book chapters and various technical reports. He is research topic co-editor of the journal Frontiers in Marine Science and member of the Scientific Committee of the European Algal Biomass Association (EABA).

MACROALGAE-DERIVED COMPOUNDS AGAINST CARDIOVASCULAR AND NEURODEGENERATIVE DISEASES

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We previously demonstrated that diet supplementation with seaweed *Sargassum fusiforme* (*S. fusiforme*), its lipid extract or pure 24(S)-saringosterol could prevent cognitive decline and neuropathology in a mouse model of Alzheimer's Disease (AD) [1-2]. Moreover, the *S. fusiforme* lipid extract could prevent cardiovascular disease (CVD). We tested a lipid extract of European seaweed *Himanthalia elongata* (*H. elongata*). Diet supplementation with *H. elongata* extract prevented cognitive deterioration in APPswePS1ΔE9 mice [3]. The cerebral amyloid-β plaque load remained unaffected. However, IHC analysis revealed that the extract lowered glial markers in the brains of APPswePS1ΔE9 mice. While cerebellar cholesterol concentrations remained unaffected, both extracts increased desmosterol, an endogenous LXR agonist with anti-inflammatory properties. The *H. elongata* extract increased cholesterol efflux and decreased the production of pro-inflammatory cytokines in LPS-stimulated THP-1-derived macrophages. Additionally, our findings suggest a reduction of AD-associated phosphorylated tau and promotion of early oligodendrocyte differentiation by *H. elongata*. RNA sequencing on the hippocampus of one-week-treated APPswePS1ΔE9 mice revealed effects of *H. elongata* on, amongst others, acetylcholine and synaptogenesis signaling pathways. In conclusion, an extract of *H. Elongata shows potential to reduce AD-related pathology in APPswePS1ΔE9 mice. Increasing desmosterol concentrations may contribute to these effects by dampening neuroinflammation.*

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Short Biography of the speaker

Dr Mulder is Associate Professor and head of the Laboratory of Vascular Medicine at the Department of Internal Medicine. Her laboratory focusses on obtaining insight into the role of lipid and sterol metabolism in the onset and progression of cardiometabolic and neurodegenerative diseases.

Because numerous age-related diseases are associated with altered lipid fluxes, we aim at modulation of lipid/lipoprotein metabolism to prevent or slow down disease progression. Our research focusses, among others, on liver X receptor-activation, using marine-derived compounds for preventing the progression of Alzheimer's disease in a long standing collaboration with the University Hasselt, University Clinics Bonn, UMC Groningen.

SEAWEED-DERIVED BIOACTIVES: HARNESSING OCEAN-DERIVED COMPOUNDS FOR SUSTAINABLE CROP IMPROVEMENT

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The rising demand for sustainable agricultural practices requires solutions that stimulate crop productivity without compromising our ecosystems. One of those solutions lie in the use of seaweed-derived bioactives, which have emerged as potent biostimulants capable of enhancing plant growth, stress tolerance and soil health.

Seaweeds synthesize a broad spectrum of bioactive molecules including polysaccharides (e.g., alginates, carrageenans, *Ulva*ns), polyphenols, betaines, and plant hormone analogues such as cytokinins, auxins, and abscisic acid. These compounds, when applied to crops have been shown to improve various agronomical traits such as germination rates, root and shoot development, and increased resistance to (a)biotic stresses. Among the possible mechanistic action of seaweed bioactives, gene expression associated with stress defence pathways, as well as improvements in nutrient uptake efficiency, have been documented following treatment with some seaweed extracts, indicating a possible priming of plant physiological responses. Lastly, the application of seaweed-derived bioactives can also impact the soil microbiome, which can lead to a modulation of agronomical performance as well as soil health in general.

While seaweed-derived crop bioactivities have been studied with some depth, another potential source of crop-acting bioactives could originate from the seaweed-associated microbiome (SAM). Indeed, the SAM has been shown to produce a plethora of compounds that could impact crop productivity. Those include phytohormones, antimicrobial compounds or defence elicitors that could be used to prime the crop's pathogen response pathways, triggering a response similar to seaweed biostimulants themselves. Despite this potential, actual uses of SAM-bioactives on crops have not been thoroughly investigated. In order to bridge that gap, our research focuses on the isolation and characterisation of the epi- and endophytic microbes associated with seaweed species present in Ireland. High-throughput screens for bioactivities on crops have been designed to quickly identify extracts, pools and fractions containing crop-acting bioactives, to be followed by compound characterisation and elucidation of their mode of action.

By systematically isolating and screening microbial communities from diverse Irish seaweed species, we aim to expand the current biostimulant toolbox beyond seaweed-derived molecules to include microbially-sourced bioactives. This dual approach not only enhances our understanding of the seaweed holobiont systems but also lays the groundwork for developing novel biostimulants formulations. Ultimately, integrating seaweed and SAM-derived bioactives into crop management strategies holds significant promise for advancing sustainable and resilient agricultural practices.

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Cost Action CA20106- SeaWheat

Short Biography of the speaker

Antoine Fort graduated with a MSc in Plant Biotechnology from Bordeaux University, France, in 2010 and later on obtained his PhD in NUI Galway on the theme of crop improvement through genetic engineering in 2013. This was followed by several post-doctoral positions, where Antoine worked on a variety of organisms ranging from crops, seaweeds, invertebrates and microbes. Antoine's expertise and research work is multidisciplinary in nature and ranges from "omics" (genomes, transcriptomes, proteomes & metabolomes) to cas9 genome editing and molecular ecology. After his work on seaweeds physiology in the University of Galway as postdoctoral researcher, he joined the Technological University of the Shannon as a lecturer in 2021. His current research interests are focused on the discovery of novel bioactives from the seaweed microbiome, funded through a Research Ireland Frontiers for the Future project

ULVA CAROTENOIDS: STUDIES ON THEIR BIOACCESSIBILITY AND GREEN EXTRACTION WITH EMERGING BIOSOLVENTS

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Ulva (sea lettuce) is a genus of green seaweeds long known as a source of compounds of interest to the agri-food industry and others (pharma, cosmetics, biomaterials). They are sources of carotenoids, including the ones present in the human macula lutea, above all lutein. These are versatile compounds of great interest as components of diverse products intended for human consumption, such as functional foods, nutricosmetics or supplements. Studies on the potential bioavailability (bioaccessibility) and extraction of carotenoids from algae in general and *Ulva* in particular are very timely considering the global challenge of producing healthier and more sustainable foods and associated policies and concepts, such as the Green Deal, the Blue Economy or the Circular Economy [1–3]. In this work, the main outcomes of recent studies of our group on these topics are presented.

In one of these studies, ultrasound pre-treatment conditions were optimized in order to enhance the macular carotenoids bioaccessible content (CBC, an estimation of potential bioavailability) of *Ulva lactuca*. Sea lettuce (kindly donated by Porto-Muíños S.L., Spain) was desalted, freeze-dried, ground and sieved. To optimize the sonication conditions, response surface methodology (RSM) was applied and a central composite design (CCD) was performed. The ratio sample: distilled water was 1g/ 40 mL. The parameters tested were amplitude (20–50%) and time (1–5 min). After the treatments, the solids were recovered and subjected to a consensus in vitro gastrointestinal digestion [4]. The micellar fractions were analyzed by RRLC-DAD [5]. The results indicated that the sonication conditions tested can significantly influence ($p < 0.05$) the macular CBC from the seaweed. The optimal conditions for maximising bioaccessibility were the use of an amplitude of 45% for 1 minute, which led to a 1.3-fold increase relative to the untreated control.

In another study, the efficacy of two green technologies, microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) in the extraction of *Ulva* carotenoids was evaluated. For this purpose, traditional food-grade solvents (acetone, ethanol, ethyl acetate, and hexane) were compared with emerging biosolvents such as 2-methyltetrahydrofuran (MeTHF) and ethyl lactate. Dehydrated *U. lactuca* was ground for 5 minutes at a frequency of 30 Hz at a sample:solvent ratio of 1:20. The conditions for UAE were 30% amplitude, 20 kHz frequency and 2 minutes of treatment. Those for MAE were 300 W of power for 5 minutes. The levels of carotenoids in the extracts were analyzed by RRLC-DAD [5]. The data revealed that the efficiency of UAE varied as a function of the carotenoids and the physicochemical properties of the solvents. In general, this intensification technology led to significantly ($P < 0.05$) higher contents of individual carotenoids with all evaluated solvents. Significant increases in the levels of total carotenoids were observed compared to the control and the UAE treatment regardless of the extraction solvent.

Hexane and MeTHF were the most effective solvents for total carotenoid extraction, with no significant differences between them. MeTHF appears as a sustainable, environmentally friendly alternative compared to other conventional solvents used for carotenoid extraction, notably hexane, which is very toxic and petroleum-derived [6].

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Short Biography of the speaker

Prof. Meléndez-Martínez is a Stanford University's World's 2% Scientist since 2020. His main research interest deals with the sustainable production of health-promoting foods, with a strong focus on carotenoids in the context of agro-food, health and cosmetics. He coordinated two large international networks, one Iberoamerican (CYTED-IBERCAROT) and another European (COST Action EUROCAROTEN). Some awards and recognitions: 1) Extraordinary Prize of Doctorate; 2) International Carotenoid Society (ICS) George Britton Award for Young Researcher; 3) Fellow of the ICS; 4) Manuel Losada Award to Excellence in Agro-food Research; 5) Iberoamerican Academy of Pharmacy Award; 6) Stanford University's World's 2% Scientist; 7) Induction into the scientific honour society Sigma Xi (USA).

COMPARATIVE ANALYSIS OF GROWTH, BIOCHEMICAL COMPOSITION, QUALITY, AND BIOREMEDIATION POTENTIAL OF *ULVA* SP. AND *GRACILARIA* SP. CULTIVATED IN PHOTOBIOREACTORS WITH DIFFERENT CULTURE MEDIA

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The cultivation of marine macroalgae is increasingly recognized as a sustainable solution to address global challenges in food security, climate change mitigation, and the development of a circular bioeconomy. Beyond serving as sources of alternative protein and bioactive compounds, macroalgae provide essential ecosystem services such as nutrient recovery and carbon sequestration. This study assessed and compared the growth, biochemical composition, and nutrient uptake of *Ulva sp.* and *Gracilaria sp.* cultivated in cylindrical indoor photobioreactors with four culture media: untreated lagoon water (WL), lagoon water enriched with anaerobic digestate (WD), lagoon water enriched with effluent from sea urchin aquaculture (WU), and lagoon water enriched with synthetic F/2 medium (WF/2).

Our results revealed that *Gracilaria* achieved its highest specific growth rate (SGR) in WU (9.45 ± 0.40% day^{-1}), outperforming *Ulva* in nutrient-poor conditions (WL and WU), while *Ulva* showed more consistent performance across treatments, with the highest growth rate in WF/2 (8.10 ± 0.75% day^{-1}). The biochemical profiles of the two species differed markedly: *Gracilaria* exhibited significantly higher levels of protein (up to 17.97 ± 2.77% DW in WF/2), carbohydrates (70.37 ± 7.63% DW in WL), and lipids (notably in WU). By contrast, *Ulva* consistently exhibited significantly higher concentrations of pigments—including chlorophyll-a, chlorophyll-b, and polyphenols—as well as higher fiber content across all treatments. In terms of bioremediation, *Ulva* consistently demonstrated greater nutrient uptake efficiency (NUE) and nutrient uptake rates (NUR), for total phosphorus in all treatments, while *Gracilaria* showed greater nitrate uptake in the WL and WD treatments.

This study shows how different nutrient sources affect biochemical composition, growth, and biomass quality, and underscores the complementary potential of the two species: *Ulva* excels in rapid nutrient removal and pigment-rich biomass production, while *Gracilaria* offers higher yields of protein and carbohydrates. Their combined use in multispecies cultivation systems could enhance year-round biomass production, diversify product applications, and improve the ecological resilience of macroalgae cultivation systems. The results provide a valuable foundation for optimizing high-value macroalgae cultivation strategies under controlled indoor conditions.

Acknowledgments

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Short Biography of the speaker

Over the past three years, my research has focused on the study of macroalgae, with particular emphasis on how their biochemical profiles vary in response to different environmental conditions, including marine heatwaves and eutrophication. I have explored how these stressors influence the composition and of valuable metabolites such as phytopigments, proteins, carbohydrates and lipids. In parallel, I have investigated the application of algal photobioreactors as a sustainable strategy for mitigating excess nutrient loads in aquatic systems, while simultaneously producing biomass suitable for the extraction of high-value biochemical compounds.

Additionally, my work has involved the extraction, quantification, and characterization of bioactive compounds from several native macroalgal species, such as *Ulva sp*., as well as from invasive or alien species including *Asparagopsis taxiformis* and *Caulerpa spp*. My research aims to support the development of integrated bioremediation and biorefinery approaches that promote both environmental sustainability and the valorization of algal biomass in biotechnology.

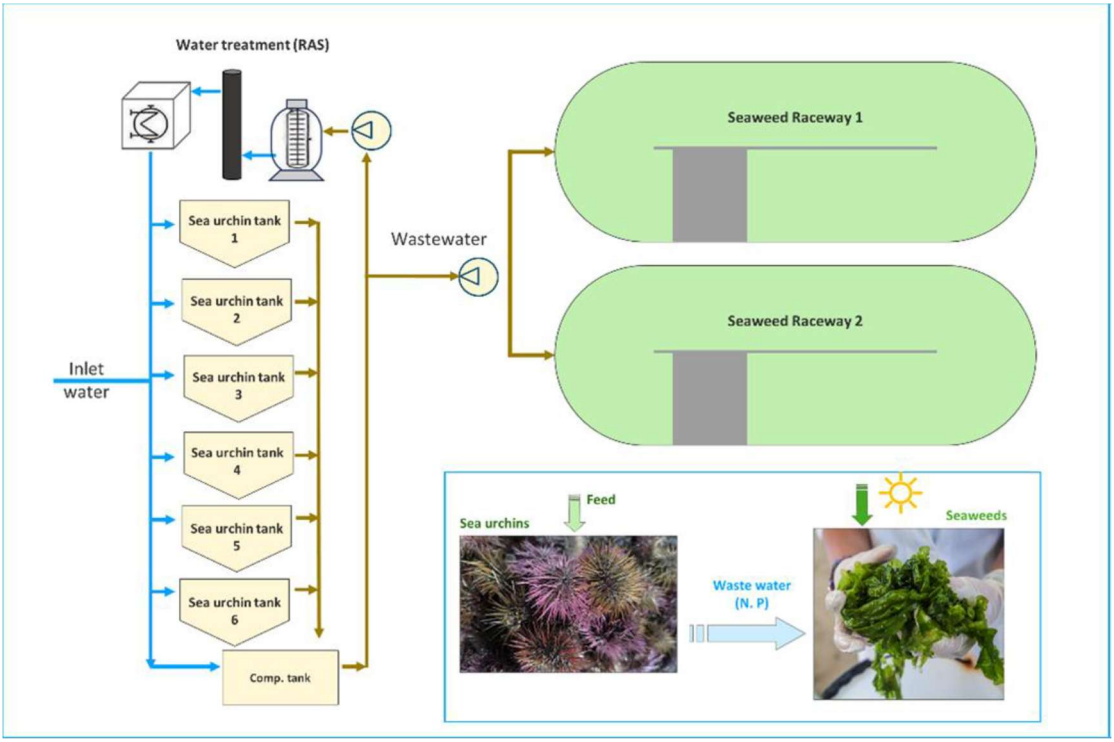
NUTRIENT REMEDIATION AND BIOMASS PRODUCTION OF *ULVA* SPP. IN AN IMTA SYSTEM WITH SEA URCHIN *PARACENTROTUS LIVIDUS*

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Integrated Multi-Trophic Aquaculture (IMTA) is a kind of co-culture where different species belonging to different trophic levels, such as fish, seaweeds, and shellfish are produced together. IMTA seeks to biodiversify fed aquaculture (e.g. finfish or shrimps) with extractive aquaculture, recapturing the inorganic (e.g. seaweeds) and organic (e.g. suspension- and deposit-feeders) nutrients from fed aquaculture for their growth (Chopin et al., 2012). The combination of fed/extractive aquaculture aims to engineer food production systems providing both biomitigative services to the ecosystem and improved economic farm output through the co-cultivation of complementary species (Chopin et al., 2012). IMTA systems offer a sustainable approach to aquaculture by co-cultivating species from different trophic levels, allowing nutrient recycling and economic benefit from the diversification of the productions. In this study, we evaluated the performance of *Ulva* sp. With foliose morphology as an extractive species in an IMTA system with the sea urchin *Paracentrotus lividus* as the fed level. The experimental setup involved culturing *P. lividus* under controlled conditions (20°C) in V shaped Tank and fed with defrosted wild sea lettuce collected from the Santa Gilla Lagoon (Sardinia, Italy). *Ulva* sp. was concurrently cultivated in 2700L raceway provided with paddle wheel and artificial light (24h) to absorb the inorganic nutrients released through urchin excretion. Every week the water from the sea urchin tank is transferred to the raceway where Ulva growth. The nutrient absorption by *Ulva* sp. was estimated each week for one month.

Water quality parameters (ammonium, nitrate, nitrite and phosphate) were monitored weekly, alongside with *Ulva* sp. biomass growth and nutrient uptake efficiency. Results demonstrated that Ulva exhibited significant growth rates (20%) and effectively reduced inorganic nutrient concentrations (90 to 99% of nitrate), indicating efficient biofiltration capacity. These findings support the integration of Ulva in IMTA systems as a viable strategy for nutrient bioremediation and increased sustainability in echinoderm aquaculture.

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Short Biography of the speaker

My research focuses on the study of commercially important marine invertebrates, such as sea cucumbers and sea urchins. For years, I have been working on developing aquaculture protocols for these species, designing innovative solutions in pilot-scale facilities.

Currently, I am a researcher within the eINS project where I am involved in the development of a pilot integrated multi-trophic aquaculture (IMTA) system. This approach aims to move beyond monocultures, diversifying production and repurposing waste products in line with circular economy principles. At the same time, I am studing the use of algal photobioreactors to reduce excess nutrient loads and utilize the produced biomass for the extraction of bioactive compounds or the formulation of plant-based feeds for aquaculture, particularly for emerging species.

INNOVATIONS IN LAND-BASED SEAWEED AQUACULTURE

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Innovations in seaweed aquaculture and particularly in land-based systems, are rapidly transforming the industry, offering new solutions for food security, environmental sustainability, and industrial applications. Recent research highlights advances in cultivation technologies, integrated systems, and the development of value-added products, positioning seaweed as a key player in the global bioeconomy and climate change mitigation efforts. Some examples of technological advancements include: industry 4.0 and automation, Infrastructure innovations, integrated multi-trophic aquaculture. The combination of these is can also be a reality in the near future and not only enhance productivity and economic viability but also deliver substantial environmental benefits, positioning seaweed as a cornerstone of sustainable aquaculture and the global bioeconomy.

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Short Biography of the speaker

Presently the Director of the Centre for Innovation in Macroalgae at A4F – Algae for Future, a Portuguese company with more than 15 years of experience in the field of algae biotechnology. Trained as a Biologist, completed his PhD in 2004, working on the life-cycle of North Atlantic Porphyra and, after a typical research career, moved to the business world in 2012. Co-founded ALGAplus, where he stayed until 2020. As researcher, including also the years within the business field, accumulates the experience of participation in >20 national and international projects, published several book chapters and >40 scientific papers in peer reviewed journals. Current professional roles also include serving as member of the Portuguese committee for standardization of algae and algae products (CT211 and CENTC454); member of the industrial committee of the European Algae Biomass Association (EABA); leading WG02 – *Ulva* in aquaculture - of the Cost Action SeaWheat; leading WP2 of the European project INNOAQUA; serving as board member of the Kelp Ark (non-profit) organization.

STUDYING BACTERIAL INTERACTIOS IN *ULVA* WITH APLICATION IN INTEGRATED MULTITROPHIC AQUACULTURE: A COLLABORATIVE RESEARCH WITHIN *SEAWHEAT* COST ACTION

Pintado J.[1]*, Qui-Minet Z.[1], Bruni L.[1], Del Olmo G.[1], Wichard T.[5], Engelen A.[3], Aires T.[3], Sulpice R.[6], Rosignoli S.[6], Bibi F.[6], Garcia-Cardesín E.[2], Pérez-González A.[2], Lastres M.[4], Cremades J.[2]

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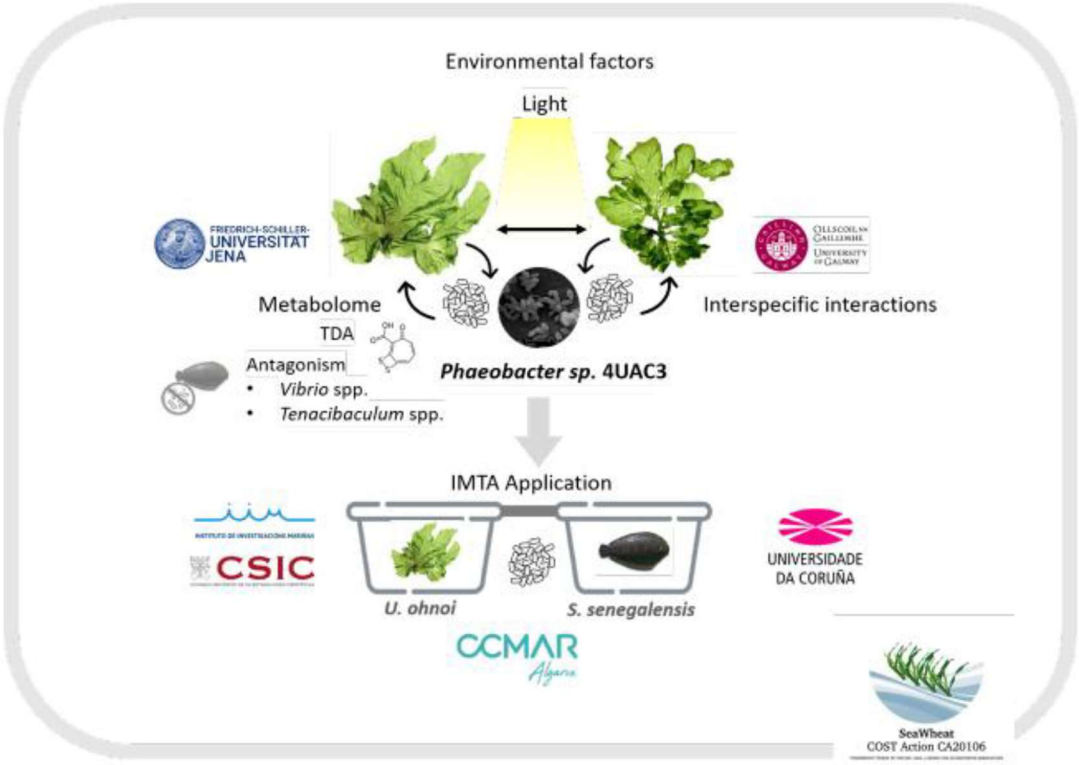
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The laminar thallus of *Ulva* provides an important niche for biofilm-forming bacteria, such as those of the genus *Phaeobacter*, which can antagonise fish pathogens such as *Vibrio* spp.and *Tenacibaculum* spp., through the production of tropodithyetic acid (TDA). *Phaeobacter* bacteria have been shown to be effective as a probiotic in aquaculture, reducing the mortality of fish larvae experimentally infected with Vibrio. Therefore, at IIM-CSIC the co-cultivation of *Ulva ohnoi* with *Phaeobacter* sp. 4UAC3, a strain isolated from *Ulva australis*, has been proposed as a strategy to tackle fish pathogens such as *Vibrio* spp. in IMTA-RAS. However, high light intensity impacts *Phaeobacter*'s persistence on *Ulva*, which did not occur in non-living surfaces. *Phaeobacter* sp. 4UAC3 colonization on *U. ohnoi* decreased in the light but remained stable in the dark [1]. To elucidate the underpinning mechanisms of this behaviour, a collaboration within the SeaWheat COST Action was established with Thomas Wichard's group at the University of Jena, to investigate how the colonization of *Phaeobacter* sp. strain 4UAC3 on *U. ohnoi* surfaces affects the alga's microbiome and metabolome dynamics. The light regime significantly altered the microbial community structure, metabolite production, and physiological responses of both the bacterium and the alga. In darkness, *Phaeobacter* sp. strain 4UAC3, produced TDA and modulated the microbiome and the exo- and endo-metabolomes of *U. ohnoi* [2]. Based on these results, a two-phase light

intensity approach to optimise antagonistic activity was developed for IMTA-RAS and scaled-up. The microbial communities are being analysed, in collaboration Aschwin Engellen's group at CCMAR, to understand the impact of *U. ohnoi* introduction and its microbial manipulation in the overall microbiome of IMTA-RAS at an industrial-scale. Whether *Phaeobacter* sp. 4UAC3 could differentially affect the physiology of different *Ulva* species, and whether the observed behavior of the influence of light on the maintenance of *Phaeobacter* on the surface of *U. ohnoi* also occurred in other *Ulva* species, a collaborative research, in the frame of the SeaWheat COST Action was established with Ronan Sulpice's group at the University of Galway. The comparative phenotyping of different *Ulva* species (cultivated and wild) revealed interspecific interactions which favoured the maintenance of *Phaeobacter* 4UAC3 even under high light intensity. These results, indicated a possible interaction between the compounds produced by some *Ulva* species with the microbiomes of other species present in the same environment. This opens a new approach to the study of bacterial interactions in *Ulva* spp., applying a populational perspective, not only applicable for IMTA-RAS but also to the understanding of mechanisms in green tides. Overall, this collaborative research, demonstrated the success of SeaWheat COST Action CA20106 to bring together researchers from different areas of expertise to tackle both fundamental and applied research related to *Ulva* species.

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REMOVAL OF PESTICIDES BY *ULVA* AND ITS ASSOCIATED MICROBIOM

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The global level of environmental pollution has steadily increased over the past years and continues to rise annually. Pesticides impact our environment, wildlife, and human health, and we need innovative ways to remove them1. It was demonstrated that the macroalgae *Ulva compressa* and its associated microbiome can metabolize xenobiotics like BPs and steroid hormones2 ,3.

High-throughput approaches were employed to investigate the potential of *U. compressa (cultivar U. mutabilis)4* for biological wastewater treatment with high temporal resolution. To ensure a high number of replicates for robust dose-response analyses, a semi-automated method for Pulse-Amplitude-Modulation (PAM) fluorometry was developed. This method enabled the assessment of the tolerance of *U. compressa* to selected xenobiotic compounds. Specifically, the impact of increasing concentrations on the maximum quantum yield of photosystem II, the electron transport rate, and oxygen production was evaluated.

Furthermore, removal experiments of selected pesticides by *Ulva compressa* and its associated microbiome were conducted. Therefore, targeted and untargeted mass spectrometry was performed to determine the removal rate, investigate the removal mechanism, and detect potential break-down products of the pesticides. We demonstrated that *U. compressa* and its associated microbiome could withstand high concentrations of a wide range of selected pesticides, up to mg/L level or higher. However, *Ulva* is less resistant against typical electron-transport inhibitors (e.g., hexazinone) but shows strong signs of a full recovery after periods with less exposure (Fig. 23A).

Furthermore, *U. compressa* can metabolize very efficiently specific hormones (e.g., hexestrol) entirely within four days into bromide derivatives (Fig. 23B). These findings highlight *U. compressa* and its microbiome as a resilient and promising system for sustainable, biologically driven wastewater treatment.

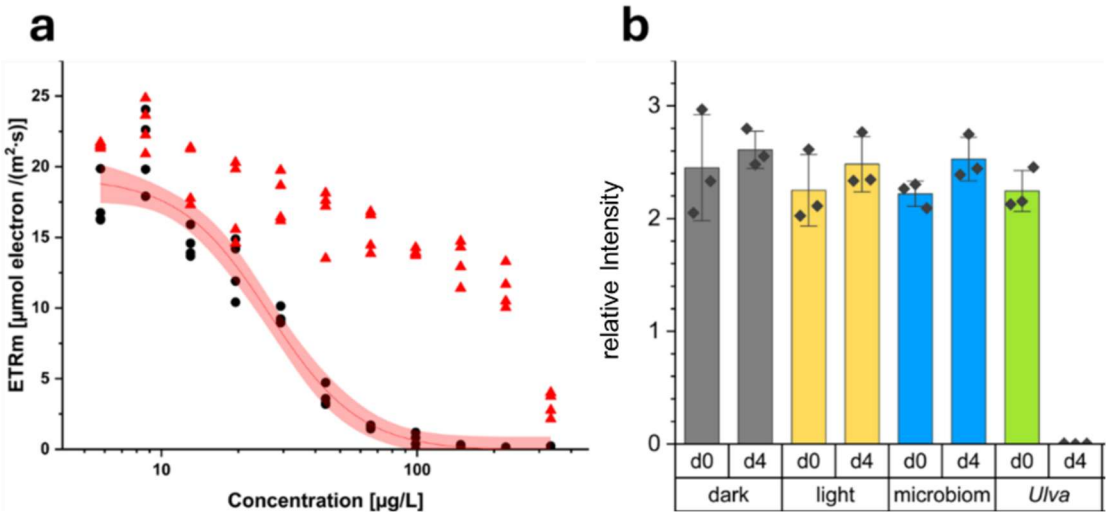


Figure 22 (a) Dose-response curve of hexazion intoxication (black circle) surveyed after 4 days by ETRm in U. compressa (EC50 = 26.5 ± 1.9 µg/L). ETRm was measured again after 4 days upon a growth medium exchange to test the detoxification potential. (b) Removal of hexestrol from the growth medium by U. compressa was compared with abiotic controls in dark or light, and the microbiome in the absence of Ulva.

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Short biography of the speaker

Simon Redlich is a PhD candidate in chemistry and a member of the Thuringian Water Innovation Cluster research group at the Friedrich Schiller University of Jena in Germany. He obtained a bachelor's degree in Biogeosciences and a master's degree in Chemistry – Energy – Environment. His research investigates the toxicological tolerance and xenobiotic removal capacity of *Ulva* and its associated microbiome.

BIOCHEMICAL DIVERSITY AMONG *ULVA* SPECIES ACROSS EUROPE

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Ulva is arguably one of the most abundant, widespread, and well-known seaweed genera in the world. However, its true species diversity and biochemical composition remain significantly underexplored, even in well-studied regions such as Europe. This study, conducted within the framework of the COST Action SeaWheat (E*ULVA*) project, investigates the metabolic diversity of green macroalgae *Ulva* spp. across a wide range of natural environmental conditions. A total of 495 samples from 15 different countries were collected from various habitats, including rocky and sandy shores, aquaculture systems, freshwater streams, lagoons, and channels, during different seasons (spring, summer and fall). Sampling sites featured in situ temperatures ranging from 13 to 30 °C and salinities from 0 to 52 ppt.

Key metabolic parameters, such as starch, *Ulva*n, chlorophyll a, chlorophyll b, carotenoids, total amino acids, and proteins, were quantified and analyzed in relation to environmental variables and species. Significant correlations between temperature/salinity and carbohydrate and pigment levels were identified. A focused analysis of sites sampled across multiple seasons further confirmed these trends, revealing site-specific and seasonal variability in the biochemical composition of *Ulva* species.

This work highlights the naturally variable biochemical profile of *Ulva* biomass and provides valuable information on species-environment associations, with future applications in biotechnology and environmental monitoring.

Acknowledgments

We would like to thank all the COST Action members who provided the samples and made this collaborative analysis possible. All those members will be co-authors in an ongoing publication.

Short Biography of the speaker

Clara Simon is a marine biologist who completed her PhD at the University of Galway (Ireland) in 2024 under the supervision of Dr. Ronan Sulpice. Her research focused on the green seaweed *Ulva* spp., studying the impact of environmental factors on its life cycle and physiology. Clara aims to understand how environmental changes affect *Ulva*'s metabolism and to explore the best ways of exploiting its valuable biomass. This research combines fundamental and applied science to understand how photosynthetic organisms like *Ulva* adapt to their environment offers valuable insights and potential uses in biotechnology.

INTENSIVE AQUACULTURE AT A TURNING POINT: FROM MONOCULTURE TO INTEGRATED SYSTEMS – A NECESSITY BUT OPPORTUNITY AS WELL

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Aquaculture practices can be broadly classified into "food" and "business" aquaculture [1]. The former, practiced for centuries in China and other Asian countries, has always been based on polyculture principles, resulting in an ecologically sustainable approach with minimal environmental impact. This form of aquaculture primarily provides food for local consumption. In contrast, business aquaculture emerged in Japan in the 1960s and expanded to the Western world over the following decades. Driven by advancements in the understanding of the biology of high-value species (such as various fish and crustaceans), innovative techniques in broodstock maturation, hatcheries, and grow-out systems (in ponds or cages) led to the development of industrial-scale practices. Business aquaculture, particularly the use of monoculture systems for grow-out, initially had low environmental impacts. However, as market competition intensified in the 1980s, the industry began to adopt higher stocking densities and more formulated feeds, which resulted in greater environmental impacts and an increased incidence of diseases caused by bacteria, viruses, and parasites. In the past decade, sectors like Penaeid shrimp farming in Asia have faced major catastrophes, with significant socio-economic consequences. It remains a hypothesis, but many farms may be operating beyond the local environment's carrying capacity. There is a pressing need to shift the focus in aquaculture from disease treatment to prevention. The current reliance on disinfection methods, which indiscriminately kill both harmful and beneficial bacteria, highlights the need for new microbial management strategies. Shrimp and marine fish cultured in recirculating aquaculture systems (RAS), where biofilters enhance microbial competition, appear to be less susceptible to unpredictable losses. Similarly, integrated farming practices such as Integrated Multi-Trophic Aquaculture (IMTA) – which combines fish, seaweed, and shellfish farming in coastal areas – or zero-water exchange intensive shrimp farming, where effluent is recirculated through tilapia and seaweed ponds before returning to shrimp ponds, seem to provide similar benefits. While still under investigation, these integrated systems may offer indirect microbial management benefits, potentially leading to improved overall performance.

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Short Biography of the speaker

Patrick Sorgeloos has a PhD in marine biology and set up the *Artemia* Reference Center at Ghent University in Belgium in 1978. He has been involved in brine shrimp *Artemia* projects and fish/shellfish hatchery developments all over the world. Until his retirement as emeritus professor in 2013 over 350 Master and 70 PhD alumni graduated at Ghent University in the field of aquaculture.

As Past-President of the World Aquaculture Society, and co-founder and board member of the European Aquaculture Technology & Innovation Platform, Patrick is a strong promoter of international networking in aquaculture and is still involved with many international aquaculture organizations. He was co-founder of the Ghent University spin-off company Artemia Systems that is now operating under the name of INVE Aquaculture. He received honorary awards in China, Egypt, Greece, India, Malaysia, Russia, Thailand, USA, and Vietnam.

FROM GREEN WAVES TO GREEN FARMS – HOW BASIC RESEARCH ON SEA LETTUCES ENABLES A SUSTAINABLE BLUE ECONOMY

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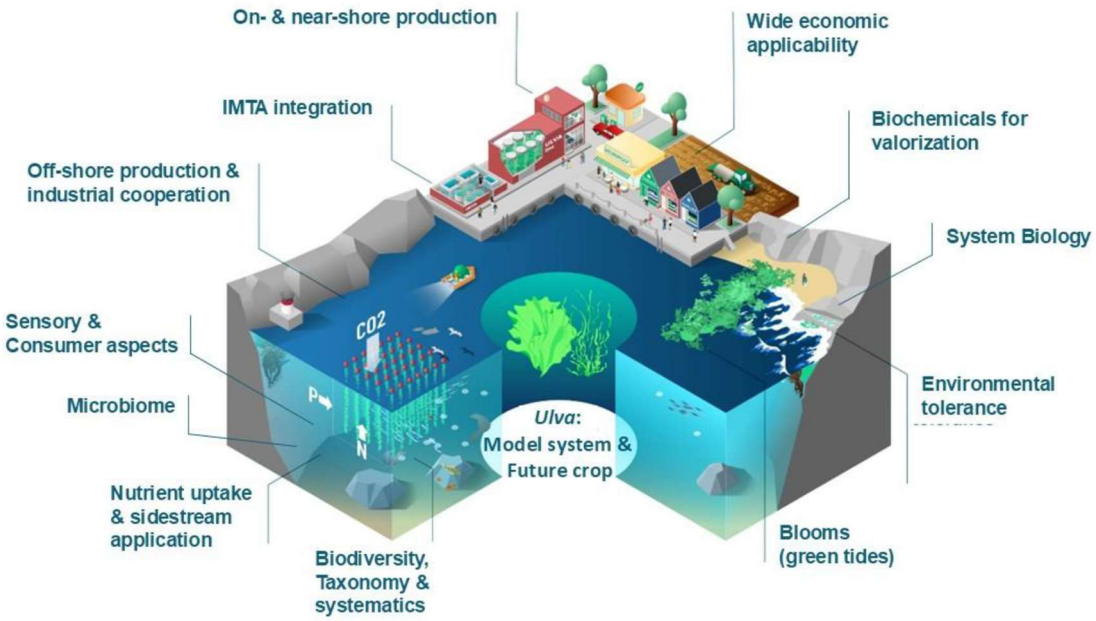


Figure 23 Graphical Abstract: Ulva as a model system and emerging aquaculture crop: Integrative research on Ulva exemplifies how basic research can drive innovation in the Blue Bioeconomy

The green macroalga *Ulva* is emerging as a powerful model system for both fundamental and applied research, bridging key questions in system biology with urgent challenges in sustainable resource production. Characterized by extreme morphological plasticity and wide ecological distribution, *Ulva* offers unique opportunities to study genotype–environment interactions, adaptation, and ecotypic variation across contrasting marine habitats. Our research integrates aquaculture studies with high-resolution genomic analyses to dissect the drivers of phenotypic variability and to identify adaptive traits critical for growth and resilience. In particular, the Atlantic-Baltic Sea transect — with its strong environmental gradients — serves as a natural laboratory for understanding how *Ulva* responds to fluctuations in salinity, temperature, and nutrient availability. These insights not only inform our understanding of plasticity and local adaptation but also guide the selection of high-performing ecotypes for industrial yet sustainable cultivation. As a fast-growing organism rich in protein and essential nutrients, *Ulva* presents exceptional potential as a future crop—offering sustainable solutions for biomass production, bioremediation (including the valorization of food industry side-streams), and the development of bio-based materials. By positioning *Ulva* as both a model organism and a future crop, this research framework supports the co-development of basic biological insights and scalable aquaculture practices. Ultimately, our work lays the foundation for a resilient blue economy—where fundamental science and applied innovation jointly shape sustainable food and resource systems of the future.

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Short Biography of the speaker

Sophie Steinhagen is associate professor at the University of Bergen (Norway) and docent at the University of Gothenburg (Sweden). She investigates seaweed biodiversity, phylogenetic relationships, and species-specific traits within seaweeds. Her current research explores the interplay between environmental factors and genomic set up of seaweeds, unravelling the secrets behind the content of high-value compounds and setting baselines for breeding programs in European seaweed crop strains to support a sustainable seaweed aquaculture.

EU SUPPORT TO ALGAE SECTOR DEVELOPMENTS

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The presentation "EU Support to Algae Sector Developments" will provide an overview of the European Commission's strategic actions to unlock the full potential of algae as a key enabler of the green and blue transitions. Anchored in the **EU Algae Initiative**, this support spans policy development, funding, and stakeholder engagement, aiming to create a more robust, sustainable, and innovative algae ecosystem across Europe. Key efforts include the launch of **EU4Algae**, a multi-stakeholder platform facilitating collaboration, knowledge exchange, and market access; substantial funding allocations to **algae-related research and innovation projects** under Horizon Europe and other EU programmes; and the development of **evidence-based studies** to address regulatory and market barriers. Additionally, the Commission has taken proactive steps to raise **visibility and awareness** of the sector, notably through the organization of the **EU Algae Awareness Summit** and tailored **BlueInvest events** designed to attract private investment and accelerate algae entrepreneurship. Collectively, these initiatives reflect the EU's commitment to positioning algae as a high-impact, low-carbon solution for food, feed, materials, and ecosystem regeneration.

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Short Biography of the speaker

Maris Stulgis holds a university-level diploma in Water Bioresources and Aquaculture from Kaliningrad State Technical University. Prior to joining the European Commission, he spent 10 years in the Latvian national administration, focusing on environmental protection and fisheries control policy development and implementation. As a Policy Officer at DG MARE, he has worked on fisheries control policy - including the development of new control technologies and leading EU inspector teams in the Baltic and North Sea regions - as well as on fisheries data management, marine pollution, biodiversity, and environmental policy. Since 1 October 2020, he has been responsible for the Blue Bioeconomy, Algae, and Marine Aquaculture.

USING MARINE FINFISH HATCHERY EFFLUENT TO GROW *ULVA SP.* IN A PILOT LAND-BASED IMTA SCHEME

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Ulva sp. seedlings, collected from a nearby natural macroalgae bed, were used to initiate a land-based pilot seaweed aquaculture system. The algae were cultivated in translucent HDPE tanks, each with a capacity of 1 m³. Water from the sedimentation tank—the final stage of the biological water treatment process of the marine finfish hatchery at Kefalonia Fisheries SA—was continuously flowing through the tanks, supplying inorganic nutrients (NH_4-N, NO_3-N, NO_2-N, PO_4-P, etc.) with a residence time of 20 minutes.

This hatchery effluent originated from the treatment of runoff water from broodstock, larvae, and fry tanks used for rearing sea bass (*Dicentrarchus labrax*) juveniles before their transfer to offshore sea cages.

Ulva sp., introduced at an initial loading of 1 kg per m³, grew well in the effluent, which had an average concentration of 0.87 mg/L total dissolved inorganic nitrogen and 0.62 mg/L inorganic phosphorus. Since January 2025, the integrated multi-trophic aquaculture (IMTA) system using *Ulva* sp. achieved an average daily growth rate of 10.870% (±7.802%), with lower rates observed in spring (4.827%, ±2.438%) and higher rates in early summer (18.927%, ±1.812%).

The success of this pilot *Ulva* sp. cultivation—effectively contributing to both additional treatment of the hatchery effluent and biomass production—supports the planned scale-up to treat the full volume of the farm's discharge. Discussions are currently underway regarding the use of the produced biomass as raw material for agro-industrial applications, such as biostimulants and biofertilisers.

Short Biography of the speaker

Eva Troianou graduated from the Department of Biological Applications and Technologies at the University of Ioannina, Greece, and went on to complete a master's degree in Evolutionary Genetics at the University of Edinburgh. She has also worked with the Population Genetics Group at the Institute of Marine Research (IMR) in Bergen, Norway.

In 2018, she returned to her homeland, Kefalonia, where she currently works in the Research and Development Department of Kefalonia Fisheries. She is involved in national and European research programs, which focus on the cultivation of *Ulva* at an industrial pilot scale, among other topics related to macroalgae and sustainable aquaculture.

A QUICK VIEW OF THE *ULVA* INDUSTRY & THE SEAWHEAT SME PLATFORM

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Europe, as a continent, lags behind other world regions in algae production. Specifically, regarding marine macroalgae (seaweeds), Europe contributes less than 1.4% to global production, with most of its supply being harvested from the wild. There are fewer than 200 companies involved in macroalgae production, and less than 20 of those either cultivate or collect *Ulva*. In contrast, over 500 companies, employing more than 2000 individuals, are engaged in the processing and product development of macroalgae, with over 40 companies incorporating *Ulva* into their product lines. The market for seaweed-based products, particularly in the cosmetics and meat alternatives industries, appears very promising despite the often negative connotation associated with the term "seaweed."

In the context of the COST action SeaWheat, we have developed a platform to facilitate interactive networking among all stakeholders involved in the research, production, and valorization value chain. Additionally, we have created a specific platform for exclusive communication between participating companies, known as the SeaWheat SME platform. Approximately 25 companies have expressed interest and joined this platform to gain access to firsthand information and foster idea exchange between experts and commercial entities.

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I am indept to Karīna Bāliņa (Latvia University) and Céline Rebours (Moreforsking) for their help in working with the SME platform

Short Biography of the speaker

Dr. Ioannis Tzovenis, with over 30 years of experience in algae biotechnology and aquaculture, founded MIKROFYKOS, a company that advises Spirulina producers and develops natural products from algae. A graduate of the National and Kapodistrian University of Athens and a Ph.D. holder from Ghent University, he taught for a decade at the Technological Educational Institute of Epirus in Greece. He is a member of several professional organizations, including EAS, EABA, ISAP, FELPS/HELPS, GEOTEE, and EBEA. Dr. Tzovenis has extensive experience in marine fish hatcheries, fish larvae nutrition, shellfish and algae technology, and integrated multitrophic aquaculture. He has delivered over 100 scientific presentations, has over 40 publications, and has participated in more than 40 EU or nationally funded projects.

UNRAVELLING DIVERSITY AND DISTRIBUTION PATTERNS IN *ULVA* THROUGH A PAN-EUROPEAN COLLABORATIVE EFFORT

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Ulva is one of the most abundant, widespread, and well-known genera of seaweeds globally. However, identifying individual species within this genus based on morphology alone is extremely challenging due to their high phenotypic plasticity and overlapping traits. As a result, the true diversity of *Ulva* species remains largely unknown—even in extensively studied regions like Europe. To address this gap, the European *Ulva* Taxonomy Initiative (E*ULVA*) is undertaking a comprehensive assessment of *Ulva* species diversity and their geographic distribution. In collaboration with 180 researchers, the E*ULVA* consortium has collected nearly 4,000 samples from diverse habitats across the Northeast Atlantic, the Mediterranean, and Macaronesia. This dataset spans 25 countries—from Morocco to Svalbard and from Iceland to Azerbaijan—and includes environments such as coastal zones, freshwater systems, and salt marshes. To efficiently analyze this large dataset, we developed a dual-barcoding approach using Oxford Nanopore Technologies. This method allows us to pool approximately 800 samples per sequencing run and target multiple full-length genetic markers (ITS, tufA, rbcL). By integrating these data with all relevant GenBank sequences from the region, we reassess species diversity and distribution, offering new insights into species concepts and biogeographic patterns. Our sequencing strategy has proven highly effective, achieving identification rates of 92–98% per 96-well plate. This represents a significant advancement in species discovery, enabling the detection of non-native and bloom-forming species, as well as the co-sequencing of small epiphytic and endophytic taxa such as *Ulvella* and *Acrochaete*.

Acknowledgments

This work makes use of resources and facilities provided by UGent as part of the Belgian contribution to EMBRC-ERIC (FWO I001621N).

Short Biography of the speaker

Olivier De Clerck is an expert on seaweed diversity, genetics and evolutionary biology. With his group at Ghent University he actively develops genomic resources, including genome editing techniques, for *Ulva*. He also studies the interaction of *Ulva* with microbiota (bacteria and viruses).

SEAWEED FEED AND FOOD SAFETY: EU POLICY AND OUTLOOK

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The EU legislation on contaminants in food, Council Regulation (EEC) No 315/93 of 8 February 1993 provides that food containing a contaminant in an amount which is unacceptable from the public health viewpoint shall not be placed on the market (food can only be placed on the market when it is safe).

Furthermore, it is foreseen that contaminant levels shall be kept as low as can reasonably be achieved by following good practices at all stages of the production chain. In order to protect public health, maximum levels for specific contaminants shall be established where necessary. Also, the consultation of European Food Safety Authority (EFSA) for all provisions which may have an effect upon public health is mandatory.

Following requests of the European Commission, the Panel on Contaminants in the Food Chain (CONTAM) from the European Food Safety Authority (EFSA) has completed in recent years several scientific opinions on contaminants, including environmental contaminants, reviewing the possible risks for human health due to the presence of these substances in food, including seaweed. As follow-up to the outcome of the EFSA opinions, maximum levels are established in foods, including seaweed by Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006.

The consumption of seaweed is increasing and the presence of contaminants in these foods might contribute significantly to human exposure to these contaminants. In order to be able to estimate that contribution, EFSA has issued in 2023 a report on "Dietary exposure to metals and iodine via consumption of seaweed and halophytes in the European population. Currently maximum levels for certain metals (such as lead, cadmium and arsenic) in seaweed for human consumption are under discussion.

Taking into account the possible animal and public health concern related to the presence of undesirable substances in feed, the undesirable substances are regulated at EU level in feed to ensure a high level of animal and human health and environmental protection.

Directive 2002/32/EC of 7 May 2002 of the European Parliament and of the Council on undesirable substances in animal feed is the framework for the European Union action on undesirable substances in feed.

Following requests of the European Commission, the Panel on Contaminants in the Food Chain (CONTAM) from the European Food Safety Authority (EFSA) has completed several scientific opinions on undesirable substances in feed, reviewing the possible risks for animal and human health due to the presence of these substances in feed and their presence in food of animal origin following the transfer from feed.

The use of seaweed as feed material is increasing also due to a favourable impact on the environment (such as lower methane emissions).

In the presentation recent developments and an outlook as regards EU policy on contaminants/undesirable substances in seaweed to ensure a high level of animal human health protection will be explained in detail. Also, more details will be provided how the outcome of EFSA opinions on contaminants/undesirable substances is followed up in EU regulation thereby referring to recent examples.

Short Biography of the speaker

Frans Verstraete graduated in 1985 as agricultural engineer at the University of Ghent (Belgium). After his studies he held positions at the University of Ghent and thereafter at the Belgian Ministry of Agriculture and he was for a period technical adviser of the Belgian Minister of Agriculture. He is working for the European Commission since 1997. In the European Commission he is working at the Directorate General Health and Food Safety. He is responsible for the elaboration, development and management of the EU-legislation on certain contaminants in feed and food

ADVANCES AND STRATEGIES FOR IDENTIFYING NOVEL MARINE BIOACTIVES WITH INSIGHTS FROM BACTERIA-ALGAE INTERACTIONS

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The marine environment harbors many organisms that produce potentially novel bioactive compounds with promising applications in pharmaceuticals, biotechnology, and industry [1, 2]. Among these, macroalgae have gained attention due to their rich bouquet of chemical compounds and ecological significance. Recent advances highlight the critical role of bacteria-macroalgae interactions in modulating the production of metabolites in macroalgae [3].

In *Ulva*, for example, microbial symbionts influence algal development, defense mechanisms, and metabolic outputs, thereby shaping the profile of bioactive compounds. This presentation explores current strategies for identifying novel marine bioactives, specifically focusing on the *Ulva* holobiont and the associated microbial consortia.

Approaches such as co-cultivation experiments, metagenomic and metabolomic profiling, and systems biology tools have deepened our understanding of the molecular cross-talk between *algae* and their associated bacteria. The selection of species, cultivation techniques, experimental design, harvesting strategies, and improved analytical approaches all work together to enhance the identification of interesting compounds.

My presentation will highlight insights that reveal the potential for discovering unique natural products in macroalgae. In this context, understanding ecologically relevant microbe-host interactions may support the identification of novel compounds, as demonstrated by the discovery of (-)-thallusin in the *Ulva* holobiont [3]. Unlocking the biochemical potential of *Ulva*-bacteria systems may pave the way for innovative applications in environmental sustainability of aquacultures and novel approaches in agriculture.

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Short Biography of the speaker

Thomas Wichard is a research group leader and lecturer in analytical chemistry at the Institute for Inorganic and Analytical Chemistry of the Friedrich Schiller University Jena (Germany). After being awarded a PhD in biochemistry (Max Planck Institute for Chemical Ecology), he investigated the metal recruitment of nitrogen fixers at the Princeton Environmental Institute (USA). His team applies various analytical chemistry, chemical ecology, and molecular biology methodologies to understand the basis of eco-physiological processes in bacteria-macroalgae interactions (cross-kingdom interactions).

Ulva

The Future Wheat of the Sea

Workshops



From Ulva aquaculture to food and feed production: state-of-the-art, bottlenecks, risks and gaps

Lisbon, Portugal

May 23-24, 2023

Ulva
The Future Wheat of the Sea

ORGANIZERS

Dr Rui Pereira, A4F – Algae for future, WG2 leader
Dr Sylvia Strauss, The Seaweed Company, WG3 leader

The COST Action SEAWHEAT (https://seawheatcost.haifa.ac.il/) and the companies A4F and The Seaweed company organized a 2-day Workshop on the technical aspects of *Ulva* cultivation (WG2) and its utilization for food and feed (WG3), from May 23-24, 2023. It was hosted by A4F, Algae for Future at the ALGATEC Eco Business Park (https://algatec.eu/), in Lisbon, Portugal.

RATIONAL AND OBJECTIVES

This workshop intended to promote a closer cooperation between *Ulva* experts and end users in the food and feed sector. Despite being one of the most common seaweed species in nature (with world-wide distribution) one of the most studied and a genus already accepted as a food ingredient in Europe, *Ulva* production and utilization is almost insignificant. Part of that can be due to a lack of coordination between the producers and the specialized end-users of food and feed biomass, with a much closer knowledge of the market needs. In other words, there is a sort of gap between what the SMEs are producing and what the market needs. The all process is often a vertical chain, with the *Ulva* producers also doing product development and commercialization.

This workshop is thought to bring together seaweed experts in the *Ulva* production and product development with experts in the food and feed sectors in general. Specific objectives are to identify bottlenecks and gaps and draft a common strategy to overcome those. The outcome would be a significant contribution towards the overall SEAWHEAT goal, which is the development of *Ulva*-based blue-biotech industries and the promotion of *Ulva* as a model organism in European algaculture.



ORGANIZATION

The workshop was part of the planned list of events for the current grant period, a joint organization between the leaders of WG2 and WG3, "*Ulva* in Aquaculture" and "*Ulva* in feed and food". The Workshop program was discussed and decided among a group of experts that constituted the scientific committee. These were (listed alphabetically): Anna Fricke (Germany); Bela Buck (Germany), Muki Shpigel (Israel); Raquel Quintã (Portugal); Rui Pereira (Portugal) and Sylvia Strauss (The Netherlands).

In addition to the organizing committee, it is also important to highlighting the role of the Chair Sessions. Anna Fricke (Leibniz Institute of Vegetable and Ornamental Crops) and Alex Golberg (Tel Aviv University) chaired the session on "*Ulva* Post-Harvesting and other emerging issues"; Mariana Doria (A4F) chaired the session on "Algae Biorefinery; Raquel Godinho (Algikey) and Sylvia Strauss (The Seaweed Company) chaired the sessions on "*Ulva* in Food and Feed" and Raquel Quintã (S2AQUA - Collaborative Laboratory, Association for a Sustainable and Smart Aquaculture) and Erick Malta (CTAQUA - Centro Tecnológico de Acuicultura de Andalucía) chaired the session on "Engineering and Production).

PARTICIPANTS *IN SITU*

The workshop was participated by 38 experts (5 of which presented online) from 15 different countries (including 32% of participants from ITC countries), affiliated to SMES (34%) or Academic institutions (66%). In figures 1 and 2 we present more statistical details of the participants.

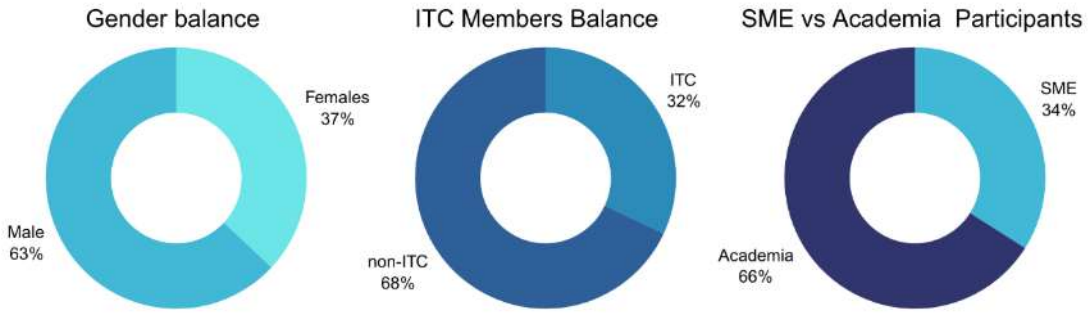


Figure 24 Distribution of the Workshop participants by gender, members of ITC countries and affiliation.

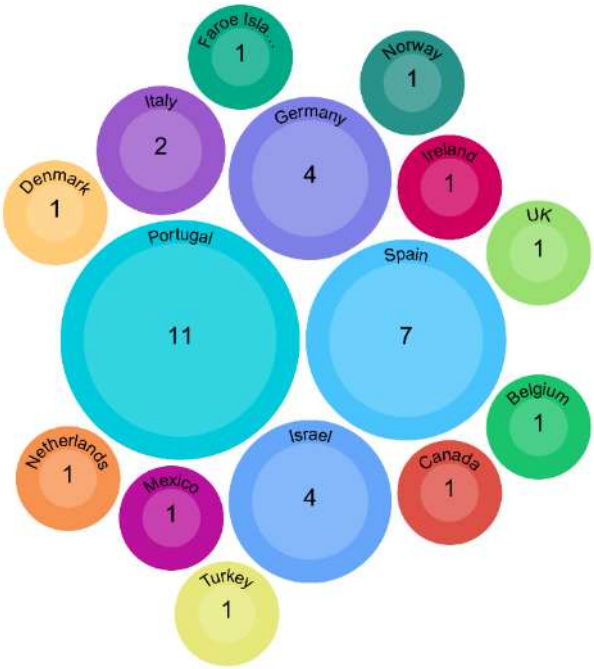


Figure 25 Distribution of the workshop participants by country according to their affiliation.



Figure 26 Group photo of the participants of the workshop held in Algatec Eco Business Park

In addition to the main group of 38 participants with interaction possibilities (including 5 online speakers), it was also possible to organize the live streaming of the presentations and final discussion moments. The access to that was completely free but required a registration. A total of 133 people registered for the live streaming during

the 5 initial sessions, divided into 79 that are already Cost Members and 54 people that were not registered Cost Members at least at that time.

WORKSHOP DESIGN AND FUNCTIONING

The workshop was designed to include presentation moments by topic, including 30 minutes for discussion of each topic, half day of discussions in parallel breakout groups spread in two 1hr sessions and a technical visit to Green Aqua's algae production facility. In summary, there were 7 sections, divided in presentation sessions and breakout groups for discussions. Topics covered during the sessions, in line with WG2 and WG3 and decided by the scientific committee were: Session *1 – Post-Harvesting processes and other emerging issue; Session 3 – Ulva in food; Session 4 – Ulva in Feed; Session 5 – Engineering and Production*. In addition, Session 2 of the program was a side-event on "*Algae Biorefinery*", organized by partners of the European Project MULTI-STR3AM and with representatives of the projects CIRCALGAE, MULTISTR3AM, SEAMARK as well as from SINTEF. For more details on the program, please see Annex I at the end of this report.

Each session comprised between 4-6 presentations and included 30 minutes for discussion of the correspondent topic. A total of 22 talks, including 2 plenary talks by our international guest speakers (Dr José Zertuche from Mexico and Dr Alan Critchley from Canada) set the stage for the discussion moments. Also relevant to note the role of the sessions chairs. Each session had 2 designated chairs, with the mission to a) keep time and moderate the discussions during the presentation moments and b) animate and manage the discussions during the group breakout sessions for each topic, including organizing and presenting the final outcome of those discussion.

Prior to the beginning of the workshop, the designated Session Chairs received an information package for their session, including a short-bio of each of the speakers as well as the abstracts for each presentation.

The afternoon of Day 2 was fully dedicated to discussions and brainstorming in breakout groups. Two parallel sessions were ongoing in different rooms and the participants were free to travel between groups and participate as much as possible. After approximately 1 hour the discussion topics changed and gave room to two more parallel sessions with the same functioning.

The workshop concluded with each session chairs presenting the main conclusions for their topic. The document summarizing those conclusions is still an "in process document", but a draft can be found in Annex II of the present report.

Finally, last but not least, the participants were taken on a guided tour to Green Aqua's algae production facility. Dr Luis Costa (A4F Board Member) explained the functional of the entire site, the technologies involved, the capacities and the synergies with other industrial partners at the site.



Figure 27 Different moments of the breakout groups for discussion.



Figure 28 Different moments of the coffee break with seaweed products degustation.



Figure 29 Different moments of the visit to Green Aqua's Algae production facility.

CONCLUSIONS

The workshop "From *Ulva* aquaculture to food and feed production: state-of-the-art, bottlenecks, risks and gaps" was a very enriching event for all participants, judging from the lively discussions (that sometimes extended beyond the planned time) and from the feedback of the participants at the end of the event. The level of participation of the online viewers was also very unexpected, since that was not in the initial plans and, therefore, was not announced but a few days before the event. An e-Book of Proceedings is also under preparation, with extended abstracts provided by the speakers and students that presented posters.

Most important, we feel that the objectives of the workshop were attained. The SME participants represented 34% of the workshop population assuming relevant roles during the different sessions (both presenting their experiences and chairing sessions) and participating actively in the discussions, thus clearly contributing to reinforce the interaction between the former and the Academia partners.

In terms of the specific objectives defined, the discussion sessions resulted in information regarding "Bottleneck & Challenges" and "Proposals for solutions" in all 4 discussion topics.

As already mentioned, Annex II of the present report, it is possible to find the draft documents produced by the session chairs, with the main conclusions of the discussion groups. Very briefly, in Session 1 (**Post-harvesting processes and other emerging issues**), the participants elected and classified 5 main topics – **Scale and price**; **Feedstock quality**; **Technology**; **Logistics**; **Market**. In addition, 2 main gaps were identified (**Technical**

Economic Analysis and **Knowledge transfer**). For possible solutions, the group pointed to **Partnerships**, **Specific *Ulva* solutions**, **Research in pilot scale** and **Mobile Processing Units**.

In sessions 3 & 4 the participant started by dividing the topics related to *Ulva* in Food into 1. Cultivation and Processing and 2. Product Development and Consumer Acceptance. For each sub-topic they identified several Bottlenecks and Challenges and proposed solutions. Some of the issues identified in the first sub-topic overlap with issue also identified in the other sessions (*e.g.* **Unstable quality**, **Production Costs**, **Investors** and **Subsidies**, **Regulation**, **Upscaling**). In the Product Development and Consumer Acceptance, the main bottlenecks identified were **Valorization** of biomass, Lack of **consumer acceptance**, Lack of **knowledge & information** about seaweed as food, **Food neophobia**, Lack of **Marketing + Communication** strategies, with proposals for solutions for all of those.

Finally, in session 5, the main problem identified was the relatively high price per biomass unit. There was also a consensus that there is a need to increase productivity. Based on this starting point, two groups of solutions are proposed, one classified as Biological Solutions (*e.g.* **strain selection**, **specialization** of the companies, ***Ulva* hatcheries**, **modelling**, bioengineering and application of **holobiont**, **optimization** of **production based** on **market study**) and Engineering Solutions (e.g. **technological solutions**, **mechanization**, strategical **partnerships**, **large scale** cultivation, **energy-saving** solutions for cost reduction, data collection and processing for **site selection**).



From Ulva aquaculture to food and feed production: state-of-the-art, bottlenecks, risks and gaps

Lisbon, Portugal

PROCEEDINGS

Ulva

The Future Wheat of the Sea

INTRODUCTION

This document compiles the information presented by the speakers and poster presenters (students) at the SEAWHEAT workshop organized by WG2 and WG3 in Lisbon, Portugal, during 23-24 May, 2023.

The workshop intended to promote a closer cooperation between *Ulva* experts and end users in the food and feed sector. Despite being one of the most common seaweed species in nature (with world-wide distribution) one of the most studied and a genus already accepted as a food ingredient in Europe, *Ulva* production and utilization is almost insignificant. Part of that can be due to a lack of coordination between the producers and the specialized end-users of food and feed biomass, with a much closer knowledge of the market needs. In other words, there is a sort of gap between what the SMEs are producing and what the market needs. The all process is often a vertical chain, with the *Ulva* producers also doing product development and commercialization.

The workshop was thought to bring together seaweed experts in the *Ulva* production and product development with experts in the food and feed sectors in general. Specific objectives were to identify bottlenecks and gaps and draft a common strategy to overcome those. The outcome intends to be a significant contribution towards the overall SEAWHEAT goal, which is the development of *Ulva*-based blue-biotech industries and the promotion of *Ulva* as a model organism in European algaculture.

The abstracts are organized in their order of presentation at the workshop.

The poster abstracts are grouped at the end and organized in alphabetic order of the first author.

PROGRESS AND CHALLENGES IN *ULVA* BIOREFINERY. HOW TO SCALE UP?

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Biorefinery, as used in the Biorefinery Euroview report, is: “Integrated bio-based industries, using a variety of different technologies to produce chemicals, biofuels, food and feed ingredients, bio materials (including fibers) and power from biomass raw material“ (Euroview, 2010). Biorefineries are the manufacturing units of bioeconomies. In a biorefinery, one or several biomass feedstocks (crops, lignocellulosic biomass, seaweed, microalgae, insects, etc.) are processed into a wide range of products targeting zero waste and minimal green house gases emissions. Furthermore, the key advantage of biorefineries is the capacity to produce multiple products from the same raw materials not only it increases the revenue per mass of feedstock, but also diversify the applications thus making the whole system economically resilient (Golberg, Liberzon, et al., 2020). This economic analysis of co-production has been recently demonstrated in seaweeds (Palatnik et al., 2023).

The co-production of multiple products usually refers to the use of multiple subsequent processing steps. Each step leads to the production of one or several different products (Golberg, Robin, et al., 2020). Since each step removes a fraction of the biomass, concentrating the leftover, the subsequent steps are simpler and more efficient. For example, in the case of *Ulva sp.* biomass, by extracting protein and water soluble molecules in a first step, the content and purity of residual water-soluble polysaccharides such as ulvan increases (Golberg, Robin, et al., 2020). This is critical since the processing to achieve a certain level of concentration and purity is a major economical and technological hurdle of biorefinery. In addition, certain processes such as crushing, milling, and other cell and tissues disruptions will usually benefit most the subsequent processes by improving the contact surface of the biomass and the accessibility of intracellular components without drying as the intermediate process. Therefore, the energy and cost of such process benefit the entire processing chain instead of a single product (Zollmann et al., 2019).

The major current challenge resides in designing the biorefinery, i.e to chose the processes, equipment and to integrate the processes into one optimized process flow (Golberg, Robin, et al., 2020). This is a challenge because biomass processes and equipment are usually designed for the sole production of one product and not the preservation of the left over for subsequent processing targeting zero waste. Their integration required the understanding of the effect of each process on each constituent of the biomass, which are the product target of the subsequent processing steps. As for today, there is no widespread and successful standard for industrial seaweed biorefinery design. Although a considerable effort is being deployed to answer those two technological gaps (better bioprocesses and integration of those processes) at the research level (Table 1), there are still gaps in industrial research and development and large scale production to fill.

Table 1 Ulva biorefinery. Co-production of various ingredients from Ulva and their transformation to additional products

Species	Directly co-extracted products	Products produced by transformation of *Ulva* derived ingredients	Reference
Ulva lactuca	water-soluble proteins and carbohydrates		(Postma et al., 2017)
U. lactuca	protein and carbohydrates	glucose, rhamnose and xylose, acetone, butanol, ethanol, and 1,2-propanediol	(Bikker et al., 2016)
U. fasciata	mineral rich liquid extract, lipid, ulvan, and cellulose	ethanol	(Trivedi et al., 2016)
U. lactuca	mineral rich liquid, lipid, ulvan, protein, and cellulose		(Gajaria et al., 2017)
U. lactuca	water-soluble carbohydrates	acetone, butanol, and ethanol (ABE)	(van der Wal et al., 2013)
U. rigida	carbohydrate, salt, concentrated protein		(Pezoa-Conte et al., 2015)
U. ohnoi	salt, pigment, ulvan, and protein		(Glasson et al., 2017)
U. lactuca	mineral rich liquid extract, ulvan, protein	methane	(Mhatre et al., 2019)
U. ohnoi	salts, starch, lipids, ulvan, proteins, and cellulose.		(Prabhu et al., 2020)
Mix of U. rigida and *U. fascia*	hydrochar, 5-HMF, monosaccharides, proteins, peptides.	ethanol	(Polikovsky et al., 2020)
U. ohnoi	Mix of monosaccharides	ethanol	(Jiang et al., 2016)
U. ohnoi	Starch, Proteins, and minerals		(Prabhu et al., 2019)
U. ohnoi	hydrochar	polyhydroxyalkanoates	(Ghosh et al., 2021)
U. lactuca	polysaccharides, proteins		(Andrade et al., 2022)
Mix of *Ulva* species	monosaccharides		(Robin et al., 2017)
Ulva sp. not defined	Polysaccharides	biodiesel	(Ruangrit et al., 2023)
U. ohnoi	hydrochar, monosaccharides	polyhydroxyalkanoates	(Steinbruch et al., 2020)
U. lactuca	antioxidants and phenolic compounds		(Rashad et al., 2021)
Ulva sp. not defined	ulvan	biogas, polyhydroxyalkanoates	(Arul Manikandan & Lens, 2023)
Ulva rigida and Ulva ohnoi.	Water soluble and insoluble protein		(Robin et al., 2018)
U. lactuca	Bio-oil, hydrochar	bioethanol	(Sharmiladevi et al., 2021)

When processing seaweeds using biorefinery approach it is possible to define two types of products (Golberg et al., 2014). The first type includes extracts directly separated from the seaweed biomass. In *Ulva* biorefinery, for example, separation of salt, cellulose, ulvan, starch, proteins, lipids, simple monosaccharides and peptides has been reported in various process configurations. The second type includes products produced by transformation of the first type using biological, chemical or thermal catalyzes. The summary of the published works which address co-production of various ingredients from *Ulva* and their transformation to additional products appears in **Table 1**.

The next critical steps in the development of *Ulva* biorefinery is demonstration of processes using common to food and chemical industry process steps and equipment, which can be scaled. The must to get output of these efforts will be a mass and energy balances for each step as well as decision on scalable equipment. Next steps should include process simulation using common industry tools such as AspenPlus or SuproPro process design software which also enable economic and life cycle analysis of processes and plants. Following steps could include integrated process demonstration for determination of products safety, stability, final applications and complete economic and environmental analysis. This is a significant effort that would require joint expertise of biomass producers, process engineers and environmental economists.

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PILOT-SCALE BIOREFINING.

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In 2010 Aberystwyth University, alongside Bangor and Swansea Universities, won a large European regional development fund grant to set up BEACON, a biorefining centre of excellence in west Wales and the valleys, a regionally deprived area within the UK. This five-year project was followed by a second five-year project, BEACON+, with subsequent later funding which included east Wales and the University of South Wales before BEACON ended in early 2023. The biorefining centre of excellence at Aberystwyth has moved from strength to strength and is now based within Aberystwyth's Innovation and Enterprise Centre adjoining the University's institute, IBERS.

There has been macroalgae processing at IBERS, Aberystwyth University, since 2007, with initial studies producing biofuels before developing into more higher value products and latterly biorefining to multiple products. Alongside the development of BEACON, the macroalgae processing has also increased from bench scale (*e.g.* 10 g wet weight feedstock) and there are now several processes that have been conducted using seaweed at pilot-scale (up to 300 kg wet weight). Following the first macroalgae pilot-scale biorefining process at IBERS, a joint publication was produced between IBERS staff and the commercial partner, Oceanium Ltd, containing pre-processing considerations, observations and recommendations when working at pilot-scale. The process conducted was multi-layered and generated three liquid products and one solid product, with the process overview shown in Figure 30.

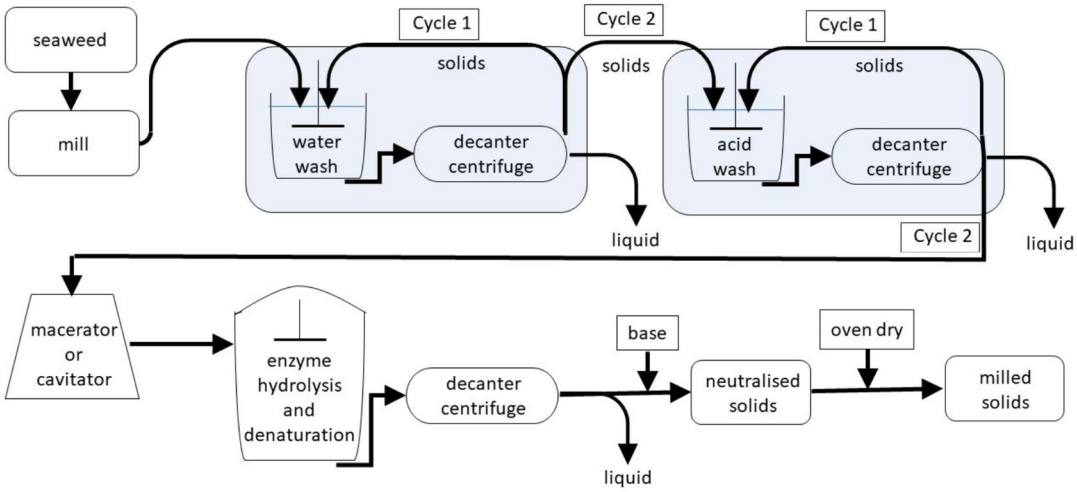


Figure 30. Schematic of the main steps involved with the macroalgae processing. Inverted 'T' indicates stirred mixtures. Material stored at 4°C overnight between steps or at -20°C longer term. From Adams et al., 2021

Key findings reported in this work include the following: the requirement for exceptionally clean macroalgae feedstock, as only a few snails or stones can damage milling machinery. Considerations should be made regarding the composition of larger equipment material, as pilot-scale is generally conducted in plasticware or stainless steel rather than glassware as in lab-scale processing. This has implications for cleaning and heating processes. Relatedly, the acid used in the process needs greater consideration as stainless steel does not have resistance to hydrochloric acid and even higher-grade steel only has a limited resistance to it. Conversely, most stainless steel has resistance to sulphuric acid at high or low concentrations, though is non-resistant at intermediate concentrations. Acids also react with metals within the macroalgae, with industrial research showing that sulphuric acid increases the lead (Pb) content in remaining solids compared to that by hydrochloric

acid by forming low-solubility lead sulphate which is thought to become trapped in the biomass rather than the more soluble lead chloride or chloro-complexes which are partly removed in the wash steps.
This presentation will reflect on this and other pilot-scale processing of macroalgae including previous pilot-scale work using *Ulva* at scale. It will discuss some of the differences between macroalgae and terrestrial biomass and how macroalgae can handle differently – not all biomass is the same. The presentation will also include the use of the pilot-scale batch steam explosion and the potential of this process to yield single sugars for subsequent bioprocessing.

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APPLICATION OF *ULVA SP.* IN PACKAGING.

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The use of single-use packaging materials has increased dramatically in recent decades in parallel with increasing trends in convenience and fast-food. Most of these packaging materials are made of non-biodegradable, petroleum-based polymers that have degradative impacts on the environment and contribute to the global plastic pollution crisis. Rethinking and redesigning packaging materials using natural resources sustainably to replace single-use plastics will therefore be an essential step to achieving the European Commission's new circular economy action plan (European Commission, 2020). Marine Resources, including seaweed, provide an enormous pool of yet unexplored and potentially valuable resources for the production of diverse biomaterials, including packaging, and interest is growing in the use of macroalgae in the packaging industry. The global seaweed-based packaging market is expected to account for $613.42 million by 2029 (Data Bridge Market Research). Screening of local macroalgae species revealed that *Ulva sp.* are good candidates for packaging materials, considering their functional qualities (*e.g*. antioxidant and antimicrobial activity) high growth rates, broad geographic range, mild taste, low iodine content, and the high abundance of biomass produced during summer green tides in many coastal areas. Therefore, several packaging films and prototypes have recently been developed using *Ulva sp.* biomass. Nevertheless, the source of biomass for packaging production continues to be a major limitation due to the risk of heavy metal contamination in harvested material, as well as the knowledge and technology gaps associated with land-based production. Here we present recent developments in the use of *Ulva sp.* in packaging materials, discuss current limitations and knowledge gaps, and provide recommendations for future research needs.

LIPIDS FROM *ULVA SP.* WITH ADDED-VALUE AS NUTRIENTS AND BIOACTIVE PHYTOCHEMICALS FOR HEALTHY FOOD AND FUNCTIONAL INGREDIENTS.

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Ulva species are green seaweeds that are found abundantly in the marine environment, and can also be produced in large scale in sustainable aquaculture, namely in integrated multitrophic aquaculture (IMTA) systems. They are some of the most widely-traded edible seaweeds with high market value. These popular green seaweeds are often commercialized as fresh or dried products, but also as extracts, either for direct or processed consumption worldwide. In fact, *Ulva* species has a lot of potential as a versatile ingredient that can be incorporated in various dishes, as salads, soups, and smoothies. In addition, it can be processed into different products like *Ulva* chips, *Ulva* pasta, and *Ulva* powder, which makes it an attractive option for both food manufacturers and consumers. The demand for plant-based foods and sustainable food sources is increasing, and *Ulva* fits the bill perfectly as it is a promising food source for healthy and sustainable diets.

The biochemical composition of *Ulva* has been extensively studied, revealing that these seaweeds are rich sources of healthy nutrients and various bioactive compounds, including carbohydrates, proteins, lipids, vitamins, and minerals, making them a valuable ingredient not just for food but also feed, nutraceuticals and pharmaceuticals (Simon et al., 2022). The major components of *Ulva* are carbohydrates, accounting for up to 70% of dry weight. These include polysaccharides as ulvan, which showed anti-inflammatory and anticancer properties, as well as various mono- and disaccharides, such as glucose, mannose, and xylose, which can serve as a primary source of energy.

On the other hand, protein also account for a significant portion of *Ulva*'s composition, making up to 20% of dry weight. Essential amino acids, like lysine and methionine, can be found in high quantities, which are vital for maintaining human health. Lipids can account for up to 5% of dry weight, comprising a large diversity of molecules such as polyunsaturated fatty acids (PUFA). Studies have shown that PUFA lipids have several health benefits, including reducing the risk of heart disease. *Ulva* also contains a wide range of micronutrients, such as vitamin C, vitamin E, iron, and calcium, that are indispensable for a good health and for preventing nutrient deficiencies. This benefits targeted populations such as vegetarians, vegans, and sports enthusiasts as *Ulva* is characterized by a high nutritional value and low-calorie content. *Ulva* can be a valuable addition to the food industry, as a sustainable source of nutrients and a versatile ingredient, and it can also help promote sustainable food system. Moreover, the consumption of *Ulva* has been linked to various health benefits, including anti-inflammatory, antioxidant, antidiabetic, and anticancer properties, that can help prevent and manage non-communicable diseases (NCDs), such as cardiovascular disease, diabetes, and cancer.

Recently algae lipids and lipidome are gaining increasing interest and thus the lipidome of some *Ulva* species has analyzed using modern OMICs approaches (Lopes et al., 2019; Moreira et al., 2020). Distinct *n*-3 fatty acids with benefits for human health were detected, such as alpha-linoleic acid (ALA) and docosapentaenoic acid (DPA). These fatty acids are mostly esterified to more complex lipids that were identified in the lipidome of lipidome, including glycolipids, betaine lipids, and phospholipids. These lipids possessed bioactive properties of interest, such as antioxidant and anti-inflammatory properties. Moreover, the lipidome of *Ulva sp.* was found to be influenced by environmental factors, as season and geographical location, which makes it a promising tool for traceability that is very usefull for the authentication and geographic origin certification of the algal product (Moreira et al., 2020; da Costa et al., 2020; Monteiro et al., 2020). Effectively, the plasticity of the lipidome and unique lipidomic features of *Ulva* species highlight its potential to ensure quality control and prospect biomass for target bioactive compounds.

Overall, the lipids of *Ulva sp.* have a great potential as functional ingredients. The knowledge on the composition and lipid plasticity of this green seaweed is relevant to optimize culture condition and and valorize specific wild production sites, as well as, maximaze production of lipids of interest.

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SEAWEEDS/SEAWHEAT CULTIVATION ONLAND – VIA COMMODITY TO SPECIALTY PHYCONOMY (TO OUTER SPACE).

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Relative to the open water cultivation of seaweeds, with a history of a few centuries, on land cultivation is in its infancy. However, this intensive and relatively expensive form of algal aquaculture has made many leaps and bounds within the past five to six decades.

Notwithstanding the on land cultivation of microscopic stages of laver and kelps and perhaps the co-cultivation of multiple-species, in coastal dugout ponds, of red and green seaweeds with shrimp and fish, pioneered in eastern countries, the west has embraced on land practices using various types of ponds for specialist applications of macroalgal biomass, and in particular products with high values.

On land cultivation of seaweeds is an expensive undertaking given the land, infrastructure and necessary technical expertise required by the operators and scientists involved. Such activities are not to be entered into lightly! First define the value of the biomass and its costs of production. It is not a field of dreams and a “build it and they will come” attitude will not cut it. A “just do it” approach does not work in the case of applied phycology, called here precision phyconomy. Indubitably the end product needs to have an inherent high value and clear path to market to bear the costs of on land operations (both capital payback and operational outlays). There has been an evolution of ponds and structures used to produce various types of seaweeds for specific applications. Consequently, the range of seaweeds currently grown on land cultivation is limited. It needs to be expanded in terms of species, cultivars, nurseries and libraries; not to do so would be a critical flaw.

There are success stories and there are those where the facility has changed hands and purposes as a crop of higher value has been identified to warrant the embedded costs of operating on land. When successful, precision phyconomy of some seaweeds provides multiple opportunities for exceptional levels of control and manipulation. Notably, on land farming of macroalgae has learned much from the operations of microalgal facilities, from staged production in the lab. and ponds, via small paddle wheel raceways to large, field-like operations of ponds relying on spargers and compressed gases for circulation of biomass. This talk presents some of the challenges, but highlights successes and provides an optimistic future for the phyconomic production of a wide range of candidate seaweed species.

DEVELOPMENT OF *ULVA* FOOD PRODUCTS AND THEIR CONSUMER ACCEPTANCE.

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Lately, seaweed is becoming recognised as an important sustainable food alternative for land crops. Although seaweed cultivation and consumption is predominant in Asia, where eating seaweed has been part of the culinary heritage for many centuries, this is not the case in Europe, where macroalgae are generally not considered as attractive food source. Until recently, the low consumer acceptance of seaweed products in the Western societies has been a hindrance for the food industry to invest in the development of new seaweed products. Although the majority of global seaweed products consist of brown and red algae species, green algae from the genus *Ulva sp.* have been identified as the most suitable macroalgae for biomass production and innovative blue biotech industries in European mariculture. The advantageous nutritional profile of *Ulva* and its high content of beneficial dietary fibres and other bioactive ingredients make this a valuable food item exhibiting not only health benefits but also functional improvements. Despite these favourable qualities, the share of *Ulva* in European mariculture and in food products is still marginal and many efforts need to be taken to exploit *Ulva*'s potential to the best.

The success or failure of a newly developed *Ulva* product is determined by several steps along the whole *Ulva* value chain. First, the choice of suitable strains, a scalable cultivation and food grade processing conditions assure sufficient quantity and quality of the biomass. As the European *Ulva* cultivation is still in its infancy, demand can be higher than the production capacity by one farmer. Furthermore, certification of the *Ulva* biomass is mandatory for the food industry to assure compliance with European food safety and quality standards. Finally, the development of the end product and its marketing strategy strongly influences consumer's purchase decision and enduring acceptance.

To date, only few *Ulva* products are existing on the European market that are scarcely available in specialised shops or online. Thereby, *Ulva* is mainly offered as algae itself for a small niche market. However, to profit from *Ulva*'s versatile potential, to better valorise the raw biomass in food and to increase it's sustainable impact, *Ulva*'s most promising applications are as enriching ingredient in familiar, everyday staples and snacks.

Cereal-based products such as bread, pasta or crackers are mainly produced from refined flours, being poor in fibres and micronutrients. Several studies have shown that enrichment of bread or pasta with *Ulva* powder or dried flakes in concentrations up to 4% can improve nutritional profiles without affecting taste. Furthermore, *Ulva* can also serve as suitable ingredient in gluten-free products when applied in concentrations up to 5%.

Another promising application of *Ulva* as sustainable ingredient could be the inclusion in processed meat and fish products, e.g. in burgers and sausages. The substitution of a certain percentage of meat content with *Ulva* can result in a healthier product with less meat, increased fibres and reduced CO_2 imprint. Additionally, due to the hydrocolloidal properties of ulvan and other polysaccharides, *Ulva* supplementation improves the water binding capacity, resulting in juicier texture with less cooking loss.

An increasing demand for alternative protein sources as meat-free analogues opens up further opportunities for *Ulva* as ingredient. Suitable strains high in protein could at least partially substitute less sustainable plant-based protein sources such as soy. Furthermore, *Ulva* can enrich dairy products such as cheese and seasoned butter as well as sauces, spreads and probiotic products. Fermentation of *Ulva* is still an underdeveloped yet efficient method to add nutritional value by enhancing digestibility and the bioavailability of bioactive compounds. Marketable applications remain marginal to date despite the fact that pre-treated *Ulva* can be a suitable fermentation substrate. Another important outcome of seaweed fermentation is the improvement of organoleptic qualities by removing unwanted sea smell which could help to increase consumer acceptance.

To increase the impact of *Ulva* as food ingredient, the current niche market needs to be extended for a broader range of consumer types. Recent food trends, such as the search for new sustainable food sources and alternative proteins, awareness for healthier diets and openness to a world cuisine are favourable conditions that might help to generally increase acceptance of seaweed in our daily diet and to promote *Ulva* products.

However, consumer groups are very heterogeneous and resistance to food innovation is a function of personality, values, attitudes and socio-economics. The most promising target group for seaweed marketers are food innovative consumers who can mostly be found among young, urban, well educated communities who are also health and environmentally conscious and appreciate new taste experiences. This group is normally highly committed and likely to spread positive feedback on choosing seaweed food. In contrast, conventional, more careless or uninvolved consumers could be targeted mostly with convenience products and snacks including seaweed more inconspicuously.

The necessity to understand the motivation of consumers to eat seaweed in order to build up a successful market is reflected by numerous studies that have been conducted in recent years in different consumer groups in the UK, Canada, Sweden, Norway and Australia. As a general tendency, these studies showed that the awareness of health benefits has a stronger influence on seaweed consumption than sustainable responsibility. However, the feeling of environmental obligation to eat seaweed can also be activated by the perception of health consequences. Interestingly, among those who are already familiar with consuming seaweed products (mainly snacks), the most important driver to eat seaweed is good flavour. In contrast, the strongest barriers to seaweed consumption is food neophobia, the fear of the unknown, as well as the lack of knowledge and unfamiliarity, followed by poor availability and choice of products. Unsustainable packaging is also a purchase barrier among eco-conscious consumers.

In order to increase the impact of *Ulva* in European food products, the hitherto niche sector should become more mainstream. After assuring constant supply and quality biomass, this can primarily be best achieved by a product co-development of seaweed farmers with the food industry, developing business cases for defined products and taking different consumer types into account. Secondly, the low acceptance in many consumer groups can be increased by providing more information about seaweed, increasing exposure in stores, media and restaurants by spreading the sustainability and health story and giving inspiration through recipes, tastings, cookbooks, etc. More trust in new products can be achieved by transparent labelling and including *Ulva* as ingredient in familiar or convenient products, for example in bread, pasta or snacks. Generally, the incorporation of *Ulva* in products will have a higher acceptance and ability to overcome food neophobia more easily than consuming the whole algae itself which most people are not familiar to handle in the kitchen. Ultimately, nowadays, the role of trendsetters and social exposure should not be underestimated. Ambassadors could be celebrity chefs, food bloggers and influencer in social media, promoting seaweed consumption not only in gastronomy and cooking shows but also in everyday life.

ULVA FOR INTEGRATIVE URBAN FARMING – THE FOOD4FUTURE APPROACH.

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Driven by the growing awareness in macroalgal products and the interest to use edible macroalgae as food, rather than only as source of food additives, macroalgal cultivation is expanding in different ways. Next to coastal-based farming approaches, there is also a rising interest to provide these fresh and healthy sea vegetables in inland areas to diversify the local food supply. In this context land-based cultivation approaches are fostering a stable and controlled production. To meet the current challenge of integrating cultivation systems within existing urban infrastructure, transdisciplinary approaches are required. Under the umbrella of the "Agricultural Systems of the Future" funding line of the German Federal Ministry of Education and Research (BMBF), the joint project "food4future" (funding code IGZ: 031B0730A, www. https://www.food4future.de) brings together novel technologies, such as lightweight materials and new LED irradiation concepts, for the co-cultivation of marine and terrestrial organisms in a saline environment. Among other promising alternative crops (*e.g.* halophytes) and animal breeds (e.g. crickets, jellyfish), food4future also considered macroalgae as ideal candidates for an integrative urban farming approach.

In this context, two species, *Ulva compressa*, formerly known as Enteromorpha sp. and *U. fenestrata*, formerly known as *U. lactuca*, were identified as suitable for the intended approach. After isolation from field material the macroalgae were exposed to different cultivation conditions to define the potential cultivation range (*e.g.* temperature, salinity and light) and to identify the most suitable cultivars. Next to testing different light regimes and intensities, the project also focused on the effect of applied construction materials and the use of different cultivation media. In addition to development, growth and biomass production, strong emphasis was given to the metabolic composition and nutritional value of the cultivated species, like pigments (chlorophylls and carotenoids), fatty acids (SFA, MUFA, PUFA), or mineral compositions of cultivation media and algal biomass. Aiming to construct viable and flexible cultivation units, the project focused on modern lightweight composite materials, able to serve for several construction purposes of the fostered cultivation systems and tested their feasibility for macroalgae (*U. compressa*, *Ectocarpus* sp.) and crickets (*Achaeta domesticus*). Considering material resistance, rigidity, and direct material-organism interactions, from the 10 initially chosen composites, the bio-based polylactic acid (PLA) was identified as most suitable material for joint farming. For macroalgae cultivation, PLA sustained the corrosive cultivation conditions and provided a suitable settlement substrate without affecting the macroalgal physiology or nutritional composition (carotenoids and chlorophylls) (Fricke et al., 2022).

With growing distance from the coast, the access to and choice of cultivation media provides a crucial and often cost-intensive aspect in marine aquaculture. Taken this in account we focused on alternative cultivation media and identified regional saline brine water sources as suitable cultivation medium for marine macrophytes (Fitzner et al., 2021). First results clearly showed that not only water treatment but also taxa identity played a role for cultivation success in the new media (Fricke et al., 2023, under review). *Ulva compressa* was successfully grown and cultivated in the brine water based medium. In addition, the project aimed to use the valuable water source in long-term experiments, cultivating *U. compressa* in artificial seawater under

greenhouse conditions over several months. This study not only showed alterations in the media composition but also differences in the carotenoid concentrations, showing seasonal alterations, as well as changes over cultivation time.

Bringing the marine algae into our artificial environment and exposing them to so far unexperienced conditions potentially not only affected the macroalgae itself, but also seem affect their associated microbiota as we experienced in different contamination events. To further study this issue and also ensure the biological safety of our developing systems we recently started to study the bacterial community associated with our cultivated organisms.

In parallel to all research activities, food4future always aims for further outreach to the public. In this respect, the project provides a modern information platform (https://www.food4future.de/en/news-events/news), using different online and also offline channels to inform different stakeholders on the project content to foster transparency and creating trust and interest in the new technologies and related products.

After four years of the project, the species *U. compressa* and *U. fenestrata* can be considered as feasible candidates to become an integrative part of urban agriculture, providing a valuable nutritional profile, able to grow in different alternative media and cope with alternative construction materials. Thus, our study shows that these sea vegetables are promising candidates for environmental controlled inland cultivation.

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THE USE OF *ULVA SP.* IN INTEGRATED MULTI-TROPHIC AQUACULTURE SYSTEMS (IMTA). AN ECOLOGICAL APPROACH FOR A SUSTAINABLE MARICULTURE

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Scarcity of fresh water, overfishing and decline of ocean biodiversity, marine eutrophication by anthropogenic activities, and the increasing demand for seafood has all required attention from a more comprehensive, global perspective. Moving from conventional aquaculture toward an ecological approach to developing and managing a sustainable aquaculture that cares for environmental and sociological aspects can relieve at least some of these problematic issues. Nutrient assimilation in aquaculture using Integrated Multi-Trophic Systems (IMTA) is a promising ecological approach for sustainable aquaculture. The rationale behind the IMTA systems is to convert the excretions of the organism cultured upstream into valuable food for the organism cultured downstream. Seaweed has a high capacity for nutrient uptake per unit of culture area and can be some important additional valuable products. *Ulva sp.* (sea lettuce) Macroalgae are important for marine ecology and have been identified as ideal candidates for biofiltering fishpond effluents. In particular, the worldwide distribution of *Ulva* in many ecological settings indicates that this is a suitable species for cultivation globally. The rapid growth of *Ulva* is attributed to its high photosynthetic activity and uptake rate of nitrogenous nutrients. In addition to using *Ulva* as a biofilter, their integration into a diet, either as feed for macroalgivores (abalone, sea urchins, shrimp, and fish) or as a supplement for human consumption, can increase the overall profitability. *Ulva* assimilation rate ranged between 1-5 g N m^{-2} d^{-1}, and *Ulva* yield ranged between 150-300 g (WW) N m^{-2} d^{-1} or 55 -110 kg N m^{-2} d^{-1}. We have to take into consideration that *Ulva* growth is light-dependent, and the production is not uniform throughout the year. During summer, when the days are longer, production reaches 300 g N m^{-2} d^{-1}; in winter, production is as low as 150 g m^{-2} d^{-1}. The area required in IMTA for seaweed production must consider the lowest yields. Excess *Ulva* in summer can be dried and incorporated into pellet feed. In the biochemical composition of Ulva, we can see that protein levels are about 34% (DW) compared to 10-12% in the wild. Although *Ulva* is an efficient biofilter, it has a low commercial value. Using this species as a biofilter alone would be expensive, as running such a system requires electricity, space, and labor. Therefore, adding macroalgivores such as sea urchins to the fish seaweed or using it as a feed supplement for marine fish is an attractive option to increase the overall profit of the integrated system. Protein derived from fishmeal is the most expensive ingredient in fish feeds. Therefore, reducing and replacing this ingredient with a less expensive protein source yielding the same fish growth performances will contribute to reducing production costs. Protein-rich *Ulva*, used as a biofilter in an IMTA system, was evaluated as a dietary ingredient for gilthead seabream (*Sparus aurata*) at a high (up to 30%) replacement of fishmeal ratio. By removing 100% of the fishmeal (i.e., replacing 260 g kg^{-1} of fishmeal with 291 g kg^{-1} less expensive animal meal and 146 g kg^{-1} Ulva), the total cost of feed containing *Ulva* was reduced by $0.25 kg-1; with an FCR of 1.7, the saving was $0.45 per 1 kg fish produced. Since fish feeds represent over 50% of the operating costs in intensive aquaculture, with protein being the most expensive dietary source, about 10% saving on the feed cost is economically significant. The somatic growth rates of two species of Abalone (*Haliotis discus hannai* and *Haliotis fulgens*) and two species of sea urchins (*Paracentrotus lividus* and *Tripneustes gratilla*) were significantly faster when fed with IMTA-based *Ulva* compared to wild *Ulva* exhibited high gonad somatic index (SGI), and high quality, bright-orange gonads. Land Based IMTA systems will increase profit for the farmer, improve food conversion rate (FCR), diversify the mariculture products, often create additional jobs, and, most importantly, reduce environmental pollution.

USE OF *ULVA SP.* IN ANIMAL DIETS: OPPORTUNITIES AND CHALLENGES.

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A considerable augmentation in the demand for food is foreseen as the world population is poised to a continual increase estimated to grow more than 9 billion by the year 2050. Concurrently, growing urban and richer population due to enhanced income levels will drive the demand for animal-origin foods especially meat and milk. These factors will impact the land use to feed the animals for food production since more affordable feedstocks and biofuel will be required. Consequently, a competition for land use might arise between agricultural production for human and animal populations. Increased human activity over the past decades has resulted in global warming, increased greenhouse gas emissions, unusual precipitation, growth of weeds, pest attacks, and disease load. These will eventually impact the global food production. Furthermore, the use of synthetic and conventional feed supplements has been discontinued or banned in whole or parts of the world. Therefore, the use of new and alternative feeds and feed additives in animal diets is necessitated to spare the conventional counterparts. Algae and algae-derived products are among these non-conventional feed components. *Ulva sp.* are chlorophytes (green algae) belonging to the Ulvaceae family that are commonly referred to as sea lettuces, marine algae, or seaweeds. Although *Ulva sp.* can grow naturally, human activity releasing nutrients to pollute the oceans has made substantial contribution in the growth of *Ulva sp.* as environmental hazard. However, seaweeds comprise of useful nutrients like protein, fats, and carbohydrates, and functional components that provide opportunity for use in animal diets. *Ulva sp.* may account for the partial replacement of major plant-based feedstuffs competing with the food crops intended for expanding global population. In addition, bioactive molecules extracted from *Ulva sp.* can be used to produce functional animal diets to improve animal health and performance. Thus, *Ulva sp.* can ensure the food security by serving as a component of diets either as a feedstuff or as feed additive. Nonetheless, the presence of certain components in *Ulva sp.* may act as antinutrients and the use of bioactive molecules of *Ulva sp.* in animal diets might be expensive thus limiting the use of these seaweeds in animal diets. This paper critically presents the opportunities and challenges of the use of *Ulva sp.* in animal diets from feedstuff and feed supplement perspectives.

Ulva sp. as feedstuff/feedstock: *Ulva sp.* possess very low content of dry matter/mass (DM), a considerable amount of cellulose and starch along with complex carbohydrate ulvan (carboxy-sulfated group; $COOH^{+}$ $SO3^{-}$), lower quantities of mostly saturated lipids, and substantial amounts of valuable micro-, macro-, and trace minerals. Given the nutrient content, the use of *Ulva sp.* looks promising for use in animal diets at higher inclusion levels. Given the protein and cellulose contents, *Ulva sp.* seem suitable for the replacement of alfalfa in ruminant diets which was further confirmed in an in vitro gas production test of *Ulva lactuca*. In situ study conducted in cows suggested that a large portion of the crude protein fraction of *Ulva lactuca* was rumen escape/undegradable protein. The use of *Ulva sp.* in poultry diets is potentially limited. Previous studies have reported that *Ulva sp.* can be used up to 3% in poultry diets without any adverse effect on the growth performance of chickens. However, recent studies have shown that the inclusion of *Ulva sp.* up to 15% does not pose harmful effects on the growth performance of broiler chickens. Although the use of *Ulva sp.* is possible to some extent in cows, it seems challenging in poultry as the digestive system of poultry neither possesses the cellulose digesting enzymes nor the big fermentation compartment like rumen. Keeping this in view, the use of fibrolytic, cellulolytic, and proteolytic enzymes is imperative to allow for greater inclusion levels of *Ulva sp.* in poultry diets. Few recent studies have reported that the inclusion of enzymes in diets containing *Ulva sp.* do improve the growth performance of broiler chickens attributable to several factors.

Ulva sp. as a source of bioactive molecules: Besides the use of *Ulva sp.* directly as feedstuff in animal diets, *Ulva sp.* can provide various bioactive molecules such as ulvan and a range of different pigments. These bioactive molecules can be extracted from *Ulva sp.* using different techniques and biorefinery approaches. Ulvan can act as a potential prebiotic that can be used in monogastric animals whereas pigments can be used

to enhance the color of the animal-origin foods and antioxidant status of cells and tissues. Biorefinery approach can also yield other high-end products like proteins and carbohydrates that have the potential to be used in monogastric and polygastric animals alike.

While *Ulva sp.* present various opportunities for use in animal diets to reduce the competition between humans and animals to use the plant-based cereals and grains as food or feed resources, it poses challenges that need to be addressed before the routine use of *Ulva* can be made possible. Since green tides and algal blooms have appeared due to man-made disaster of pollution and release of effluents to water and oceans, there are risks of heavy metals and undesired impurities in Ulva. Additionally, *Ulva sp.* harvested from the ocean generally contains greater ash content that creates hinderance in the use of *Ulva sp.* in animal diets. Over the past few years, tank cultivation has seen an upward trend, however, most of tank cultivated *Ulva sp.* goes for human consumption. Therefore, scaling-up of *Ulva* production is a major challenge as animals consume large amount of feed and *Ulva* will be required in larger quantities. Studies have shown that protein fraction in *Ulva sp.* might have been overestimated because most studies used the nitrogen-to-protein conversion factor of 6.25 that is not true. Standardization of analysis of *Ulva* is imminent. Either a universal conversion factor of 5 or a conversion factor of 4.6 specific to *Ulva* has been proposed in recent studies. This will allow greater transparency in the use of *Ulva* to balance the nutrient requirements of animals. Moreover, seaweeds in general and particularly *Ulva sp.* are not a part of the reference feed databases of different countries and world-wide popular organizations. This should be taken into consideration after careful studies to enhance the use of *Ulva sp.* in animal diets. A recent study has reported that the fermentation of ulvan in the gut can liberate the hydrogen sulphide that affects the mitochondrial function of the cells and tissues. Hence, it is necessary to address this question before proposing the use of *Ulva sp.* in animal diets.

COMMERCIAL CULTIVATION OF *ULVA* IN MEXICO: FROM THE LABORATORY TO COMMERCIAL PONDS.

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From the late fifties to the early eighties, the Mexican seaweed industry was concentrated on the utilization of seaweeds for the production of phycocolloids and more recently, the use of seaweeds as fodder in abalone aquaculture and seaweed extracts for agriculture. All the seaweeds used in these activities are harvested from natural beds. In the mid 90´s, the first studies to develop the cultivation of seaweeds in ponds took place at the Instituto de Investigaciones Oceanologicas (IIO) of the Universidad Autonoma de Baja California (UABC). These studies were focused on the cultivation of carregenophytes in ponds following the principles of "tumbling culture" published in the 80´s by JS Craigie, RGS Bidgell, and collaborators. Although the results for the commercial cultivation of carragenophytes at a pilot scale were technically successful, the reduction of carrageenan demand worldwide and the abundance of commercial natural beds halted its commercial scalation. These studies, however, provided the opportunity to develop essential infrastructure at the UABC-IIO that included an outdoor twelve 1m^3 tanks facility and pilot ponds (40 to 100 m^2) to continue seaweed aquaculture research at a commercial pilot scale.

In the Spring of 2015, efforts to develop the commercial cultivation of *Ulva* spp were initiated at the IIO-UABC. This was the first effort for land cultivation of seaweed focused on human consumption. *Ulva* strains were collected from Bahia San Quintin on the Pacific coast of the Baja California Peninsula, 200 km south of Ensenada and 300 km south of the US-Mexican Border. Basic studies for light and temperature tolerance, optimal initial density, and pilot culture in 100 m^2 ponds were performed on these strains for six months including winter and summer. The best strain was selected to be cultivated in ponds for two and a half years in 100 m^2 ponds (1 m depth). Cultures were initiated with 3 kg per m^2 and fully harvested after 21 days and re-seeded with the initial density. Seaweed production was maximum in the Spring (290 g m^{-2} d^{-1} ww) and minimum in Winter (40 g m^{-2} d^{-1} ww). The annual average production throughout the study was 174 g $m^{-2}d^{-1}$ (equivalent to 24.8 g m^{-2} d^{-1} dw considering a ratio of 7:1 ww:dw).

These studies provided the bases for the development of the first commercial *Ulva* farm in Mexico. The farm presently has 1920 m^2 of ponds with a capacity of 100 to 121 wet tons per year. Studies now in progress have been focusing in testing the possibility for improving the productivity per unit area following three lines of research; increasing light and temperature in winter and cooling water in summer by artificial means; adding CO2 and; a strain selection. The increase in temperature and light in winter can increase the Dec-Feb yield by 44 % while maintaining the water temperature in summer at 23°C and 20°C can increase the yield by 55 and 72 %, respectively. Although the pH in the pond cultures often reach values above 9.3 indicating a lack of bicarbonate, the addition of CO_2 does not increase productivity significantly due to the alternative mechanisms of *Ulva* to concentrate CO_2. Finally, a more comprehensive selection strain program, now in progress, indicates the feasibility to find a strain, even of the same species, that can perform better as a cultivar providing consistently, higher yield and properties.

Due to its high growth rate, and the feasibility to grow vegetatively, *Ulva* cultivation has been considered for multiple uses including; biofuels, bioplastics and, as a CO_2 sink. These markets require huge amounts of biomass at a very low cost. Certainly, onshore cultivation by tumble culture, regardless of the scale, may never be economically feasible for these uses. Ulva, however, has also been considered for its nutritional, nutraceutical, and pharmacological uses. The main challenge to reach these markets is to provide seaweed with consistent properties and quality. Pond cultivation, where fertilization can be controlled and, to some extent, environmental conditions of light and temperature can be influenced, or partially controlled (by managing biomass density, water exchange or other means) may be the proper cultivation approach. Although these markets can afford seaweed at a higher cost, production cost is still a major challenge. The overhead cost of producing seaweed for human consumption, for example, where sanitary regulations required expensive infrastructure and multiple certifications, is one of the main barriers for the industry to succeed. Productivity per

unit area is one of the main factors that influence cost in onshore seaweed cultivation. Therefore, studies to increase productivity are essential in order to have financial success.

Proper infrastructure design and engineering are also essential to optimize seaweed productivity. Farm operation can be improved by mechanization and automatization of processes such as fertilization and harvesting. However, the investment necessary for installing or improving mechanization and automatization is tied to an increasing scale of production. So, even if the technology is available, the necessary investment may not be justified or affordable by small farms. On the other hand, the increase in productivity by the development of improved strains can increase productivity at any scale. This is the reason for the importance of proper

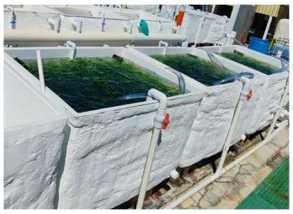
Field studies and collection



Laboratory characterization



Outdoor tank cultivation



Pilot scale cultivation



Commercial cultivation



ALGAPLUS: TEN YEARS OF PRODUCTION AND EVOLUTION.

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ALGAplus is a private Portuguese company that initiated its activity in 2012, in a property located at Ria de Aveiro with 14 ha. ALGAplus produces customized seaweed and seaweed-based products mainly for the food and well-being markets, reaching the B2B (companies & restaurants) and B2C (retail) sectors with different brands. ALGAplus seaweed are organic certified, assuring warranties of sustainability, quality standards through time and traceability to the customers. At ALGAplus, the production is done under the Integrated Multi-Trophic Aquaculture (IMTA) concept, with a strong focus in R&D and continuous support to the customers.

All seaweed production phases are carried out in-house: biomass production in a land-based modular IMTA system, processing (washing, drying, salting, cutting, milling), and packaging. Besides, ALGAplus works with their customers on tuning the seaweed biomass to the desired characteristics or on the domestication of new species of interest, giving them the product differentiation needed in highly competitive markets. The main species in production at this Portuguese company is *Ulva sp.*, however, other species native from the Atlantic Ocean are also farmed and commercialized year-round, such as *Porphyra dioica*, *Porphyra umbilicalis*, *Gracilaria gracilis*, *Codium tomentosum*, among others.

In 2016, the company reached a milestone with 600 m2 of surface of production, however, this meant still a small production in comparison with the market demand and the upscaling production/processing was mandatory. Since 2020, ALGAplus is actively working on the process's optimization and upscaling, that goes from the nursery to outdoors tanks, including the update of protocols of cultivation. In 2022, ALGAplus reached another milestone with 0.4 ha of production surface due to the implementation of 15 raceways for seaweed production under the same IMTA concept, that were designed together with INEGI in the frame of the European project GENIALG. In 2023, the company expects a production of 80 tons of seaweed (fresh weight), however, this production can reach 165 tons fresh weight with the raceways fully operational.

Currently, ALGAplus is optimizing the system in terms of energy and labour costs, manipulation of production factors according to downstream and upscaling needs. These keystone tasks are being carried out in the frame of different national and international collaborative projects, such as SeaMarK (Horizon Europe) and Pacto da Bioeconomia Azul (02/C05-i01/2022); and also together with strategic partners with expertise in the field of macro- and microalgae, such as GreenCoLab.

ALGAplus works on the development of new seaweed-based food products in partnership with already established food companies. Some products already in the market are: a seaweed bread with no salt, a salted honey, a ready-to-eat canned meal, frozen ready-meal, and a mayonnaise with sea-lettuce that adds an umami flavour to the sauce, having also the claim of 50% less salt. In terms of outreach, the company is strongly related with the local community (associations, schools, governmental bodies), is part of national associations of the sector (BlueBioAlliance, PROALGA, Associação Portuguesa de Aquacultures) and integrated in international networks (International Seaweed Association, Global Seaweed Coalition, EU4Algae). The company has an open-door policy, being also partner of the Portuguese program Escola Azul, since education part of its strategy.

TECHNICAL CONSIDERATIONS FOR THE INTENSIVE TANK CULTIVATION OF ULVA: THE ROLE OF NITROGEN AVAILABILITY.

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During the last 30 years, native *Ulva* species were the main assayed organism for the development of intensive seaweed aquaculture in the Canary Islands. In particular, biomass of *Ulva rigida* C. Agardh (nowadays identified as *Ulva lactuca* Linnaeus) have been produced year-round from laboratory experimental setups to tubular photobioreactors, tanks and raceways at pilot plant scale in our facilities at Taliarte (east coast of Gran Canaria). As far as successful outdoor productivities, reaching seasonal values higher that 100 g DW m^{-2} d^{-1}, and biomass qualities were obtained during the first experimental trials, integrated multi-trophic aquaculture methodologies were evaluated and established as sustainable and environmental-friendly principles for the semi-industrial development of the sector in the region.

In some of our studies, the availability of inorganic nitrogen and dynamics on tank-cultivated biomass, grown under N-enriched and N-depleted conditions, deeply affected growth, physiological and biochemical characteristics of *Ulva*.

Chlorophylls, ash, caloric content, fatty acids, dietary fibres and colouration varied significantly depending on the nitrogen conditions. C:N ratios (and protein contents) correlated significantly with biochemical parameters. Fatty acid (FA) synthesis continued during the N-starvation period; saturated and mono-unsaturated FA increased to a maximun of 72.2 %, while poly-unsaturated fatty acids (PUFA) decreased to 27.7 %. During the N-enriched recovery period, the reverse was found. C:N ratios above 10 correlated with carbohydrate synthesis as shown by the dietary fibre level. Under nitrogen enriched conditions, C:N ratios decreased along with a decrease in fibre level. Under controlled conditions, nitrogen represents a major influence on the development of intensive tank cultivation of *Ulva rigida*, not only by affecting parameters closely related to nitrogen metabolism but also some clearly influenced by carbon uptake.

Ulva has been widely used as a biofilter because of its high efficiency to remove nitrogenous inorganic compounds (up to 90 % in the form of ammonium) from wastewaters. *Ulva* species show the capacity to utilize, quickly absorb and metabolize different forms of inorganic nitrogen, mainly nitrate and ammonium, depending on their availability. However, ammonium ($N\text{-}NH_4^+$), which can be toxic or inhibitory for some seaweeds at concentrations higher than 30–50 µm, is the preferred nitrogen form for *Ulva* and other species with interest in seaweed aquaculture. Moreover, some authors demonstrated that $N\text{-}NH_4^+$ availability controls ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and carbonic anhydrase (CA) activities in *Ulva rigida*, confirming that there is a close relationship between inorganic nitrogen, photosynthesis and carbon metabolism. Therefore, excess and depletion of nitrogen sources in the culture medium causes important cellular responses in both wild and cultivated algae.

More recently, intensive tank produced *Ulva* biomass have been evaluated for multiple applications related to sectors as food and feed or those related with the use of high value metabolites as the cosmeceutical or nutraceutical. It is particularly relevant the characterization of biomass with different biochemical profiles,

obtained under specific growth-controlled conditions, as feed for mollusks such as the abalone or as novel dietary ingredients for aquafeeds improving the immune response in aquaculture.
All this knowledge on how *Ulva* intensive cultivation and biomass quality are strongly related will be reviewed and considered in the near future as the basis for the development of new projects with the aim to evaluate the carbon sequestration and footprint reduction in a scenario of global change.

ULVA PRODUCTION; CAN WE IMPROVE ENGINEERING SOLUTIONS TO CULTIVATE AND PROCESS AT LOWER PRICES?

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Over the last 2 decades large blooms of the opportunistic macroalgae *Ulva sp.* has been witnessed with the epiphany in 2009 in the yellow sea with a recorded 1.9 million tonnes caused by eutrophication (Chuanmin Hu et al, 2010). Several companies have tried to capitalise on this, drying the biomass using solar and wind to make fertilisers (high N and P content in tissue) and animal feed or process it as fresh. However, this brings many issues from contamination with sand and plastics to high metal content or other impurities which are a signature of the site harvested. However, for food production these issues severely limit the use of algae blooms and cultivation of *Ulva* is the only viable alternative, either through rope culture (Sweden), tank cultivation (e.g., Israel, France and others) or pond cultivation (Netherlands) or in IMTA systems using tanks and ponds as side product of a higher value species like shrimp. Nevertheless, food grade material also means a stringent drying and processing protocol, all factors making cultivated *Ulva* extremely expensive and not a main stream product. This presentation will discuss the current status in cultivation and processing and will suggest options to make the process cheaper by lowering the carbon footprint and energy input. If we truly want to make *Ulva* the wheat of the sea, we need to improve or cultivation ad processing protocols in a more economic and sustainable way.

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ALTERNATIVE AND POTENTIAL APPROACHES IN THE MAINTENANCE OF VEGETATIVE GROWTH IN *ULVA* AQUACULTURE AND ENHANCED DELIVERY OF ALGAL PRODUCTS.

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Many *Ulva* species proliferate in nature and form green algal blooms ("green tides"), which can occur when nutrient-rich wastewater from agricultural or densely populated areas is flushed into the sea. The seaweed growth rate and chemical content are hardly predictable and are affected by environmental factors, including epiphytic bacteria. Bacteria are necessary for the adhesion of *Ulva* to its substrate, its growth, and the development of its blade morphology. *Ulva mutabilis* develops into a callus-like morphotype in the absence of certain bacteria. However, with the addition of the necessary marine bacteria, the entire morphogenesis can be restored. Surprisingly, just two bacteria isolated from *U. mutabilis* are sufficient for inducing morphogenesis and establishing the reductionist system of a tripartite community such as *Ulva mutabilis*- *Roseovarius* sp.- *Maribacter* sp. (Wichard, 2023). The reductionist approach has enabled the study of the bacteria-induced morphogenesis and the life cycle of *Ulva under standardized conditions: The presentation will highlight two approaches relevant to Ulva´s aquacultures.*

(1) Control of changes in the life cycle: Blade cells of the green macroalga *Ulva mutabilis* Føyn (Chlorophyta) excrete regulatory factors into their cell wall and growth medium to control the status of the cell. These unknown sporulation inhibitors, a glycoprotein (SI-1) and a small molecular weight compound (SI-2) located within the bilayer of *Ulva* control the vegetative status of the cell. Upon removal of the sporulation inhibitors by mincing the mature thallus into small single-layered fragments and washing intensively, the gametogenesis of the thalli can be artificially onset. The gametogenesis is subdivided into a determination phase (36 h), within vegetative growth could be resumed by the addition of SIs, and a differentiation phase afterwards, insensitive to SIs, when gametogenesis occurred (Figure 1) (Stratmann et al., 1996; Wichard and Oertel, 2010; Vesty et al., 2015). At this time of no return, the formation of progametes starts, and the gametes are duplicating up to 32 gametes in one gametangium during the last night. The swarming phase occurs on the third day, when the pore caps are opening, and the gametes can leave the gametangia upon the removal of an additional swarming inhibitor.

It is tempting to assume that those inhibitors allow us to manipulate the life cycle of land-based aquacultures applying a non-genetic approach. A protocol is suggested for the direct application in bioassays and aquacultures (Kessler et al., 2017; 2018). As sporulation inhibitors are produced and released depending on the age of *Ulva* cultures, mixed-age populations might help to prevent sporulation events in aquacultures (Obolski et al., 2022).

(2) Applications of microbiome leveraging and engineering for promoting macroalgal growth and bioactives in aquaculture: Our study found that IMTA conditions significantly affected the structure and composition of the *U. rigida* microbial community (Califano et al., 2020) Morphogenetic activities were detected in sterile-filtered water samples tested in bioassays with *U. mutabilis* because of the clustering of species known for their morphogen production (e.g., *Roseovarius* sp., *Sulfitobacter* sp., and *Maribacter* sp.). We hypothesized that microbiome engineering could control the chemical composition of *Ulva* biomass and constituents. We demonstrated that the engineered *Maribacter* sp. and *Roseovarius* sp. consortium modulated *Ulva mutabilis* photosynthate content such as amino acids, polyols (Polikovsky et al., 2020) and fatty acids. Further microbes colonising macroalgae should be isolated under controlled, reproducible conditions. Hereby, multiple omics technologies should be used to determine the phenotypes of the designed holobiont and its new traits. As bacteria can influence algal development and other bacteria in many ways, a diverse inoculum may improve aquaculture development. Therefore, functional groups of bacterial strains should be defined based on a suite of similar traits (Wichard 2023).

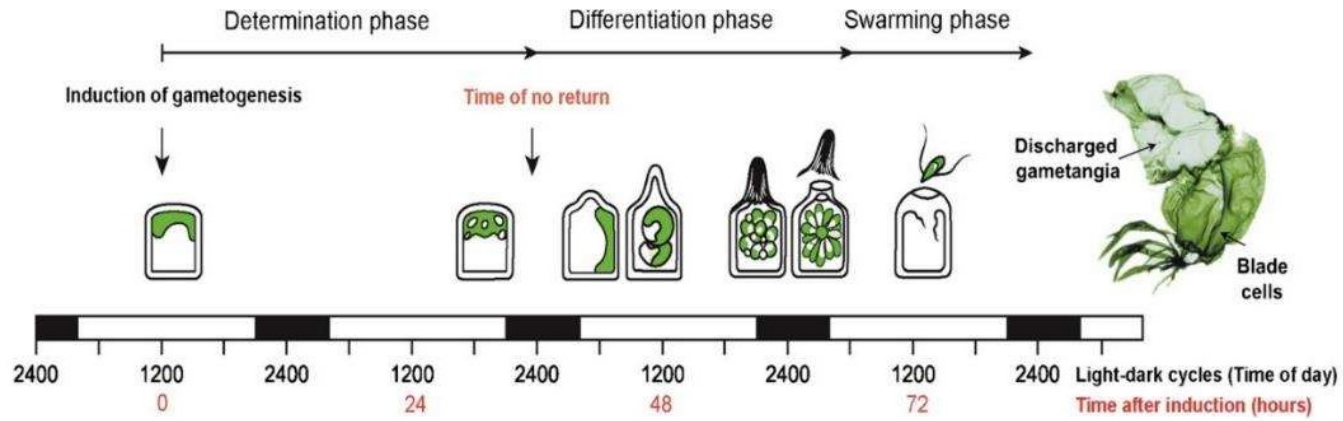


Figure 31 Regulation of gametogenesis in Ulva mutabilis under the control of sporulation and swarming inhibitors (adopted from Wichard and Oertel (2010) and Kessler et al. (2017))

In summary, understanding the chemical ecology of *Ulva* and its controlled life cycle will improve the maintenance of aquaculture and improve their biorefinery products.

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From Ulva aquaculture to food and feed production: state-of-the-art, bottlenecks, risks and gaps

Lisbon, Portugal

POSTER SESSION

Ulva
The Future Wheat of the Sea

NATURALLY OCCURRING PROTOPLASTS IN A "GREEN TIDE" STRAIN OF *ULVA LACINULATA.*

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The *Ulva* genus has proved to be a suitable candidate for large-scale cultivation and can serve multiple purposes in several industries (e.g., pharmacy, cosmetics, food, and feed) (Mantri et al., 2020; Leyva-Porras et al., 2021). However, to guarantee the success of its large-scale production, it is important to find solutions to the currently existing biological and technical limitations. Some *Ulva* species reproduce in predictable ways, and their reproduction can be induced by following established methodologies (see Mantri et al. 2020 for recent review). However, attempts at inducing the reproduction of the commonly cultivated species *Ulva lacinulata* (Kützing) have been unsuccessful, and large-scale cultivation of this species requires re-stocking from the wild or limiting the harvest to maintain a starting stock of biomass. While this species is often sought for cultivation because it grows well unattached and it does not sexually reproduce, which results in the loss of biomass, this species often degrades, which also contributes to a loss of biomass. The cause of this biomass degradation has until now not been understood. Here, we present evidence that tissue degradation in *U. lacinulata* occurs as a result of natural protoplast production and release. These protoplasts can then develop in several different directions. New blades can be formed, which either develop into adult thalli or undergo gametogenesis very early in their development, resulting in the release of gametes and the germination of new germlings. These results contribute to a new understanding of the life cycle of *U. lacinulata*, and provide the first evidence of natural protoplast production in this species. Because the induction of protoplasts in *Ulva sp.* is time-consuming and expensive, finding the trigger for this process will be an important step to accelerating the success of large-scale cultivation of this species.

The biological material (previously molecularly identified as *Ulva lacinulata* by Cardoso et al. 2023) was collected in 2021 in a "green tide" area in Lagoa de Óbidos, Portugal (following the Nagoya Protocol) and then brought to the Alfred Wegener Institute, Germany where it has been cultivated in a closed system. Throughout its cultivation, it was kept in 5 L glass vessels at 15 °C (± 1 °C) and an irradiance of 70 µmol photons m^{-2} s^{-1} with a 16:8 h light:dark photoperiod (LD) in pasteurized artificial seawater (ASW) (30 PSU) supplemented with half-strength Provasoli in a concentration of 10 mL L^{-1}. The medium was replaced once per week and an aeration system guaranteed the continuous tumbling of the material inside of the vessels.

Upon observation of the onset of biomass degradation, fresh biomass (0.72 g) was collected. The biomass was distributed equally into four 1 L beakers. The experiment ran for four weeks under the previously mentioned cultivation conditions. Every week, the water in each beaker was filtered and centrifuged to collect the protoplasts. After filtering the water, the debris and small pieces of the original biomass were collected, weighed, and placed back into the beaker with newly added culture media. Calcofluor white (CFW) was used to observe the presence/absence of cell walls and disposable hemacytometers were used to quantify the protoplasts yields obtained in each beaker each week. A defined amount of the collected protoplasts were isolated in individual Petri dishes and their development was observed under the microscope. The number of protoplasts that germinated into new blades were counted after five weeks.

Our observations under the fluorescent microscope confirmed the absence of cell walls in the cells dyed with CFW, thus confirming that these cells are naturally occurring protoplasts. The total protoplast yield obtained in our first experiment was 3.21 x 10^7 cells per gram of fresh biomass, which is comparable to those reported in studies where the protoplast formation of different *Ulva* species was enzymatically induced (Reddy et al. 2018). Approximately half of the protoplasts regenerated (40-60 %) and grew into discs or germlings in a similar fashion to what has been described in the literature for induced protoplasts of *Ulva* spp (Reddy et al. 2018; Figure 32). Moreover, sexual reproduction was found to occur during gametogenesis in protoplasts, rather than in adult blades.

Additionally, by measuring the weight of the initial biomass each week, we observed that the original tissue did not completely degrade after protoplast formation and release, and a final total fresh weight of 5.79 g resulted in a 7 % daily relative growth rate. Furthermore, there was a clear recovery in the last week of the experiment with the average RGR reaching 12.3 % day^{-1} (Figure 33). Thus, revealing that degradation does not necessarily result in a total loss of the culture.

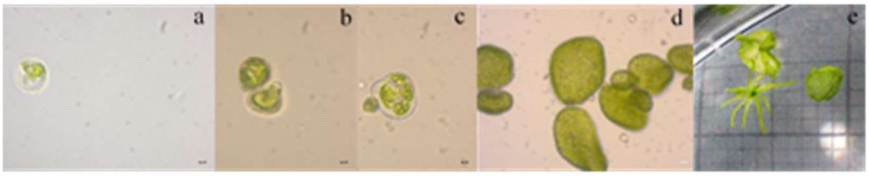


Figure 32 Development of the regenerated protoplasts from U. lacinulata obtained during a "degradation" event. a: single protoplast with no visible cell wall; b: first cell division; c: second cell division; d: Ulva discs originated from cell division of protoplasts; e: discs and germlings originated from protoplasts. A, b and c at a 400 x amplification, d at an amplification of 100 x, e taken with a binocular (each square = 1 x 1 mm).

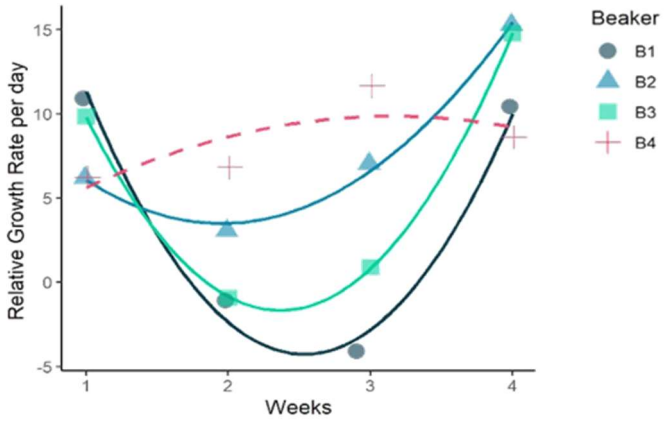


Figure 33 Relative growth rates (RGR day-1) of the degraded/protoplast-producing pieces of Ulva lacinulata in each beaker over a period of 4 weeks.

Our observations of natural protoplast production in *U. lacinulata* close an important knowledge gap in understanding this species' reproductive cycle. This new knowledge can be beneficial when trying to understand the formation of "green tides" and the differences between "green tide" strains and non-"green-tide" strains. Additionally, the production of natural protoplasts can potentially be exploited to improve the efficiency of *Ulva* cultivation methods in the future and remove the bottlenecks existing today for safe and profitable large-scale production.

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USING *ULVA LACTUCA* AS A FEED INGREDIENT IN WEANED PIGLET DIETS: EFFECTS ON NUTRIENT AVAILABILITY AND GUT HEALTH.

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Seaweeds are novel feed ingredients with the potential to at least partially replace conventional feedstuffs. *Ulva lactuca* is a green seaweed with bioactive properties that can enhance animal gut health and product composition. The objective of this study is to evaluate the effect of 7% dietary *U. lactuca*, with or without carbohydrases supplementation, on nutrient availability and gut health of piglets. Growth performance was not affected by dietary treatments. Dry and organic matter digestibility decreased with dietary seaweed inclusion and ulvan lyase supplementation. Hemicellulose digestibility increased with seaweed inclusion, suggesting a high fermentability of this cell wall fraction, independent of carbohydrases supplementation. Some beneficial microbial populations increased as a consequence of enzymatic supplementation (*e.g. Prevotella*), while seaweed diets as a whole led to increased abundance of *Shuttleworthia*, *Anaeroplasma* and Lachnospiraceae_NK3A20_group, all related with a healthier gut.

Seaweeds such as *Ulva lactuca* are potential sources of nutrients in animal diets, while providing important bioactive substances, such as n-3 polyunsaturated fatty acids and fermentable polysaccharides. Weaned piglets are a particularly sensitive group of animals that could benefit from such feeding. They endure severe changes, mostly nutritional, that cause dysbiosis and compromise their immune system. They require high-quality and highly digestible feedstuffs, preferably with health-promoting properties. This is where seaweeds could be advantageous. However, the cell wall of these novel feedstuffs can have antinutritional effects for monogastrics. Our team has previously reported how a single recombinant ulvan lyase can break their recalcitrant bonds, in *in vitro* conditions. The objective of this study is to evaluate how dietary *U. lactuca*, and the supplementation with either commercial carbohydrase mixture or ulvan lyase, can impact nutrient availability of weaned piglet diets and their microbiota.

The experimental trial took place at the Animal Production Department of the School of Agriculture (ISA) of the University of Lisbon, Portugal. Forty weaned piglets (Large White × Duroc) were randomly divided across four experimental diets: control (standard diet, wheat-maize-soybean meal based), UL (with 7% *U. lactuca* replacing the control diet), ULR (UL + 0.005% Rovabio® Excel AP, a commercial carbohydrase mix bought from Adisseo, Antony, France) and ULU (UL + 0.01% ulvan lyase). Each piglet was allocated to a metabolic cage. After an adaptation period of five days to the experimental conditions, the trial lasted two weeks (14 days). Piglets were weighed at the beginning and end of each week and faeces were collected daily. At the end of trial, piglets were slaughtered and samples of colon contents were sampled for microbiota analysis.

The experimental diets had no effect on the growth performance of piglets. The total tract apparent digestibility (TTAD) of dry matter and organic matter were reduced in the ULU diet compared to control. The TTAD of the cell wall fractions, namely neutral detergent fibre and acid detergent fibre, were increased and reduced by seaweed incorporation, respectively (Figure 1). This could demonstrate that enzymatic supplementation was not required at this level of incorporation, and that the hemicellulose fraction is easily fermented by the piglets' microbiota, with positive repercussions on the gut ecosystem. In fact, the piglets fed with *U. lactuca* had a higher relative abundance of *Prevotella* in the colon, in addition to others including *Shuttleworthia*, *Anaeroplasma* and Lachnospiraceae_NK3A20_group, which have been related with beneficial fermentation in weaned piglets. The lack of major negative effects could be attributed to the pre-treatments of the seaweed biomass (low particle size - <250 µm, and micronization). Furthermore, the high availability of minerals should be considered given possible negative repercussions of mineral imbalances, that include osmotic diarrhoea.

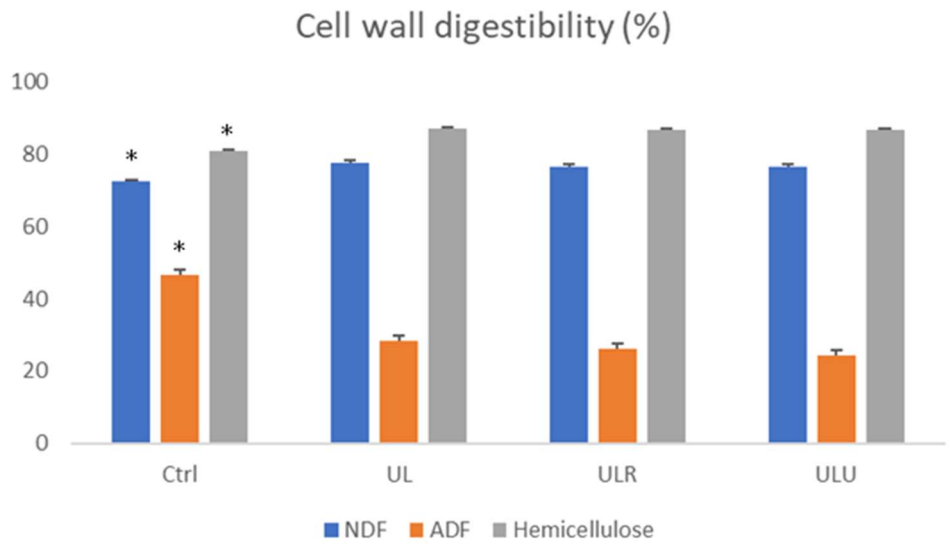


*Figure 34 Total tract apparent digestibility of neutral detergent fibre (NDF), acid detergent fibre (ADF) and hemicellulose of four experimental diets: control (standard diet, wheat-maize-soybean meal based), UL (with 7% Ulva lactuca replacing the control diet), ULR (UL + 0.005% Rovabio® Excel AP, a commercial carbohydrase mix bought from Adisseo, Antony, France) and ULU (UL + 0.01% ulvan lyase). * indicates statistically significant differences from other groups (P<0.05).*

Ulva lactuca shows potential to be used as a feed ingredient at this level of incorporation in weaned piglet diets, without the need for enzymatic supplementation. In the future, testing starch digestibility would be highly interesting given its relevance as a storage polysaccharide in green seaweeds.

Keywords: *Ulva lactuca*, piglet, digestibility, microbiome

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PRODUCTION OF FOOD GRADE LIPID EXTRACTS FROM *ULVA SP.* AND A *ULVA SP.* BASED-ALGAE BLEND USING ULTRASOUND-ASSISTED EXTRACTION.

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Algae are a rich source of bioactive compounds, such as healthy lipids with potential commercial uses (Djuricic et al. 2021; Ullmann et al, 2021). Lately, the interest in lipids from algae for application in food, nutraceutical, and feed industries, due to their great nutritional quality and attractive bioactivities, is rising. This has sparked the hunt for innovative food-grade and sustainable extraction approaches in alternative to the traditional lipid extraction that use toxic chlorinated-solvents. Instead, ethanol (EtOH) and ethyl acetate (EtOAc) is a non-toxic green extraction solvent approved to produce algae lipid extracts to be incorporated into food products and supplements. However, EtOH and EtOAc have associated with low lipid yield and/or low lipid purity (Lopes et al, 2021). To overcome this drawback, the extraction assisted by ultrasound has been investigated showing encouraging results (Zhou et al., 2021).

The purpose of this study was to evaluate EtOH and EtOAc as food-grade extraction solvent in combination with ultrasound (UAE) to obtain lipid extracts from *Ulva sp.* and a *Ulva sp.* based-algae blend.

While in *Ulva sp.* the lipid content was low (≈40 %), EtOH+UAE extract from blend was lipid-rich (≈60 %). Both *Ulva* and blend EtOH extracts showed very low lipid yield, possibly due to the non-disruption of cells walls. However, better results were obtained with EtOAc. All extracts obtained by EtOAc+UAE and EtOAc showed to be almost exclusively lipids, purity ≈90 %, while EtOAc+UAE has the advantage of provided a higher yield compared with EtOAc. The FA profile of Folch and EtOH+UAE extracts was comparable, but EtOH extract from blend was significantly different. Highest content (%) of *n*-3 and *n*-6 FA were achieved in *Ulva sp.* extracts. Fatty acid analysis of EtOAc+UAE and EtOAc extracts is still in progress. The results of DPPH antioxidant assay revealed that the extracts with the higher scavenging capacity were obtained by Folch (65.5±1.8 %) for *Ulva sp.* and by EtOH+UAE (93.2± 03%) and EtOH (94.0±0.4 %) for blend. Concerning anti-inflammatory potential, *Ulva* EtOH+UAE extract (71.7±3.1 %), and blend EtOH+UAE (77.6±1.4 %) and EtOH (82.9±1.8 %) extracts showed the best ability to inhibit COX-2. Evaluation of bioactivities of EtOAc and EtOAc+UAE extracts is ongoing.

Overall, our results showed that EtOH or EtOAc assisted with UAE is more promising to obtain food-grade extracts enriched in *n*-3 and *n*-6 lipids with health promoting properties for food, feed, and nutraceutical applications.

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PHAEOBACTER AND *ULVA* INTERACTIONS WITH IMPLICATIONS IN INTEGRATED MULTITROPHIC AQUACULTURE.

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The biofiltration of fish effluents using macroalgae has gained significant interest in Integrated Multi-Trophic Aquaculture (IMTA) systems. *Ulva* species, such as *Ulva ohnoi*, possess favourable characteristics such as high nutrient uptake rates or adaptability to various environmental conditions. Moreover, its high surface/volume ratio provides a niche for the development of epiphytic bacteria (Oca et al., 2019). These bacterial communities play crucial roles in the growth, development, and defence mechanisms of *Ulva* (Egan et al., 2008). The composition and activity of macroalgal microbial communities are strongly influenced by biological, chemical, and physical processes occurring on the surface of macroalgae. Light, in particular, plays a crucial role due to the photosynthetic nature of macroalgae, which strongly influences their metabolism and, consequently, their microbiome (Coelho-Souza et al., 2017).

Understanding the interactions between macroalgae, their associated microbial communities, and environmental factors like light intensity provides insights into the physiological processes and ecosystem roles of algae. It also opens up possibilities for developing biotechnological applications. By manipulating the microbiota of macroalgae, it may be possible to improve aquaculture practices by identifying microbial communities that promote robust growth of the algae and enhance overall health (Li et al., 2022). Moreover, microbial manipulation of algae in IMTA systems can also have an effect on water quality or the health of the organisms in co-culture (*e.g.* fish).

Among the bacteria associated with *Ulva*, *Phaeobacter* spp. stands out for its ability to inhibit fish pathogens. Some members of *Phaeobacter* genus produce tropodithietic acid (TDA), an antimicrobial compound effective against fish pathogens like *Vibrio* spp (Bruhn et al., 2007). This has led to the isolation and investigation of *Phaeobacter* as a potential probiotic in aquaculture systems. Studies have shown that introducing *Phaeobacter* to fish larvae, either through live prey or biofilters, can significantly reduce mortality caused by *Vibrio* infections (Planas et al., 2006; Prol-García et al., 2014; Prol-García & Pintado, 2013). In the context of IMTA, recent research (Pintado et al., 2023) has assessed the possibility of co-culturing of the algae *Ulva ohnoi* with *Phaeobacter* to mitigate mortality in fish larvae during *Vibrio anguillarum* infections. However, the study also suggested that the persistence of *Phaeobacter* on the surface of *Ulva ohnoi* may be influenced by light intensity.

Our research aimed to investigate the effect of light intensity on the colonization and maintenance of *Phaeobacter* sp. 4UAC3 on *Ulva ohnoi* surfaces under controlled in vitro conditions. We hypothesize that light intensity may influence epiphytic bacterial communities of *U. ohnoi* (*e.g.* in terms of diversity or functionality), and may also influence the colonization and maintenance of *Phaeobacter* sp. 4UAC3. The effect of light could be due to i) a different response of *Phaeobacter* bacteria to light, that could also have an indirect effect on algae physiology and bacterial communities, or ii) the response of the algae to light intensity, promoting growth and the production different metabolites that would influence the microbial communities and *Phaeobacter* or iii) a combination of both factors.

The results showed that the inoculated *Phaeobacter* disappeared from the surface of the *Ulva* as the light intensity increased. However, light itself did not have a detrimental effect when *Phaeobacter* was cultured on inert surfaces (glass and autoclaved *Ulva* discs) (Figure 35).

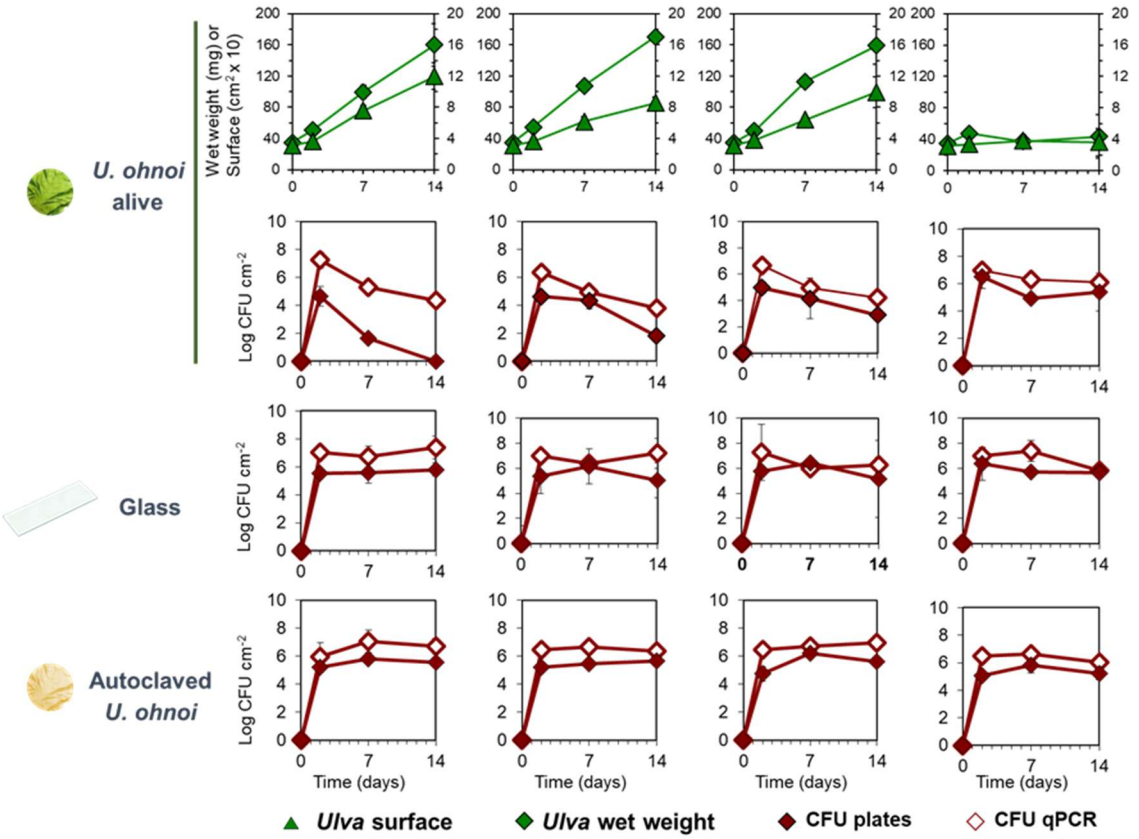


Figure 35 Phaeobacter maintenance on diferenternt surfaces under different light intensities. The concentration of Phaeobacter on different surfaces (live Ulva disc, glass and autoclaved Ulva disc) is shown under different light intensities (143, 75, 45 and 0 µmo/m2 s-1). Phaeobacter concentrations are expressed in CFU/cm2 and quantified by qPCR and Marine Agar culture plates. Also, it is shown the growth of Ulva throughout the experiment expressed in wet weight (mg) and disc surface area (cm2).

Considering this, the negative effect of light could be due to the development of bacterial taxa that would compete with *Phaeobacter*. However, bacterial communities on *Ulva* surfaces did not significantly differ for the different light intensities tested (high light, 143 µmol m^{-2} s^{-1}, medium light, 75 µmol m^{-2} s^{-1}, low light, 45 µmol m^{-2} s^{-1} and darkness), although high light intensity favoured higher species richness initially. It is possible that larger differences in light intensity are required to observe significant effects on bacterial communities. The addition of *Phaeobacter* altered bacterial communities on *Ulva* surfaces initially, but this effect diminished after a week. We observed a slight increase in the growth of *Ulva* when *Phaeobacter* was inoculated compared with control situations, nevertheless, this was not statistically significant. Certain bacterial taxa correlated positively with *Phaeobacter* (like *Litorimonas*, *Henriciella* or *Deinococcus*), suggesting potential synergy or interdependence, while others showed negative correlations (such as *Alteromonas*, *Caldicellulosiruptor* or *Roseobacter*), indicating possible antagonistic or competition interactions. Previous studies have reported similar correlations between light intensity and specific bacterial taxa (Paix et al., 2020; 2021). Additionally, we can consider the possibility that the disappearance of *Phaeobacter* could be influenced by competition and cooperation dynamics involving non-bacterial taxa and the production of specialized metabolites by the *Ulva* holobiont. However, those specific factors contributing to the disappearance of *Phaeobacter* and the interactions between different microbial taxa will require further investigation.

Based on these results, we designed an IMTA-RAS with a tripartite system Sole-*Ulva*-*Phaeobacter* and evaluated the capacity of *Phaeobacter* to control *Vibrio anguillarum* outbreaks. A small-scale prototype system was developed, consisted of three 3L-tanks connected in series: the first contained *Ulva* exposed to light with actively growing algae, the second contained *Ulva* maintained in darkness with the algae being inoculated with *Phaeobacter* (10^7 CFU/mL), and the third tank contained seawater with nutrients added to a level corresponding to the ones in the presence of fishes (Oca et al., 2019). This last tank was inoculated with *Vibrio anguillarum* (10^4 CFU/mL) to simulate an infection in the system. During the experiment, the presence of *Phaeobacter*, *Vibrio* and bacterial communities in water and algal surface were assessed by colony counting in Marine Agar

plates, qPCR and 16S rRNA gene sequencing. The results indicated that *Phaeobacter* colonised the entire system, and as in the previous experiment a slight improvement of algal growth was observed. The presence of *Phaeobacter* reduced *Vibrio* concentration in water, being undetectable at the end of the experiment, compared to the control with non-inoculated *Ulva*. Considering this, a foundation has been laid for the design of a larger-scale system for industrial production of sole an IMTA-RAS which is currently been developed.
This research paves the way for the implementation of sustainable aquaculture strategies, based on the use of *Ulva sp.*, that harness the potential of beneficial microbial communities to improve fish health and production.

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USE OF SEAWEED BIOMASS IN FOOD PRODUCTS BASED ON TECHNO-FUNCTIONAL, NUTRITIONAL AND SENSORIAL PROPERTIES

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Seaweed has interesting nutritional and techno-functional properties for the use in food, which are currently undervalued. An intelligent use of seaweed should be targeted: making product-dependent, well-informed choices for specific seaweed species based on their techno-functional, nutritional and sensorial properties. The research groups from KU Leuven and VIVES have a strong track record on the use of innovative raw materials like microalgae, insects, duckweed and brewer's spent grain in food products, with focus on techno-functional, nutritional and sensorial properties related to proteins, lipids and other constituents.

We combine expertise from three research groups:

I. Meat Technology and Science of Protein-rich Foods (MTSP) from KU Leuven: with knowledge and expertise on meat products and its vegetarian or vegan alternatives, covering ingredients quality, additive functionality and processing in relation to end product quality;
II. Centre of Expertise Agro- and Biotechnology from VIVES: with knowledge and expertise on development and industrial implementation of new food products and their processes;
III. Food & Lipids from KU Leuven: with knowledge and expertise on the nutritional and stability properties of lipids, antioxidants and color components relaying on 15 years of research on microalgae for food applications.

The collaboration between the research groups allows to cover the whole innovation chain, translating fundamental insights into applied research and covering a broad TRL scale to target optimal and diverse valorization trajectories. Exploiting and combining the strengths of the research groups allows intelligent use of full biomass of raw materials in food products. The expertise on proteins of MTSP is combined with that of lipids and antioxidants of F&L and merged with the recipe development expertise of VIVES. This is supported by the analytical infrastructure of KU Leuven and VIVES allowing a complete characterization of composition, quality and stability of seaweed and food products. Pilot equipment for preparing protein-rich food products (such as vegan meat analogs) is available at MTSP. VIVES has full access to industrial kitchen pilot infrastructure in which real conditions of cooking, packaging, cooling and regeneration in (semi) industrial environments are simulated, and to microwave technology which can be combined with vacuum for drying at low temperatures. Taste labs to perform sensorial analysis with both trained or consumer panels are used to evaluate the food products.

We are looking for partnerships aiming at an increased use of seaweed in food products. We want to achieve this by using seaweed not only for its nutritional and sensorial quality, but also for its techno-functional contributions to food products, e.g. water binding, emulsification, gelation. By focussing on using the full biomass rather than extracted components, we want to valorize the full potential of the seaweed while limiting side streams. This way, we can lower or even exclude the need for additives while maintaining the quality of the food product.



Emissions, capture, and utilisation: Principles and frameworks for Ulva

Kurresaare, Estonia

May 13-14, 2024

Ulva

The Future Wheat of the Sea

ORGANIZERS

Dr Annette Bruhn, Aarhus University, WG5 leader
Dr Celine Rebours, Møreforsking, WG6 leader
Dr Professor Georg Martin, Estonian Marine Institute, local organiser

The COST Action SeaWheat (https://seawheatcost.haifa.ac.il/) and the research institutions, Estonian Marine Institute, Tartu University; Møreforsking and Aarhus University organized a 2-day Workshop on *Ulva* ecosystem services (WG5) and the regulatory aspects of *Ulva* ecosystem services (WG6), from May 13-14, 2024. The workshop was hosted by The Estonian Marine Institute at the GO Spa hotel in Kuressaare, Estonia.

RATIONAL AND OBJECTIVES

The purpose of the workshop was to stimulate the interdisciplinary knowledge exchange and cooperation between experts from different fields of sciences from the academia and the industry. The common goal was to promote an up-scaled *Ulva* production as a means for providing Ecosystem Services, with specific focus on the removal and recycling of nutrient and carbon emissions, and documenting of the related effects of such aquaculture production on the environment.

Despite the use of *Ulva* for capture of nutrients in land-based aquaculture systems for decades, the methods to quantify the Ecosystem Services of *Ulva* (regulating and maintenance, provisioning and cultural) are under debate, and the regulatory frameworks for valorising and utilising the Ecosystem Services from seaweed aquaculture are still not in place.

The workshop was facilitated to bring together experts in *Ulva* production and ecophysiology, sustainability and regulatory frameworks, with the specific objectives to identify knowledge gaps and barriers, and to draft a common strategy to address and overcome those.

The outcome intends to be a significant contribution towards the overall goal of the 20106 COST ACTION SEAWHEAT project, which is to make a step-change towards a green economy based on *Ulva* mass production and utilization within the European community and beyond.

ORGANIZATION

The workshop was part of the planned list of events for the current grant period, a joint organization between the leaders of WG5 and WG6, "*Ulva* Ecosystem Services" and "Social, legal and regulatory aspects", and the Estonian Marine Institute, where professor Georg Martin, Jack Hall and Kristina Tivel constituted the local organizing committee.

The Workshop program was discussed and decided among a group of experts that constituted the scientific committee. These were (listed alphabetically): Annette Bruhn (Denmark); Celine Rebours (Norway), Georg Martin (Estonia); Jack Hall (Estonia); Muki Shpigel (Israel); and Karel Keesmann (The Netherlands).

In addition to the organizing committee, it is also important to highlighting the role of the Social Media and dissemination group, in particular Karina Balina, who provided the graphical design of the abstract book and program, as well as she promoted the Workshop via all SeaWheat social media channels.

PARTICIPANTS

The workshop was participated by 46 experts (3 of which presented online) from 19 different countries (Figure 36). Hereof, 43% of the participants were from ITC countries. Of the 46 participants, 13% were affiliated to SMEs, 80% affiliated to Academic institutions and the final 7% were affiliated to other organizations. The gender balance between participants was 50-50 (Figure 37). The organizing committee prioritized physical attendance at the workshop to optimize the networking between attendants. For this reason, the works shop was not offered as a hybrid solution for on-line participants.

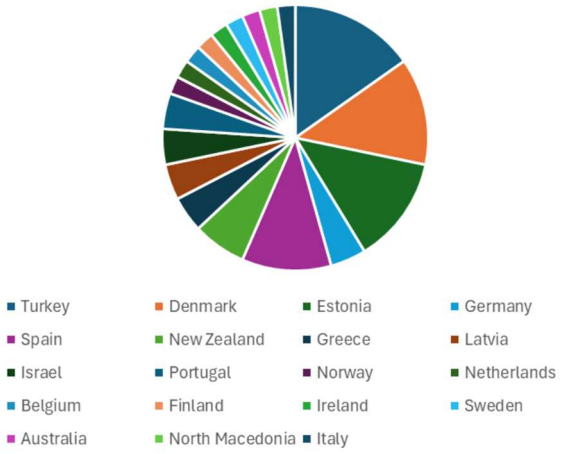


Figure 36 Distribution of the workshop participants by country according to their affiliation

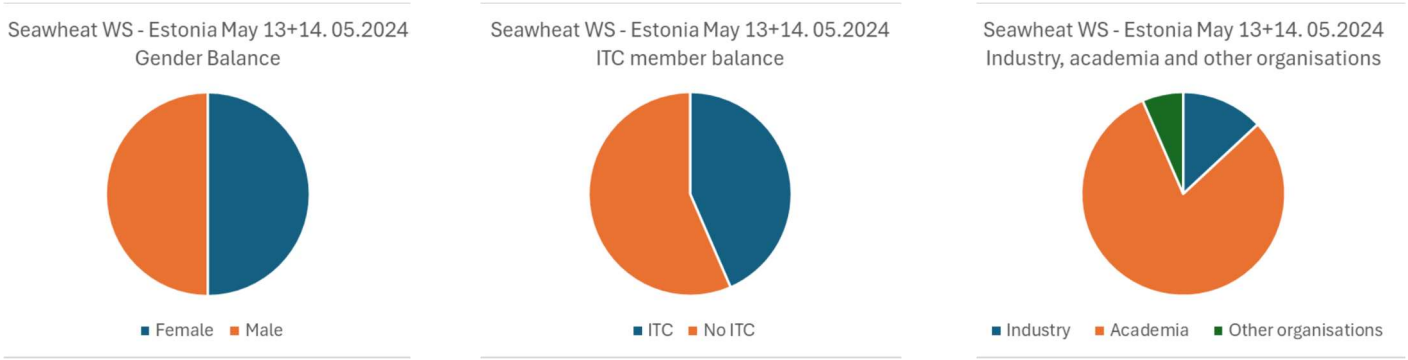


Figure 37 Distribution of the Workshop participants by gender, members of ITC countries and affiliation



Figure 38 Group photo of the participants of the Seawheat workshop outside the venue, the GO Spa hotel, Kuressaare, Estonia

WORKSHOP DESIGN AND FUNCTIONING

The workshop was designed to include presentation arranged in three sessions by topic, including 30-60 minutes for discussion of each topic by the end of each session, and an industry visit to the hydrocolloid factory, Estagar, producing furcellaran from the local *Furcellaria lumbricalis*, and the land-based trout producing company, Ösel Harvest OÜ, in cooperation with which, the Estonian Marine Institute has installed a test facility

for *Ulva* production for investigating and optimizing the emission capture of nutrients and CO2 from the fish production. In summary, there were 15 presentations, divided into 3 presentation sessions with discussions. Topics covered during the sessions, in line with WG5 and WG6 and decided by the scientific committee were: Session *1 – Algae production policy and regulatory settings; Session 2 – Quantifying emission capture – challenges and valorisation; and Session 3 – Environmental accounting (Ecosystem services & LifeCycle Assessment) challenges of applying to seaweeds*. For more details on the program, please see Annex I at the end of this report.

Each session comprised between 3-6 presentations and included 30-60 minutes for discussion of the correspondent topic. A total of 15 talks, including 3 keynote talks by our international guest speakers (Dr Catriona Hurd from Australia, Maris Stulgis from the EU commission and Kátlin Aun from Estonia) set the stage for the discussion moments. Also relevant to note the role of the session chairs. Each session had 1 designated chair, with the mission to a) keep time and moderate the discussions during the presentation moments and b) animate and moderate the discussions during the discussion sessions for each topic.

Prior to the beginning of the workshop, the designated Session Chairs received an information package for their session, including a short-bio of each of the speakers as well as the abstracts for each presentation.

Finally, last but not least, the workshop offered time and opportunity for networking between the participants. The participants were taken on a guided tour to visit two local companies: First to the Estagar production facilities, where Dr Mart Mere (Co-owner and development leader) explained the function of the entire site, the technologies involved, and the capacities of the site. Following the WS participants visited the land-based trout production company, Ösel Harvest OÜ, where Henry Ots gave a full tour of the production facility, and Dr. Professor Georg Martin and Jack Hall introduced the participants to the experimental work of using Ulva for bioremediation of the fish farm effluents.

Networking was also very intensive, stimulating and fruitful between sessions and during the dining and cultural events, emphasizing the benefits of physical meetings between participants from science and industry.



Figure 39 Different moments of the workshop: presentations, part of organizing team and networking



Figure 40 Industrial site visits - trout farm and EstAgar



Figure 41 Local culture moments of the WS - Kuresaare Castle, the Kaali crater and dining at the historical wind mill restaurant

CONCLUSIONS

The workshop “Emission capture and utilization – principles and frameworks for Ulva” was a very enriching event for all participants, judging from the engaged discussions, the networking between sessions and from the feedback of the participants at the end of the event. A proceedings of the work shop is under preparation in the form a special issue of the peer-reviewed journal Botanica Marina (impact factor: 2.2), where presenters and participants can submit their presentations or related scientific work for peer-review.

The organizing committee has a strong impression that the objectives of the workshop were attained, as interdisciplinary knowledge exchange was achieved between experts from different fields of sciences and from academia and industry, and future cooperations were stimulated. A common understanding, across standing points, of the challenges of quantifying and documenting the ecosystem services of seaweeds, and particularly Ulva, with focus on the removal and cycling of nutrients and carbon through aquaculture, was taking form, emphasizing the need for interdisciplinarity for fully meeting the targets of environmental, economic and societal benefits of a future up-scaled Ulva production.

In terms of the specific objectives defined, the discussion sessions resulted in information regarding “Challenges and Barriers” and “Proposals for solutions” in all discussion topics.



Emissions, capture, and utilisation: Principles and frameworks for Ulva

Kurresaare, Estonia

PROCEEDINGS

Ulva
The Future Wheat of the Sea

This abstract book compiles all the work presented by the speakers and poster presenters at the Seawheat workshop: **'Emissions, Capture, and Utilisation: Principles and Frameworks for *Ulva*'** organized by WG5 and WG6 in Kuressaare, Estonia, during the 13-14th of May 2024. The purpose of the workshop is to stimulate the interdisciplinary knowledge exchange and cooperation between experts from different fields of sciences from the academia and the industry. The common goal is promote an up-scaled *Ulva* production as a means for providing Ecosystem Services, with specific focus on the removal and recycling of nutrient and carbon emissions, and documenting of the related effects of such aquaculture production on the environment. Despite the use of *Ulva* for capture of nutrients in land-based aquaculture systems for decades, the methods to quantify the Ecosystem Services of *Ulva* (regulating and maintenance, provisioning and cultural) are under debate, and the regulatory frameworks for valorising and utilising the Ecosystem Services from seaweed aquaculture are still not in place. The workshop facilitates to bring together experts in *Ulva* production and ecophysiology, sustainability and regulatory frameworks, with the specific objectives to identify knowledge gaps and barriers, and to draft a common strategy to address and overcome those. The outcome intends to be a significant contribution towards the overall goal of the SEAWHEAT COST Action (CA20106), which is to make a step-change towards a green economy based on *Ulva* mass production and utilization within the European community and beyond.

The venue of the event has been chosen to widen the geographical coverage of the experts dealing with *Ulva* cultivation as the northern Baltic Sea region represents somewhat harsh conditions for *Ulva* species to thrive (low salinity, seasonality, ice conditions). The host institution, Estonian Marine Institute, is a recognised centre of Baltic Sea research doing its first steps in low trophic aquaculture research.

The abstracts are organized in their order of presentation at the workshop.

EUROPEAN COMMISSION'S SUPPORT TO EU ALGAE SECTOR DEVELOPMENTS

Maris Stulgis[1]*

[1]Policy Officer, European Commission, DG Maritime Affairs and Fisheries (DG MARE)

In recent years, European Commission has increased its support to the algae sector developments in the EU. Based on the gap analysis done Commission has come up with a 23 policy actions plan for the EU in a form of the EU Algae Initiative. More and more algae-related project acquire EU funding, ''algae" is appearing in more and more new or existing EU policies as an emerging opportunity, more and more investors and businesses are turning towards algae based businesses. To boost the collaboration within the EU algae sector, an EU4Algae – European Algae Stakeholder Platform (with 900+ registered members) has been launched. During the presentation you will learn about these and other EU actions and future plans in supporting EU algae sector.

REGULATIONS AND POLICIES FOR THE *ULVA* PRODUCTION: IMPORTANCE OF RRI, CONVENTION OF BIOLOGICAL DIVERSITY, NAGOYA PROTOCOL, ACCESS AND BENEFIT SHARING CLEARING HOUSE.

Céline Rebours[1]*

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Blue Biotechnology (BB) is a multidisciplinary, knowledge- and capital-intensive technological area that significantly contributes to innovations in the pharmaceutical, medical, cosmeceutical, nutraceutical, aquacultural, agricultural, and energy sectors. The BB sector in Europe includes non-traditionally commercially exploited marine organisms and their biomass applications. Seaweeds are identified as one of the more valuable sources of structurally diverse bioactive compounds among the marine organisms, and their utilization would support society to gain various health benefits. Seaweed cultivation in Europe is still in its early stages, with a main emphasis on kelp species. These species face challenges in penetrating the market, calling for a need to diversify the cultivated species. The food and feed sectors (i.e. human food, food supplements, nutraceuticals, animal feed) are the main markets for macroalgae biomass (up to 60%) in Europe, with other minor applications being cosmetics (18%) and fertilisers and biostimulants (11%)[1]. The green species of the genus *Ulva* can effectively assimilate dissolved nutrients with high efficiency and biomass yield[2]. Only a limited number of *Ulva* species are currently cultivated on a pilot scale, predominantly in land-based systems, and in some cases under IMTA systems[3]. In northern Europe, high biomass yields in land-based systems have been demonstrated with *Ulva* biomass that can be valorized as food and feed ingredient[4]. As the quest for marine-derived compounds with pharmacological and biotechnological potential upsurges, the importance of following regulations and applying Responsible Research and Innovation (RRI) principles also increases. This presentation aims at: 1) presenting important regulations and policies that apply to the *Ulva* production at the EU level; 2) highlighting the importance of the application of the RRI principles in biodiscovery, and 3) identifying gaps and providing recommendations on how to improve compliance of novel BB compounds with guidelines on ethical and benefit-sharing of genetic resources.

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FRAMING THE ECOSYSTEM SERVICES OF ULVA

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Green macroalgae species belonging to the *Ulva* genus provide a range of ecosystem services (ESS) worldwide – categorised as 'regulation and maintenance', 'provisioning' and 'cultural' ESS, adapting the Common International Classification of Ecosystem Services (2018). The ESS derive from 1) *Ulva* being part of natural balanced ecosystems, 2) *Ulva* being harvested and removed from unbalanced ecosystems and 3) *Ulva* being cultivated as a bioresource, and/or as a mitigation or restorative ecological engineering tool capturing and utilising carbon and nutrients. The ESS provided all contribute to achieving several UN Sustainable Development Goals – directly and in an integrative manner of which SDG 3, 6, 12, 13, 14 and 15 are closely related to the planetary boundaries quantifying the ecological ceiling of the safe operating space of humanity. In natural ecosystems, *Ulva* provides regulation and maintenance ESS such as photosynthesis, nutrient cycling, food and habitat. *Ulva* harvested from unbalanced ecosystems along with the cultivated *Ulva* allow for bringing into play the regulation and maintenance ESS as mitigation or restorative ecological engineering tools capturing and utilising carbon and nutrients, while providing a bioresource for food, feed, nutraceuticals and fibres. Cultural ESS are provided by *Ulva* through contribution to science and education.

EU STANDARDIZATION ON ALGAE AND ALGAE PRODUCTS

Susan Løvstad Holdt[1,2,3]*

[1]DTU Food

[2]The National Food Institute

[3]Technical University of Denmark.

The markets of algae are increasing, and European Commission realized that we need to know what we are trading; how do we know what we are trading and how we describe it? The challenges are e.g.:

- The green flakes that we say is Ulva, how was that species identified?
- The protein content of 12% how was that analysed and calculated?
- What unit do we use on productivity and emission capturing, and can we compare to other crops?

A group of experts across EU from companies and research work on standardizing definitions, sampling, analyses on nitrogen, amino acids, sugars, lipids, fatty acids, for cosmetic use and also energy purpose. Using the standards are not mandatory, but ensuring that the best methods are sed and a “license to trade”. We know that when e.g. companies refer to that the laboratories are working according to ISO standard ## that optimized procedures are implemented. This presentation will introduce a selection of relevant standards and standards being developed that are of interest for *Ulva* and its applications.

NOVEL DPSIR FRAMEWORK WITH THE ECOSYSTEM SERVICES APPROACH FOR SEAWEED PRODUCTION: FROM PRESSURE TO PROGRESS AND FROM REDUCE TO RESTORE

Thomsen, M.[1,2], Ecole, M.[1,2], Anwar, I.[1,2], Bruhn, A.[3,4]

The emerging seaweed aquaculture in Europe and Americas is expanding, delivering system value from engineered ecosystem services (EES) such as eutrophication and climate change mitigation. The use of seaweed as a nature-based emission capture and utilisation technology turning emissions into revenue streams while delivering non-profit value makes seaweed aquaculture a promising eco-industrial system fitting well the global agenda of green economic transitions and ecosystem health restoration. However, aquaculture activities may in some cases cause unwanted engineered ecosystem disservices (EDS), which should be avoided. For this reason, we propose an emission accounting and ecosystem-based framework with an extended scope I and IV to include circular nutrient management in the biobased product service system starting at scope I (emission capture), through scope II (energy-related emissions associated to the operation of the aquacultural production system), to the scope III, including material consumption and avoided emissions from transitioning into a biobased economy) and ending at scope (IV) which the potential services (or disservices) by the seaweed aquaculture. The emission accounting and ecosystem-based framework consisting of a triadic interrelationship between academia, industry and government with the common objective of implementing a sustainable blue economy delivering provisional (biorefinery products), supporting (nutrient recycling), and regulating (climate change mitigation) services, thereby restoring the balance between marine ecological integrity and ecosystem service utilization. We argue that an adaptive and cross-sectoral policy framework approach, with coordinated instruments, is imperative to a design of blue circular bioeconomy, from primary production to final output products (i.e. phyconomy), that restores, regenerates and respects the carrying capacity of our aquatic ecosystems. A novel DPSIR framework is presented that shifts from focusing on reducing pressure to making progress towards a restorative blue economy, emphasizing the integration of the ecosystem-based monitoring and reporting system which enables multi-governance actors from institutions across the involved sectors and scales to collaboratively develop a blue bioeconomy that delivers engineered ecosystem services that contributes to the restoration of marine ecological integrity.

HOW TO TURN EMISSIONS INTO REVENUE STREAMS

Esben Rimi Christiansen[1]*

[1]Founder of Pure Algae

Scarcity of resources increase while linear nutrient flows from land to sea contribute to marine eutrophication, and consequently loss of biodiversity. It is imperative to change the linear nutrient flows from land to sea into circular nutrient flows in order to conserve bioavailable nitrogen and phosphorus in the technosphere. Land-based fish production in recirculated aquaculture systems (RAS) provide opportunities to capture and utilise emissions of nutrients and CO2 for cultivation of macroalgae in Integrated Multitrophic RAS (IMRAS). Hereby emissions are turned into revenue streams generating a double crop production, while alleviating the biosphere from negative impacts of nutrient and CO2 emissions. *Ulva* – opportunistic green macroalgae, grows explosively under favourable conditions, generates high biomass production, and are attractive for European and global food and feed markets. Pure Algae has found a unique method and developed a technology for increasing the yield and bioremediation capacity of *Ulva* (figure 1) and keep this process stable over a long period of time (figure 2), while monitoring the quality of the seaweed as a result herof (figure 3) Results prove that *Ulva* biomass can be produced in high output and high quality when integrating a vertical seaweed cultivation system into the RAS industry.

SEAWEED SUSTAINABLE FARMING: THE EXAMPLE FROM AN EU ORGANIC CERTIFIED PRODUCER

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Aquaculture has been mentioned as a solution to the exponential population growth, but the conventional methods of finfish effluent treatment are complex and expensive. Cultivation of seaweed, responsible for more than 40 ton of nitrogen removal from coastal waters, seems to be a solution in the mitigation of environmental impact of finfish aquaculture. IMTA systems are a combination of these: they consist in the incorporation of different trophic levels on the same system in a balanced ecosystem, where finfish nutrient-rich effluents are used by the other species, converting waste into valuable commercial crops. Since 2012, ALGAplus, a Portuguese company located in Ria de Aveiro, dedicates to organic certified Atlantic seaweed species and fish farming in a land-based IMTA system. All seaweed production phases are carried out in-house: biomass production, processing and packaging. Besides, ALGAplus works with their customers on tuning the seaweed biomass to the desired characteristics or on the domestication of new species of interest. Nowadays, the main species in production is *Ulva sp.*, but species as Porphyra spp., Gracilaria gracilis, Codium tomentosum are also farmed year-round. With a strong focus in R&D, in 2023, AlGAplus reached an annual production of 210 ton (fw) of *Ulva* with the new 15 raceways-unit, designed in the frame of the European project GENIALG. Currently optimizing the system in terms of energy and labor costs and manipulation of production factors, the company is tackling these keystone tasks in the frame of different collaborative projects, such as SeaMarK (Horizon Europe) and Pacto da Bioeconomia Azul (02/C05-i01/2022).

Acknowledgments

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QUANTIFICATION OF CARBON DIOXIDE REMOVAL AND CARBON SEQUESTRATION BY SEAWEED

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[2]University of Tasmania

Seaweeds are an extremely important marine resource in coastal systems globally, with substantial potential for human food, feed additives, methane reduction from ruminants and novel products. Increasing seaweed stocks, particularly via aquaculture including into the open ocean, has been widely promoted as a natural, safe and simple solution to climate change via the photosynthetic fixation of atmospheric carbon dioxide and the subsequent sequestration of carbon as living biomass or into sediments:seaweed carbon credits are being sold on the voluntary carbon market on this basis. However, the optimism and enthusiasm afforded by seaweed 'afforestation' for carbon dioxide removal (CDR) is not currently supported by underpinning science. Here I consider how a very complex solution for CDR has gained such popularity, then outline best scientific practices required to assess CDR by seaweeds using a Forensic Carbon Accounting framework.

GROUNDING THE HYPE AROUND CARBON REMOVALS WITH SYSTEMS THINKING, AND LCA PRIORITIES TO SUPPORT A MORE SUSTAINABLE BLUE ECONOMY

Jean-Baptiste Thomas[1]*

[1]KTH Royal Institute of Technology, Sweden

Have you noticed that over the past year, we aren't seeing quite as many headlines claiming that seaweed is a silver bullet to solve the climate crisis? Confidence in this rhetoric has been undermined, and no one seems sure that climate benefits through carbon credits, removals and offsets is trustworthy anymore. In an attempt to restore trust and guarantee climate benefits, the EU is developing the carbon removal certification framework and the associated Qu.A.L.Ity criteria, and green claims may soon also be regulated. So how will this affect the algae sector? During this talk, we will explore the role of systems thinking and life cycle assessment (LCA) in grounding the hype around carbon offsets/removals, in shedding light on the environmental performance of seaweed value chains, the current state of LCA research in the sector, and what we urgently need to focus on. A critical review of the life cycle climate impacts of seaweed value chains is presented, providing a systems perspective to complement ongoing primary research that is being undertaken. LCAs strengths and weaknesses as a methodology are discussed in the context of seaweed and marine value chains, and priorities are suggested for how to evolve the method and improve its practice to better serve the growth of a sustainable blue economy.

SEAWEED CULTIVATION OR BEACH WRACK HARVESTING AS A MEASURE FOR NUTRIENT UPTAKE – A POSSIBLE BASE FOR FUTURE NUTRIENT TRADING SYSTEMS IN THE BALTIC SEA?

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[3]Environmental Science and Engineering

The Baltic Sea's low salinity means that both animals and plants invest a lot of energy in internal salinity regulation, which means that they are smaller than their relatives in a saltier sea area like the Skagerrak. Thus, when establishing a maritime aquaculture in the Baltic Sea by e.g. cultivation of blue mussels or seaweeds (Ulva), the individuals will be smaller and not economically competitive compared to their more maritime and bigger relatives from high salinity sea areas, especially if they are to be used for profitable food production. Thus, the concept of environmental mussel or environmental seaweeds has been introduced where it is argued for the cultivation of these for the uptake of fertilizing substances such as nitrogen or phosphorus from the eutrophic marine environment in the Baltic Sea. A cheaper alternative to the cultivation of seaweeds could be to take advantage of the enormous amounts of seaweed biomass washed up on the shores of the Baltic Sea as beach wracks (casts) and then use the biomass for e.g. biogas production or land-based fertilizer. The result is the same, remediation of nutrients from the Baltic Sea. The question is whether this harvest of biomass, which generates a positive effect for the environment and currently does not have a market price, should be subsidized with some form of market-based support from society. This paper/talk will discuss payments for nutrient uptake in the blue bioeconomy and when its well-motivated and when we should be careful to use it.

ECOSYSTEM ACCOUNTS IN ENVIRONMENTAL ECONOMIC ACCOUNTING – IMPLEMENTATION EXPERIENCE IN ESTONIA

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In recent years ecosystem accounting has seen a lot of development. UN Statistical Commission adopted System of Environmental-Economic Accounting - Ecosystem Accounting (UN SEEA EA) in 2021. The amendment of Regulation (EU) No 691/2011 is ongoing to include ecosystem accounts as one of the modules. More and more research is done on the topic which contributes to the better understanding of the concepts and application. Statistics Estonia began work first with ecosystem extent and monetary values in year 2019 following the guidance in SEEA EEA (EEA Ecosystem Experimental Accounting) and available country experiences. During the years the work has shifted to include physical ecosystem service flows and ecosystem condition indicators according to the amendment of Regulation (EU) No 691/2011. The presentation gives an overview of ecosystem accounts in environmental economic accounting system and describes Statistics Estonia's experience in ecosystem accounting. The work done includes mainly terrestrial ecosystems but the experience of mapping marine ecosystems and the concept of (marine) thematic accounts is also discussed.

EU4ALGAE IN ACTION – ANALYSING BIOREMEDIATION POTENTIAL OF ALGAE

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EU4Algae Stakeholder Forum is the first implementation action of the EU4Algae Initiative. The ongoing work on ES aims to assess available tools methods and scenarios for quantifying bioremediation services, including disservices of production, set state of play on environmental carrying capacity, and make suggestion towards further designing a monitoring framework. The work includes macroalgae cultivation both at sea and on land, as well as microalgae. In this session, Efthalia will present current work done and next steps, and take the opportunity to gather and share insights and recommendations to include in the upcoming publications.

INTEGRATING *ULVA* INTO IMTA SYSTEMS FOR ENHANCED NITROGEN BUDGETS AND ECOSYSTEM SERVICES

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The scarcity of freshwater, overfishing and declining ocean biodiversity, marine eutrophication by anthropogenic activities, and the increasing demand for seafood have all required attention from a more comprehensive, global perspective.Moving from conventional aquaculture toward an ecological approach to developing and managing a sustainable aquaculture that cares for environmental and sociological aspects can bring relief to at least some of these problematic issues. Nutrient removal using Integrated Multi-Trophic Aquaculture (IMTA) systems is a promising ecological approach for sustainable aquaculture. The rationale behind the IMTA systems is to convert the excretions of the organisms cultured upstream into valuable food for the organisms cultured downstream. In marine IMTA systems, algae and halophyte plants have a high capacity for nutrient uptake per unit of culture area and can be an essential additional valuable product. In addition to nutrient removal by the green seaweed *Ulva sp.*, the IMTA system proved to be a reliable source of sustainable biomass for human consumption, animal feed, and high-value by-products for the food additive industries. IMTA systems in offshore cage cultures and land-based facilities will improve the food conversion rate (FCR) and diversify the mariculture products, ultimately increasing profit for the farmer. In addition, they will often create additional jobs and, no less importantly, will reduce environmental pollution.

MODELLING AND IDENTIFICATION IN AQUACULTURE

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Mathematical models are crucial in many scientific fields. Nowadays, computer-aided model simulations are used more and more to support (i) the understanding of the dynamic behavior of water, nutrients and energy flows in food (Ulva) production processes and (ii) the design, optimization and control of reactors and reactor networks in these technological applications. Mathematical modelling that starts from first principles can be a good start. For operational use in real practice, however, the model should be in good accordance with experimental data, which is the main objective of system identification. Yet, in the past decade data-driven modelling approaches become more and more popular, thus using a limited amount of prior knowledge. However, for understanding and design of new (production) processes the use of prior knowledge, in terms of mass and energy balances, is of paramount importance.



Bioactives in Ulva

Aveiro, Portugal

June 24-25. 2024

Ulva

The Future Wheat of the Sea

ORGANIZERS

Prof. Dr. Rosário Domingues, Departament of Chemistry, University of Aveiro, Portugal
Ana Moreira (University of Aveiro, PT),
Cláudia Nunes (University of Aveiro, PT),
Margarida Martins (ALGAplus, PT),
Muki Shpigel (University of Haifa, IL),
Rosário Domingues (University of Aveiro, PT),
Thomas Wichard (University of Jena, DE)

The COST Action SeaWheat, the University of Aveiro, the Friedrich Schiller University Jena, and the Company AlgaPlus joined forces to organize a 2-day Workshop on "Bioactives in Ulva" (Working Group 4), a topic of significant importance in the research field. This collaborative effort, held from 24.06 – 25.06. 2024 with the Department of Chemistry of the University of Aveiro graciously hosting the workshop, demonstrated a shared dedication to advancing the understanding of macroalgal bioactives.

RATIONAL AND OBJECTIVES

The green seaweed *Ulva* (Chlorophyta) is a valuable source of diverse bioactive compounds with significant potential in health, nutrition, cosmetics, and pharmaceuticals. Its rich content of polysaccharides, proteins, phenolics, vitamins, minerals, fatty acids, and pigments offers a wide range of potential biological activities that can be harnessed for promoting health and well-being. The workshop aimed to bring scientists together to uncover the full potential of these compounds, as *Ulva* is assumed to play an increasingly important role in various industries. Established and young scientists and representatives from Small and Mid-sized Enterprises came together to discuss the status quo of the current research on macroalgal bioactives.

ORGANIZATION

The workshop was part of Working Group 4's (WG4) planned list of events for the current grant period. The local organizing committee, led by Prof. Dr. Rosário Domingues, Dr. Ana Moreira, and Dr. Cláudia Nunes, played a pivotal role in ensuring the workshop's smooth operation. They were supported by Dr. Thomas Wichard (University Jena, DE). The workshop program was discussed and decided upon by experts who constituted the scientific committee. These were (listed alphabetically): Rosário Domingues (PT), Thomas Wichard (DE), Céline Rebours (NO), and Muki Shpigel (IL). Ana Moreira and Rosário Domingues edited the abstract book (Annex of the report). News and information about the workshop were posted on the SeaWheat COST Action social media channels (such as LinkedIn) and the Action websites.
The local host of the workshop, the University of Aveiro (Universidade de Aveiro), located in Aveiro, Portugal, is a prominent institution known for its academic excellence, research contributions, and innovative approach to education. Established in 1973, the university has become one of Portugal's leading educational and research institutions. The University of Aveiro hosts several research centers and institutes focusing on areas such as, e.g. marine sciences, environmental studies, materials science, and education and was a perfect location for the COST Action Workshop on "Bioactives in Ulva".

PARTICIPANTS

The COST Action workshop was well received, with 60 registered participants from 14 countries (6 ITC countries). Combining it with the subsequent Training School in Aveiro at the same location proved excellent. In addition to the COST Action registered participants, further scientists attended the presentations throughout the workshop.

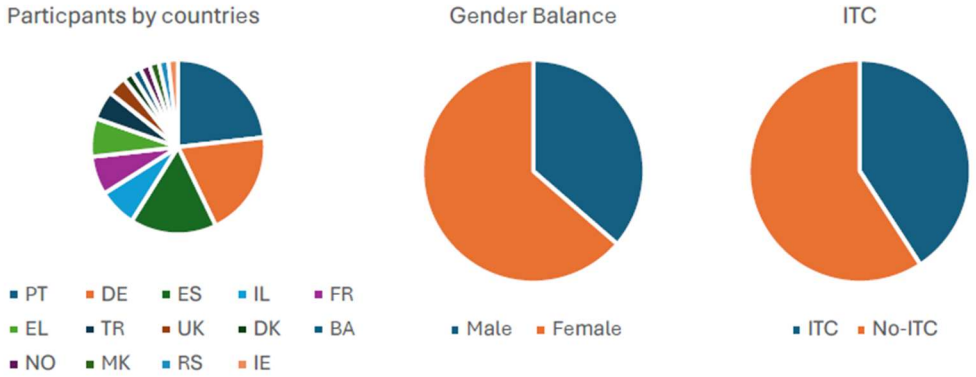


Figure 42 Distribution of the Workshop participants by countries, gender, and members of ITC

WORKSHOP DESIGN AND FUNCTIONING

Amongst the participants, current research topics on bioactives in macroalgae and their applications were presented in four sessions chaired by experts on the topic (total of 20 talks, each 30 min) : (i) "Extraction Approaches," (ii) "Omics Analysis," (iii) "Bioactive Compounds," and (iv) "Targeted Analysis of Bioactive Phytochemicals". Each session comprised 4-6 presentations by experts in the field as an overview, including discussions after each presentation.

Within these sessions, the following topics were addressed by the speakers (examples are given):

- **Extraction Techniques:** Methods for extracting bioactive compounds from Ulva
  (e.g., Hugo Pereira, Greencolab, Portugal).
- **Bioactive Properties:** Health benefits and potential applications of bioactives.
  (e.g., Dieter Lütjohann, University, Hospital Bonn, Germany)
- **Industrial Applications:** Use of *Ulva* bioactives in pharmaceuticals, cosmetics, and nutraceuticals.
  (e.g., Pi Nyvall Collén, Olmix, France)
- **Research Advances:** Latest research findings and future directions in the study of *Ulva* bioactives.
  (e.g., Vassilios Roussis, University of Athens, Greece)

As can be seen from the list of speakers, proximity to the application of bioactives was sought through reports from industry (AlgaPlus (PT), Omix (Fr), GreenCoLaB (PT). The breaks between the sessions were used for further discussions and exchanges on other activities of the COST Action participants. Networking was used to establish new projects and existing co-operations were deepened. The status quo of various COST Action activities of the WG4 were discussed (e.g., the ongoing research exchange via STSMs, the review initiative about bioactives in Ulva).

Notably, on the afternoon of the event's second day, the young scientists (Master's students, PhD students, and Postdoctoral students) who participated in the subsequent training school gave short presentations. These 15 5-minute presentations (pitch sessions) again demonstrated the growing interest in bioactives in macroalgae and provided an exciting overview of the ongoing European research.



Figure 43 Group photo of 65 participants of the Seawheat workshop outside the venue

CONCLUSIONS

This workshop focused on the bioactive compounds found in Ulva, a genus of green algae commonly known as sea lettuce. The event brought together researchers, industry professionals, and students interested in extracting and applying bioactives from Ulva species. An overview of *Ulva* bioactives, extraction techniques, and initial research findings was given. The organizing team resumed that the workshop promoted research and the cohesion of research, often scattered throughout Europe.



Bioactives in Ulva

Aveiro, Portugal

PROCEEDINGS

Ulva

The Future Wheat of the Sea

HYDROTHERMAL EXTRACTION AND DEPOLYMERIZATION OF ULVANS

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Ulvan composition and structural features, closely related to the biological properties, are influenced by the extraction and purification stages. Water extraction at temperatures higher than 100 °C allowed shortened operation time [1]. Acidic conditions aid in the de-stabilization of aggregates enhancing the yields [2] and bioactivity due to partial reduction of molecular weight [3], although coextraction of other undesirable components may occur [4]. Hydrothermal extraction with water under subcritical conditions offers advantages derived from the change in physicochemical properties of the solvent, such as the lowered relative dielectric constant, which enhances the extraction of nonpolar substances. However, the ionic product increases converting water is an interesting reaction medium acting as an acidic or basic catalyst. The release of sulfate and carboxylic acid groups in the aqueous phase generates an acidic medium promoting autohydrolysis and depolymerization of polysaccharides to oligomers and to monomers without externally added chemicals. Operation times could be additionally shortened during operation with microwave assisted heating [5].

In this study the potential of hot pressurized water or autohydrolysis was studied with conventional or with microwave heating for solubilising the ulvan fraction from *Ulva sp.* The initial defatting stage was avoided due to the low lipid and extractives content and to conform a simpler process. Different recovery strategies were tried, including the conventional cold precipitation with ethanol, with bioionic liquid and the fractionation with ultrafiltration membranes. Further characterization of the chemical, structural, rheological and cytotoxic properties were assessed.

The operation temperature strongly influenced the process performance and up to 60% overall extraction yield was attained. The maximum ulvans yield recovered by ethanol precipitation was 23% from the extract obtained during heating up to 160 °C, which required 23 min during conventional heating up to the maximum temperature, and was 32% after 6 min of isothermal operation at the same temperature with microwave heating and precipitation with chloline chloride. The lowered pH induced by hydrothermal operation, due to the presence of organic acids in the liquid phase, influences the extraction efficiency of ulvan [2], but the decrease was less pronounced than with a strong acid leading to pH below the pKa of glucuronic acids and facilitating its selective extraction over glucuronan, xyloglucan, and soluble protein [4].

The crude ulvan recovered after ethanol precipitation exhibited carbohydrate and uronic acids contents in the range 66-77%, with 7-11% sulfate and a protein content under 1%. The apparent viscosity decreased with increasing shear rate, exhibiting a clear pseudoplastic behaviour. The highest values of apparent viscosity were observed for the ulvan extracted at 160 °C, followed by those extracted at 140 and 120 °C, and were comparable to those of commercial products. The viscoelastic features of representative ulvan-based films exhibited mechanical profiles corresponding to a typical gel behaviour. The product obtained at 160 °C presented two peaks, corresponding to 150 kDa and to 24 kDa, comparable to those attained with acidic extraction, but the enzymatically obtained extract are mainly formed by high molecular weight fractions [3, 6]. Cell viability assays with the mouse fibroblasts NCTC clone 929 was not affected by ulvan samples at concentrations up to 1.0 mg/mL in the growth medium.

The ulvan free extract showed a low protein and phenolic content, this latter correlating with the reducing and antiradical properties. The hydrothermal treatment caused changes in the microstructure of the residual solid phases, from the smooth, compact and regular morphology of untreated seaweed to the shrinkage of the cell wall in extracted biomass, particularly at treatments beyond 180 °C. These residual solid fractions are enriched in protein, suggesting potential applications, i.e. for food or feed. The ash content in the residual solids was progressively reduced with increasing temperature, and also the sulfate content. The lipid content slightly followed this trend. However, a lowered carbohydrate content, and the reduction in the glucose content confirms that only 20% of the initial glucose remained in the solid suggesting that this fraction corresponded to the cellulose.

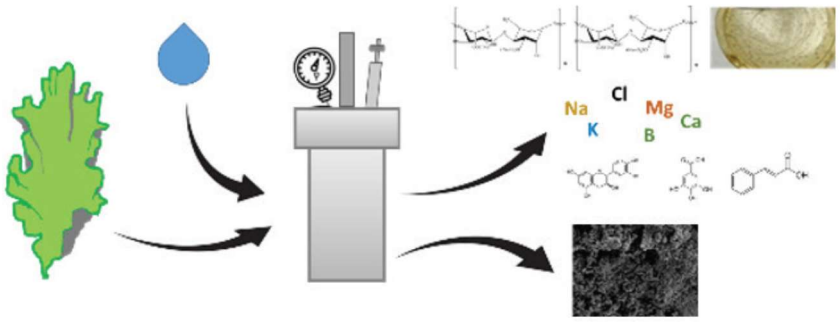


Figure 44 Hydrothermal extraction and depolymerization of ulvans

In summary, hydrothermal processing of *Ulva* sp is an efficient, rapid and tuneable technology valid to fractionate the wet algal biomass in less than 20 min with conventional heating and in 6 min with microwave assistance. No previous drying, solvent extraction and chemical addition were needed. The ulvan yields could be enhanced and the mechanical properties of the recovered ulvan were comparable to those of commercial counterparts.

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Acknowledgments

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SCALABLE BIOREFINERY PIPELINES FOR THE PRODUCTION OF ALGAE-BASED BIOACTIVE INGREDIENTS

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Algae feedstocks offer remarkable potential for diverse biotechnological applications. However, the current production costs of algae biomass in industrial-scale facilities often limit its use in high-value markets like food, nutraceuticals, and cosmetics. Although significant advances are currently being undertaken to improve the cost-effectiveness of algae production at the industrial scale, one of the most promising approaches to fully exploit the potential of algae biomass is the development of cost-effective biorefineries. Multiproduct biorefineries are an innovative approach that promotes the exploitation of the different biochemical components present in algal biomass through their sequential extraction, fractionation, and processing into high-value ingredients. In this context, GreenCoLab and associated partners have developed and optimized several biorefinery pipelines to process different algal biomasses (micro and macroalgae) into multiple ingredients at laboratory scale in the last few years. The established pipelines followed a logical sequential extraction and separation from high-value to lower-value compounds, using an array of different techniques for the valorization of the different fractions, including biomass pre-treatment, membrane filtration, enzymatic hydrolysis, and green chemistry extractions. More recently, a pilot-scale biorefinery platform - ALGAEREFINE prototyping center - was implemented at GreenCoLab to validate the scalability of the developed biorefinery pipelines and generate relevant quantities of ingredients to be tested in multiple applications. Results demonstrate the feasibility of different biorefinery processes in establishing different ingredients and upgrading the value of algae biomass. In addition, the potential of these ingredients and their valorization for different biotechnological applications (e.g., aquafeeds, cosmetics, agriculture) was also assessed, highlighting that the biological activities can be enhanced in the ingredients. The environmental and economic performance of the most promising biorefinery routes are currently being evaluated using life cycle assessment (LCA) and techno-economic analysis (TEA). Overall, the implementation of scalable biorefineries is key to maximizing the market value of the different biochemical components contained in algae biomass and ensuring their valorization in new value chains.

WATER SOLUBLE EXTRACTS OF *ULVA* USE AS PLANT BIOSTIMULANT

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Seaweed-derived extracts are promising sources of novel biostimulants [1,2] that could increase crop yields sustainably. The objective of this work was to determine whether ulvan crude extract could be used as a biostimulant for terrestrial plants. Herein we showed that crude ulvan extract, derived from cultivated local to Israel *Ulva sp.*, promotes the growth of Arabidopsis thaliana in its early germination stage. This study addressed the challenges of identifying an appropriate extraction method for ulvan, examining its bioactive concentration and timing of supplementation, and testing its effect on A. thaliana seedlings and mature plants. We identified and addressed the following knowledge gaps: (i) specification of a reliable and repeatable method for ulvan extraction from a local *Ulva* species based on a nondestructive procedure, (ii) determination of the bioactive ulvan concentration and its effect on inducing the highest stimulation to A. thaliana sprouts, (iii) examination of the effect of the timing of ulvan supplementation during the early-stage germination of A. thaliana sprouts, and (iv) determination of the impact of ulvan supplementation at the early stage of plant development on the final seed yield of mature A. thaliana. Our attempt to fill these gaps began with examining the chemical composition of our extracted ulvan and comparing it to compounds identified in the literature. We then observed the specific bioactivity effects of the biostimulant on A. thaliana seedlings. We measured morphological characteristics after three weeks under two light conditions. We divided early germination into two stages (at sowing and one week after sowing) to examine the effect of adding ulvan in those two steps separately and together. We also determined several photosynthetic parameters. Finally, we ascertained whether ulvan supplementation at the early stage of plant development affected the final seed yield of mature A. thaliana. This research identified the ulvan extraction method and the bioactivity concentration required to enhance A. thaliana seedling development, We identifying 0.2 mg/ml as the optimal ulvan concentration for stimulating A. thaliana germination. We showed that the timing of ulvan supplementation during early germination of A. thaliana is essential. We revealed that the positive effects of our ulvan extract on A. thaliana germination (enhanced root length and shoot area, recorded at the end of the three-week germination period) occurred if we added the ulvan at the beginning of this first week (ulvan present at sowing). The roots of the treated sprouts were significantly longer (+13.6%, mean 5.55 cm) than the control (mean ~4.88 cm). The treated sprouts also had a higher shoot area (+45.2%, 58.06 mm2) than the control (33.98 mm2). Adding ulvan at the beginning of the second week (after sowing) had no significant effect on these parameters. Finally, we found that the stimulation in the early-stage germination positively affected the final seed yield of mature plants. We show that the seed yield was ~43% higher for the ulvan-treated group than the control, untreated group. These results demonstrate that ulvan biostimulants could potentially reduce the use of harmful agrochemicals, thus mitigating their negative environmental impacts while increasing crop yield and contributing to global food security.

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INNOVATIVE APPROACHES FOR THE SELECTIVE RECOVERY OF NUTRIENTS AND BIOACTIVE COMPOUNDS FROM ULVA: BENEFITS AND LIMITATIONS

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Over the last years, a growing interest has shown in the use of *Ulva* from different points of view such as food, cosmetics, pharmaceuticals, etc. In this sense, one of the possible uses is through its use as such, for example, incorporating it into food, but another possible use is through the recovery of high-added-value compounds, and in some cases their subsequent purification. Some of the main advantages of this strategy are the concentration of the active ingredients, as well as an improvement in acceptance by consumers, who can reject the consumption of *Ulva* as such, but not in the form of its derivatives (i.e, additives). In this sense, the use of solid-liquid extraction techniques for the recovery of *Ulva* compounds is one of the most followed strategies, but it has different drawbacks such as the use of solvents, which in many cases are toxic, consumption of a large amount of solvents, high temperatures and extraction times, totally in disagreement with current standards regarding green extraction and sustainability [1]. Thus, different green and eco-friendly strategies have been proposed, such as pulsed electric fields, supercritical fluids and pressurized liquid extraction, among others, as possible alternatives to conventional processes. However, although the use of these technologies is of great interest and has many advantages, it also has some important disadvantages, mainly related to the scalability of the processes and other limiting factors of the process. The following presentation will evaluate some of the main advantages and disadvantages derived from the use of innovative extraction processes such as pulsed electric fields, supercritical fluids and pressurized liquid extraction.

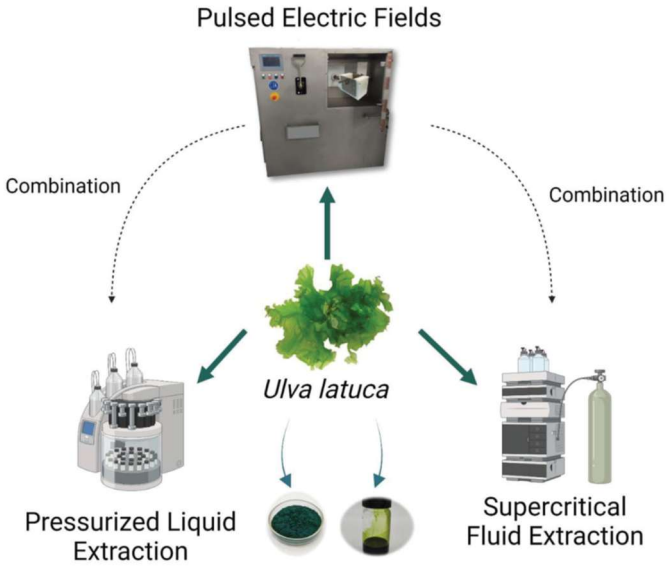


Figure 45 Innovative approaches for the selective recovery of nutrients and bioactive compounds from ulva: benefits and limitations

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EXTRACTION STRATEGIES OF BIOACTIVE COMPOUNDS AND ENVIRONMENTAL CONTAMINANTS FROM ULVA

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Seaweed constitutes an important group of marine organisms with a vast potential as sources of bioactive compounds, which have been gaining attention from the scientific community due to the broad range of biological effects they display, including antimicrobial, anti-inflammatory, antiviral, and antifungal activities. However, marine species have lower concentrations of bioactive compounds than terrestrial organisms, sometimes only trace amounts [1]. In another hand, the possible use of seaweed as bioindicator of environmental contaminants, has also the constraint of dealing with residual concentrations [2,3]. Thus, it is paramount that the extraction techniques available reflect the nature of the organism from which the extract will be obtained, as well as the characteristics of the compound to be isolated. The efficiency of the extraction methods is influenced by several factors, such as seaweed species, solvent selection, extraction temperature, pressure, and time, with optimization of these parameters crucial to maximizing the yield and quality of the bioactive compounds extracted. While traditional methods such as solvent extraction, maceration, and Soxhlet extraction have been widely employed, the concern with more clean and environmentally safe methods has led to the development of more efficient techniques. Innovative extraction methods like supercritical fluid extraction (SFE), ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and enzyme-assisted extraction have emerged as promising alternatives, offering higher extraction yields, shorter extraction times, and better preservation of bioactivity.



Figure 46 Extraction strategies of bioactive compounds and environmental contaminants from ulva

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AN INORGANIC ANTIOXIDANT IN A LIVING SYSTEM IMPACTING ATMOSPHERIC AND MARINE CHEMISTRY: IODIDE IN SEAWEEDS (KELP)

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Brown algae of the Laminariales (kelps) are the strongest accumulators of iodine among living organisms. They represent a major pump in the global biogeochemical cycle of iodine and in particular, the major source of iodocarbons in the coastal atmosphere. Nevertheless, the chemical state and biological significance of accumulated iodine have remained unknown. Elucidation of these questions was the objective of this study. Using an interdisciplinary array of techniques, chiefly relying on synchrotron X-ray absorption spectroscopy, we show that the accumulated form is iodide, which readily scavenges a variety of reactive oxygen species (ROS). We have shown that its biological role is that of an inorganic antioxidant, the first ever to be described in a living system [1]. Upon oxidative stress, iodide is effluxed. On the thallus surface and in the apoplast, iodide detoxifies both aqueous oxidants and ozone, the latter resulting in the release of high levels of molecular iodine and consequent formation of hygroscopic iodine oxides leading to particles, which are precursors to cloud condensation nuclei. When kelp thalli are submerged, this process impacts iodine speciation in seawater. In several aspects, iodide is unique as a biological antioxidant. Among the halides, it has by far the best antioxidant properties; yet, bromide complements it for the detoxification of superoxide. This paper will also report on the latest tomography- and micro-XANES-based findings concerning the subcellular localization of iodine in cells, with implications for its mechanism of uptake, storage and efflux.

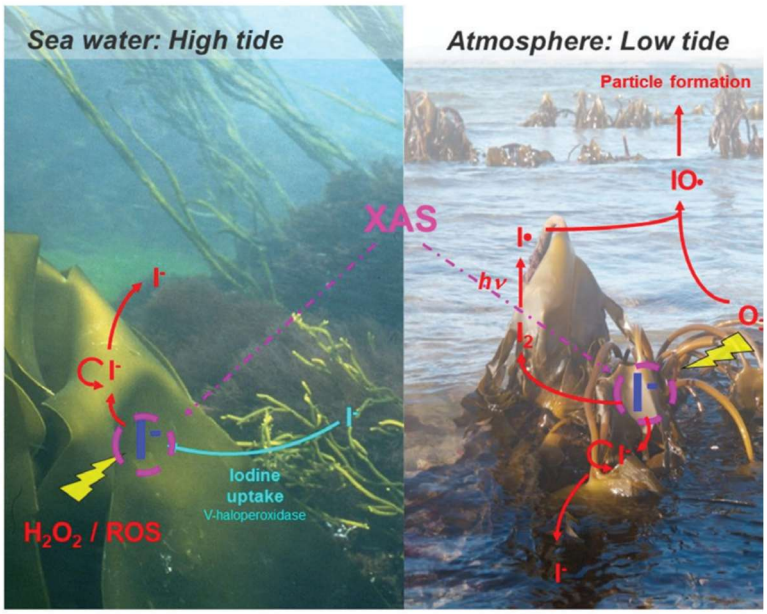


Figure 47 An inorganic antioxidant in a living system impacting atmospheric and marine chemistry: iodide in seaweeds (kelp)

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ADVANCES AND PERSPECTIVES ON SEAWEED METABOLOMICS

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Seaweed metabolomics is an emerging field with immense promise for understanding seaweed biochemical processes, metabolic pathways, and bioactive compounds [1-4]. Metabolomics, which involves the comprehensive analysis of small molecules present in a biological sample, will enable researchers to delve deeper into the metabolic profile of seaweeds and unlock their potential for various applications in industries such as food, pharmaceuticals, agriculture, and bioenergy. For example, perspectives on seaweed metabolomics can be identified in elucidating pathways, identifying bioactive compounds, or the environmental stress response of seaweeds such as the green macroalga *Ulva* [4]. Many recent studies have identified metabolic changes in the thallus of *Ulva* (endo-metabolomics) because of diurnal growth, gametogenesis, proliferation, and environmental stresses [e.g., 3,5,6]. Identifying metabolites affecting Ulva's development in the culture medium (exo-metabolomics) [1] is an additional challenge, considering the low concentration of bioactives. The metabolomics experiment design will be a specific focus of the talk. It requires careful consideration of multiple factors to ensure reliable and interpretable results. For example, it includes sample preparation, which involves extracting metabolites from biological samples while minimizing degradation and contamination. After all, finding unknown compounds is common in metabolomics, although "known unknowns" can be utilized to make descriptive comparisons between treatments like stress stimuli. Under laboratory conditions, metabolomics can detect any changes (e.g., caused by reintroduced bacteria or abiotic stresses) in *Ulva* [7] and benefits from a combined approach with transcriptome data. Finally, multivariate methods must exploit the high covariation between omics measurements. Hereby, the metabolome is of special interest since it integrates all molecular and environmental effects, which will be illustrated in case studies.

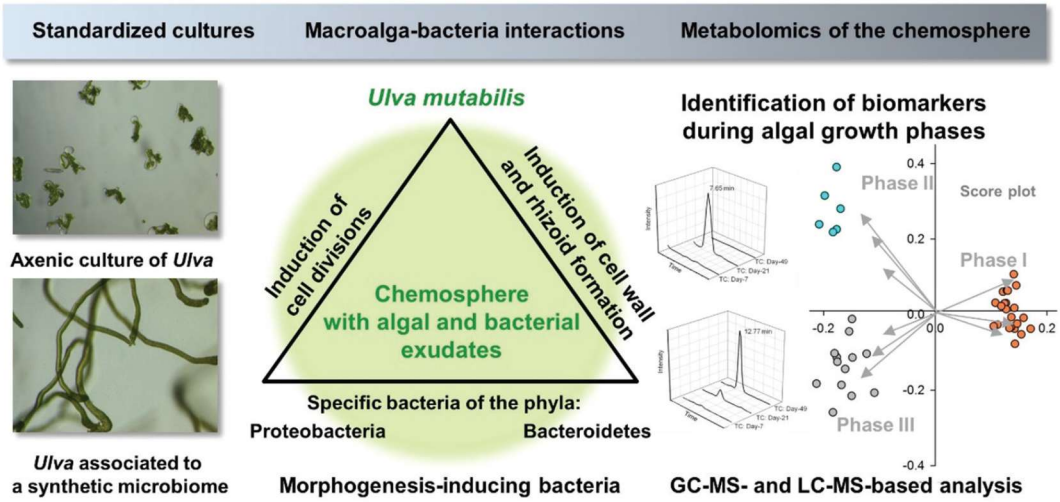


Figure 48 Advances and perspectives on seaweed metabolomics

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Acknowledgments

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LIPIDOMICS OF ULVA: UNVEILING NOVEL INSIGTHS INTO ADDED VALUE AND BIOACTIVE PROPERTIES

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Macroalgae are rich sources of valuable compounds, including polysaccharides, proteins, lipids, phenolic compounds, and pigments. Among these, lipids stand out as essential nutrients, some with notable bioactive properties, making algae a valuable resource for enhanced health [1]. Lipidomics has significantly contributed to comprehending the diverse lipid profiles of *Ulva* species, unraveling their potential as sources of omega-3 fatty acids and membrane polar lipids with promising bioactive properties, as anti-oxidant and anti-inflammatory potential [2]. Also the added value and the lipidomic profile of *Ulva* species may be modulated under varying environmental conditions and seasonal changes. Recently, it was shown that *Ulva sp.*exhibited clear seasonal variations in its lipid profile, with highest abundance of unsaturated fatty acids, including omega-3 polyunsaturated fatty acids (PUFAs), during winter and lowest during summer [3]. Moreover, regardless of seasonal fluctuations, *Ulva sp.* consistently maintained a low n-6/n-3 ratio, associated with health benefits. Analysis of polar lipids revealed variations in glycolipids and phospholipids, with glycolipids, often associated with anti-inflammatory activities, showing higher content during spring and winter, when compared to summer. The phospholipids are increasing notably during autumn and spring. Understanding the modulation of lipid composition and bioactive properties of *Ulva* lipids is crucial for selecting optimal biomass suited for diverse applications, ranging from functional foods to pharmaceuticals.

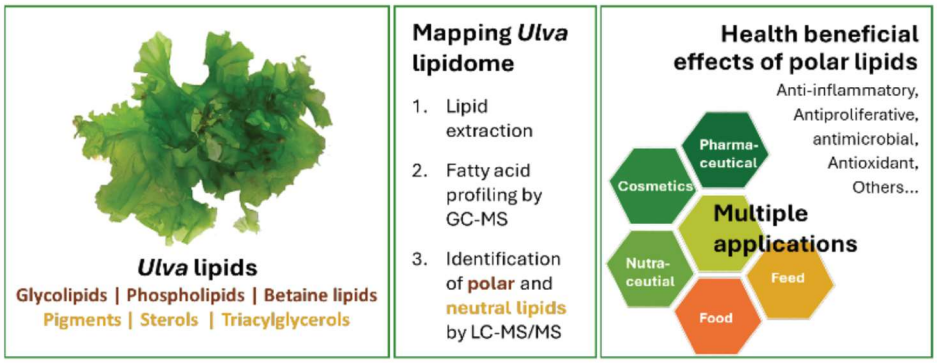


Figure 49 Lipidomics of Ulva: unveiling novel insigths into added value and bioactive properties

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WHEN TOTAL SYNTHESIS MEET LIPIDOMICS...

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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.

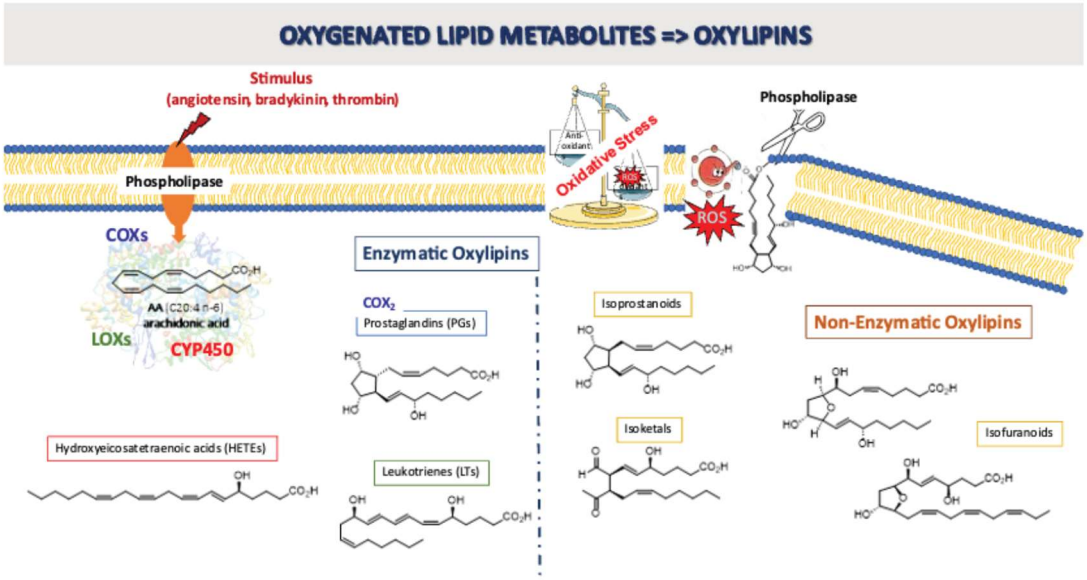


Figure 50 When total synthesis meet lipidomics...

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ALGAPLUS: A PERSPECTIVE FROM A LAND-BASED SEAWEED PRODUCER

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ALGAplus is a private Portuguese company that initiated its activity in 2012, in a property located at Ria de Aveiro with 14 ha. ALGAplus produces customised seaweed and seaweed-based products mainly for the food and well-being markets, reaching the B2B (companies & restaurants) and B2C (retail) sectors with different brands. ALGAplus seaweed are organic certified, assuring warranties of sustainability, quality standards through time and traceability to the customers. At ALGAplus, the production is done under the Integrated Multi-Trophic
Aquaculture (IMTA) concept, with a strong focus in R&D and continuous support to the customers. All seaweed production phases are carried out in-house: biomass production in a land-based modular IMTA system, processing (washing, drying, salting, cutting, milling), and packaging. Besides, ALGAplus works with their customers on tuning the seaweed biomass to the desired characteristics or on the domestication of new species of interest, giving them the product differentiation needed in highly competitive markets. The main species in production at this Portuguese company is *Ulva sp.*, however, other species native from the Atlantic ocean are also farmed and commercialized year-round, such as Porphyra dioica, Porphyra umbilicalis, Gracilaria gracilis, Codium tomentosum, among others.
Since 2011, many of the achievements of the company have been reached in the frame of internal and collaborative innovation projects. In 2016, the company reached a milestone with 600 m2 of surface of production, however, this meant still a small production in comparison with the market demand and the upscaling production/processing was mandatory. Since 2020, ALGAplus is actively working on the processes optimization and upscaling, that goes from the nursery to outdoors tanks, including the update of protocols of cultivation. In 2022, ALGAplus reached another milestone with the implemtation of 15 raceways for seaweed production under the same IMTA concept in the frame of the European project GENIALG. Currently, ALGAplus is optimising the system in terms of energy and labour costs, manipulation of production factors according to downstream and upscaling needs. Today, these keystone tasks are being carried out in the frame SeaMark (Horizon Europe) and Pacto da Bioeconomia Azul (02/C05-i01/2022); and also together with strategic partners with expertise in the field of macro- and microalgae, such as GreenCoLab.
Wordwide there has been a significant interest of the academic institutions and companies not only on the upstream processes associated to the cultivation/harvesting of seaweed (and algae in general) but also related to downstream and application of these resources. In this regard, the Algae Vertical (part of the project Pacto da Bioeconomia Azul) was created intending to develop i) methodologies to obtain more sustainable biomass, and also ii) innovative algae-based applications for the nutraceutical and cosmeceutical markets, ready-made meals and novel foods for human consumption, new functional foods for aquaculture and the development of

new agricultural solutions (i.e. biofertilizers, biopesticides and microbiota stimulants).

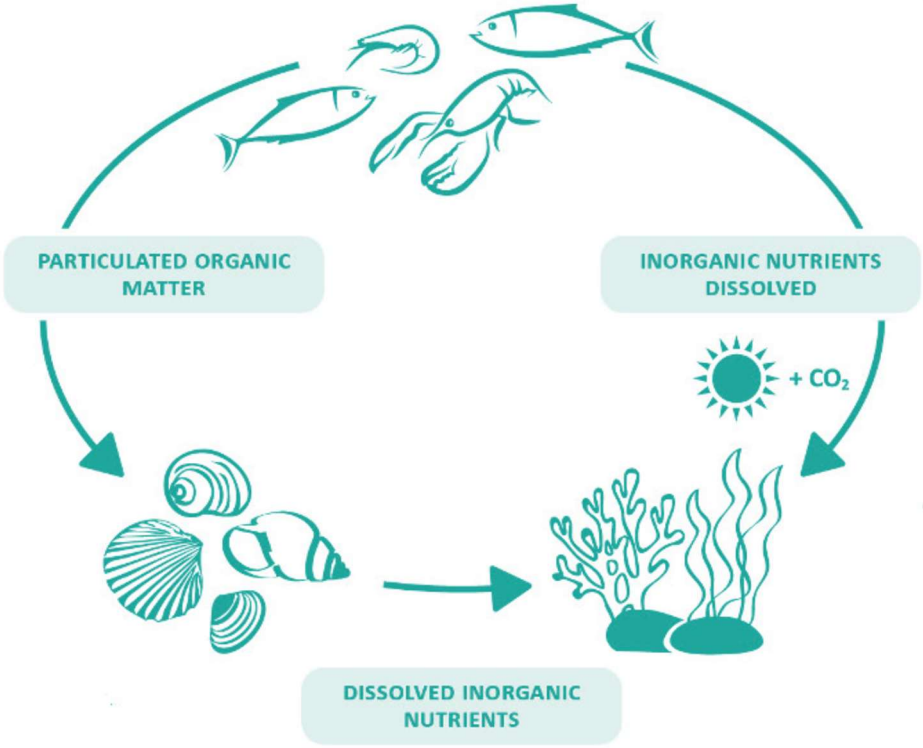


Figure 51 Algaplus: a perspective from a land-based seaweed producer

Acknowledgments

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BRITTANY ULVAN: A MALEDICTION OR A GOLDEN RESOURCE?

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Ulva species present a dual nature: Responsible for numerous strandings, green tides, or blooms worldwide, *Ulva* species are perceived negatively by coastal populations. Algal blooms and strandings have gained significance worldwide due to climate change and anthropogenic activities. They are considered a nuisance due to decomposition and the production of toxic vapors for the tourism industry in coastal areas and marine ecosystems (inhibition of seaweed zygote germination, decrease in algal species growth rates). They result in financial losses for resort operators, who must also cover the costs of removing and disposing of the thousands of beached algae.

Conversely, they have been utilized as vegetables for decades in certain Asian countries and also find numerous applications in the food, feed, or biofuel sectors. Indeed, *Ulva sp.* contains commercially valuable components such as bioactive value-added products (Ulvan, nutrients, starch, PHA). The aim of this presentation is to highlight the potential benefits of harnessing the biomass of *Ulva* species. Various strategies for valorization have been investigated in the domains of human health and material science in our laboratory. Ulvan and oligo-ulvan fractions obtained after Extraction Assisted by Enzyme have been shown to stimulate human dermal fibroblasts proliferation. An increase in the synthesis of extracellular matrix components such as type I and III collagens, and also of glycosaminoglycans has been demonstrated. Fractions also stimulate the expression, synthesis and MMP-1 enzymatic activity. At skin microbiota level, the fractions do not alter the growth of commensal bacteria S. epidermidis, S. aureus and C. acnes, but may alter the biofilm formation. Ulvan and oligo-ulvan fractions also reduce the inflammatory potential of HaCaT keratinocytes induced by C. acnes. In a distinct domain, we have investigated the utilization of *Ulva* and ulvan in materials science, specifically focusing on their impact on the fresh and hardened properties of raw earth materials

24(R,S)-SARINGOSTEROL – FROM ARTEFACT TO A BIOLOGICAL MEDICAL AGENT

Vanbrabant K.[1,2,3], Van Meel D.[3,4], Kerksiek A.[3], Friedrichs S.[3], Dubbeldam M.[5], SCHEPERS M.[2,6], Zhan N.[3,6,9], Gutbrod K.[7], Dörmann P.[7], Liu H-B.[8], Mulder M.T.[9], Vanmierlo T.[2,6], Lütjohann D.[3]*

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Enhancing the cholesterol turnover in the brain via activation of liver x receptors (LXR) can restore memory in a mouse model for Alzheimer's disease (AD) [1]. The edible Asian brown alga Sargassum fusiforme (Hijiki) contains high amounts of oxysterols such as (3β, 24ξ)-stigmasta-5, 28-dien-3, 24-diol (24[R, S]-saringosterol) that are a potent liver x receptor (LXR) agonists. We aimed to find native European seaweed species with contents of 24(R, S)-saringosterol that are comparable to those found in S. sargassum fusiforme. Additionally, we hypothesize that seasonal variations modify the amount of 24(R, S)-saringosterol in seaweeds. Sterols and oxysterols were extracted with chloroform/methanol from various seaweed species harvested in the Eastern Scheldt in different seasons between October 2016 and September 2017. Identification and quantification of the lipids was performed by gas chromatography- mass spectrometry and gas chromatography - flame ionization detection. We confirmed that brown algae Undaria pinnatifida harvested in February and S. muticum harvested in October contained the highest amounts of 24(R, S)-saringosterol (32.4 ± 15.25 µg/g, mean ± S.D. and 32.95 ± 2.91 µg/g, respectively) and its precursor fucosterol (1.48 ± 0.11 mg/g), higher than S. fusiforme (20.94 ± 3.00 µg/g, mean ±S.D.), while Ascophyllum nodosum and Fucus vesiculosus and F. serratus contained amounts of 24(R, S)-saringosterol (22.09 ± 3.45 µg/g, 18.04 ± 0.52 µg/g and 19.47 ± 9.01 µg/g, mean ± S.D., respectively) comparable to S. fusiforme. In other algae only minor amounts of these sterols were observed. The green algae *Ulva lactuca* contained only 0.29 mg/g fucosterol and 10.3 µg/g 24 (R, S)-saringosterol, while all investigated red algae did not contain any 24(R, S)-saringosterol or fucosterol. In the Eastern Scheldt algae harvested in September/October delivered the highest yield for 24(R, S)-saringosterol, with the exception of U. pinnatifida that showed the highest levels in February. We showed that exposure of lipid extracts of *Ulva lactuca* to sunlight at room temperature or in the presence of oxygen to UV-C light lead to the quantitative conversion of fucosterol into 24(R, S)-saringosterol. Exposing pure fucosterol to UV-light did not convert any fucosterol into 24(R, S)-
saringosterol underscoring the requirement of seaweed constituents in the conversion of fucosterol into 24(R, S)-saringosterol. In conclusion, we showed that brown seaweeds harvested from the Eastern Scheldt contain amounts of 24(R, S)-saringosterol comparable to S. fusiforme, varying per season and showing the highest amounts in spring. In accordance with these observations the amount of 24(R, S)-saringosterol in the brown seaweeds can be modulated by light.

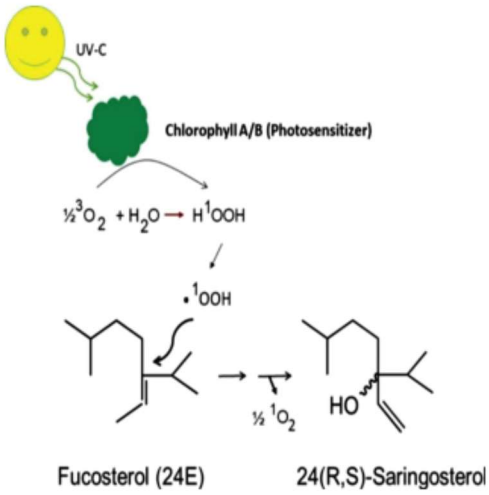


Figure 52 24(R,S)-Saringosterol – from artefact to a biological medical agent

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IMMUNE MODULATING PRODUCTS FROM *ULVA* FOR ANIMAL CARE

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The valorization of opportunistic seaweed biomass arriving in thousands of tons on the coasts of Brittany have allowed OLMIX to develop natural solutions for Agriculture and Animal husbandry based on seaweed. Today the green seaweed *Ulva sp.* containing MSP (Marine Sulfated Polysaccharides) are used in a range of animal-Care products designed to manage mycotoxin risk, digestive health and efficiency as well as immunity.
Scientific proof of efficacy, knowledge of the mode of action and dose effects are all important for regulatory compliance, to define product positioning and provide sales arguments. For these and many other reasons Olmix invests extensively into research on it's actives.
The immune system is continuously challenged in livestock animals and is a key factor of animal performance. This is why this was one of the first issues Olmix addressed with its seaweed extracts and products. Today, demonstration have been made, of the capacity of an ulvan extract from *Ulva sp.* to modulate the immune system in pig, poultry as well as aqua species, both in vitro [1 ,2, 3] and in vivo [2, 4, 5]. Using both reporter cell lines and specific inhibitors, TLR2 and TLR4 have been identified as the main target receptors in different cell types (2, 6). The in vitro activation of heterophils and monocytes by the ulvan extract leads to a temporary release of pro-inflammatory cytokines, including interleukin1-β, interferon α and interferon γ, through partly identified pathways. When ulvan is given per os to animals in vivo the activation of heterophils and monocytes is induced in a similar manner. The same protocol was used to evaluate the impact of a formulated version of this extract, called Searup®. A single oral administration at the dose recommended for use in the farm, results in heterophils and monocytes activation in vivo similarly to the purified extract. The products are also evaluated in field conditions to confirm positive effects on animal performance.
Together our results confirm the capacity of the ulvan extract to modulate immunity in several species that the formulated solution, Searup®, similarly to the purified extract allow to activate monocytes and heterophils and may provide protection against a larger variety of pathogens including virus. This may explain the recently identified capacity of the ulvan extract, and Searup®, to limit dissemination of Marek's disease virus in vitro and to promote viral reactivation in lymphoid cells.

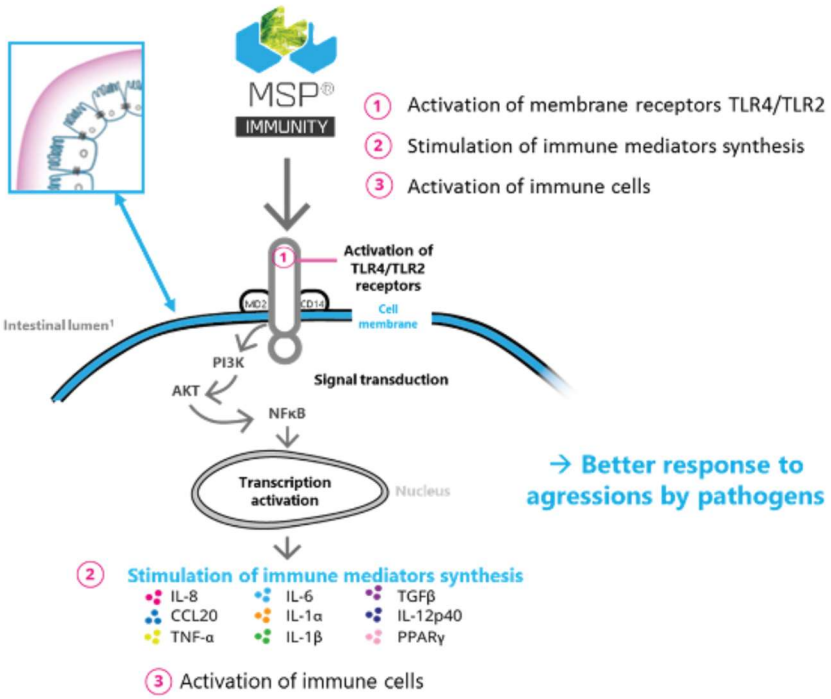


Figure 53 Immune modulating products from Ulva for animal care

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EXAMPLES OF ELISA AND REPORTER ASSAY TO ASSESS HEALTH RISKS AND BENEFITS OF DIETARY MACROALGAE

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There is an increasing interest in using macroalgae as food ingredients and the consumption of macroalgae is considered healthy due to their content in numerous bioactive compounds [1]. However, the safety of seaweed products and the health effects of its consumption are less understood. For example, correct labelling of allergens is an important aspect of food safety to provide consumers with safe food products [2]. Of the established food allergens, which must be labelled according to food regulations, cohabitant fish, mollusc and crustaceans are the most likely source of possibly contaminating allergens that could be found in seaweed products. ELISA (Enzyme-linked Immunosorbent Assay) is the most common and internationally accepted detection method for established allergenic proteins in food by direct antibody recognition of the protein and easy to perform in any laboratory. The principle of the method will be explained, followed by examples of detection of crustacean, mollusc and fish allergens in samples from Norwegian farmed seaweed [3]. Complementary methods for allergen detection will also be discussed. Further, cell cultures are interesting models to simulate conditions of health and disease. ELISA is one of the methods to assess the level of target proteins, mostly in case of secretion from the cells, such as for inflammatory signalling molecules. Here we will discuss the effects of supplementation with extracts from *Ulva* fenestrata and Saccharina latissima on the human monocyte cell line THP-1 with focus on the used methods such as ELISA and others [4].

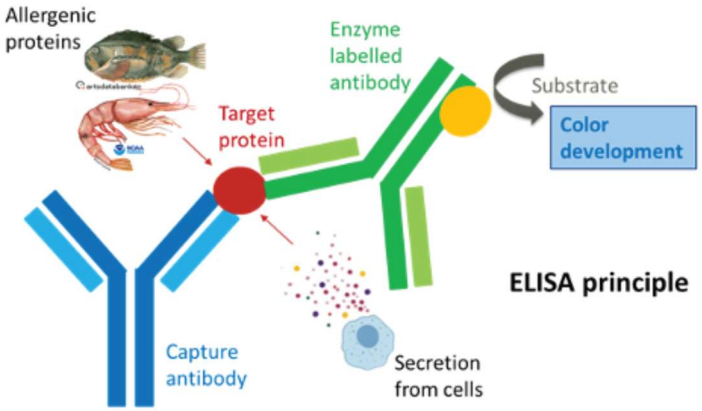


Figure 54 Examples of elisa and reporter assay to assess health risks and benefits of dietary macroalgae

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Acknowledgments

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ULVANS AS VERSATILE BIOACTIVE BUILDING BLOCKS FOR THE DEVELOPMENT OF HYBRID BIOMATERIALS FOR BIOMEDICAL APPLICATIONS

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Polysaccharides are highly appreciated as valuable ingredients of biomaterials and are widely exploited in the biomedical field due to their biocompatibility and biodegradability. Marine polysaccharides, incorporating various functionalities and exhibiting interesting physicochemical properties and significant biological activities, are attractive materials for the development of novel systems for bioapplications, such as drug delivery and tissue engineering.

Algal polysaccharides, in contrast to those of animal origin, are regarded as safer and non-immunogenic. Among them, the sulfated polysaccharides ulvans located in the cell walls of green algae of the order Ulvales (Chlorophyta), due to their physicochemical properties and the wide range of biological activities they exhibit, are increasingly studied for applications in the pharmaceutical field [1].

The utilization of ulvans as a biopolymer has not yet been comprehensively investigated. In the recent years, our group has successfully prepared and characterized a number of ulvan-based hybrid scaffolds of various structures, such as membranes, particles, hydrogels, 3D porous structures or nanofibers, and has utilized them for drug delivery, tissue engineering and wound healing, among other applications [2-13].

Our current results highlight the potential uses of ulvans as bioactive building blocks towards the design of hybrid biomaterials for biomedical applications.

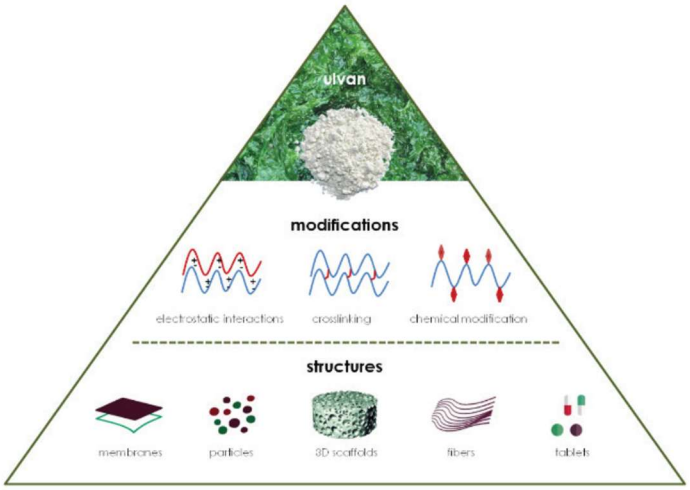


Figure 55 Ulvans as versatile bioactive building blocks for the development of hybrid biomaterials for biomedical applications

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MICROBIAL CONTRIBUTION TO FOOD DIGESTION IN THE GUT OF ALGIVOROUS SEA URCHIN: A NOVEL RESOURCE OF ALGAL POLYSACCHARIDES DEPOLYMERIZING BACTERIA

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An ecological insight into the spatial arrangement in the gut bacterial community of the algivorous sea urchin *Tripneustes gratilla elatensis* will improve our knowledge of host-microbe relations concerning the involved taxa, their metabolic repertoire, and the niches of activity. We hypothesized that alterations in the bacterial compositional structure under different diets and along the gut are associated with the potential contribution to food digestion. Toward this goal, we investigated the bacterial communities in the esophagus, stomach, and intestine of the sea urchin when fed a mono-specific diet of either *Ulva* fasciata or Gracillaria conferta, or an algal-free aquafeed. The study combined 16S rRNA amplicon sequencing, followed by bioinformatics analyses of community structure, interactions, and the prediction of their functional genes.
Sea urchins fed with *U. fasciata* grew faster and their gut microbiome network was rich in bacterial associations (edges) and networking clusters [1]. Bacteroidetes was the keystone phylum in the gut with few microbes of this phylum being central hub nodes that maintained community connectivity, while others were driver microbes that led the rewiring of the assembly network based on diet type [1]. Spatial distribution was evidenced by communities with distinct features in the esophagus and intestine. Bacteria that can contribute to *Ulva* digestion are common in the stomach and intestine and consist of genes for carbohydrate decomposition, fermentation, synthesis of short-chain fatty acids, and various ways of N and S metabolism [2]. Bacterial enrichment was performed in a selective medium with *Ulva* polysaccharides extract or ulvan. Among the isolated bacteria, a novel strain of *Alkalihalobacillus sp.* was found capable of in vitro degradation of several algal polysaccharides [3]. Carbohydrate-active enzymes of this and other bacteria are being used for the development of a bacterial based process for depolymerization of seaweed polysaccharides in a designated bioreactor.

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BASICS, DOS AND DON'TS IN CAROTENOID ANALYSIS

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Carotenoids are widespread and versatile isoprenoid compounds that intervene in many processes in Nature. They are essential in photosynthesis, acting as secondary light harvesters but also in the assembly of the pigment-protein complexes and in photoprotection. They are also important in the communication between species through colour, which is relevant for plant pollination, seed dispersal and for the mating of some animals, such as flamingoes. Some carotenoids are precursors of vitamin A, which is an essential nutrient. Besides, a large body of evidence indicates that these compounds can intervene in biological actions and help reduce the risk of diverse diseases. Importantly, carotenoids can be cleaved into derivatives (collectively termed as apocarotenoids) including aromas, important hormones or many signals that have been shown to play important roles in plants. Taken together, carotenoids and their derivatives are very important for food security and health promotion through the diet [1,2].

The analysis of carotenoids is challenging for different reasons. On one hand, with very few exceptions, they are lipophilic compounds that are located in lipidic environments (membranes, lipid globules, plastids) that make their extraction more difficult. Besides, their structure, featuring a long system of electron-rich conjugated double bonds, make them very susceptible to oxidation and geometrical isomerization. Some carotenoids, especially those with 5,6-epoxide groups, can be easily transformed upon contact with even traces of alkali [3,4].

Rather than dealing with state-of-the-art methodologies for the analysis of carotenoids, this work deals with basics and advice about their handling and choice of methodological aspects. In this sense, advice about sample preparation and storage, extraction, saponification or choice of stationary phases, among others, is given. As a general rule, carotenoid analysis should be done as soon as possible, although this is not always possible. Then, samples need to be stored. Ideally, they should be freeze-dried and kept as intact as possible, as the damage of the cells increase the risk of degradation. The approach for extraction depends largely on the purpose of the analysis. If this is to identify as many carotenoids as possible from a source, large amounts of samples and solvents will be needed. If the goal is to analyze the major carotenoids of a source, microextractions can be performed, with the consequent gain in throughput and savings in solvents. Saponification is usually done to reduce the lipidic content, get rid of interferent compounds (for instance chlorophylls) and/or to simplify the chromatograms when carotenoid esters are present. However, this step can lead to important losses of carotenoids, so it is important to think carefully if it is really a necessary step in our methodology. For many years, C18 reverse-phase columns were the stationary phases of choice for carotenoid separation in HPLC. With the advent of C30 reverse-phase columns, these gained much popularity. The choice of either type of stationary phase is another aspect that can help us optimize our analyses and workflow [3–6].

Last, but not least, it is important to start replacing traditional methodologies for carotenoid analysis with more environmentally-friendly alternatives. In this sense, studies on the replacement of typical solvents for carotenoid analysis (some of which are petroleum-derived and are toxic) for more greener alternatives (2-methyl oxolane, natural deep eutectic solvents, etc.) appear as a timely topic. The recently presented AGREEprep tool, which helps assess the greenness of sample preparation can be very helpful for this purpose [7].

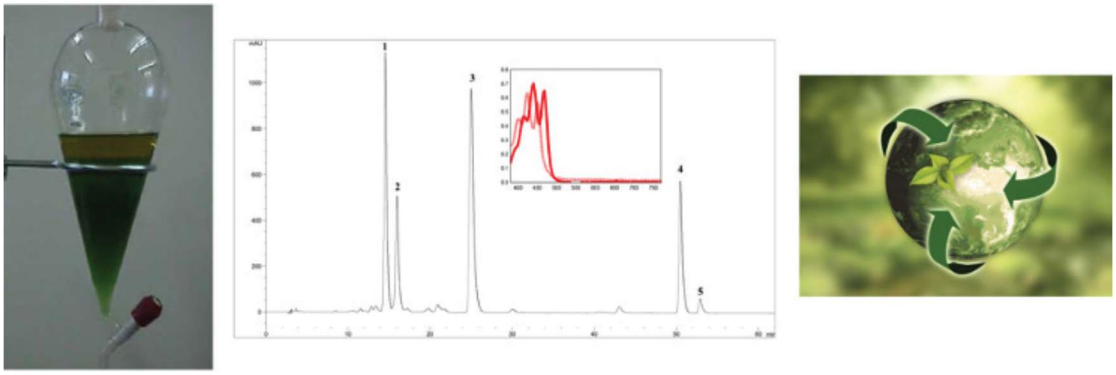


Figure 56 Basics, dos and don'ts in carotenoid analysis

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SEASONAL DYNAMICS OF NUTRITIONAL COMPOSITION AND NUTRIENT DIGESTIBILITY OF *ULVA LACTUCA*: A PERSPECTIVE OF A MEDITERRANEAN LAGOON IN MOROCCO

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Continuously growing world population has increased the demand for plant and animal origin foods thus enhancing the competition for land use and crops between food and feed. This requires the addition of new resources without shifting the balance between the competing forces. Algae growing due to the water pollution in response to the increased human activity present such resources. *Ulva lactuca*, a green algae belonging to Ulvaceae family, has been the specie of great interest for food and feed since its abundant availability in the oceans around the world. Wild growing *U. lactuca* in the oceans is not suitable for use in food because of the bioaccumulation of impurities and toxic materials in *U. lactuca*. Therefore, wild growing *U. lactuca* can be used in animal diets provided a continuous supply of uniform biomass is maintained. Marchica lagoon of Nador, Morocco receives green tides that brings a considerable amount of *U. lactuca* every year that can be valorized in animal diets. However, great discrepancies in the nutritional content of *U. lactuca* have been reported most likely arising from varying abiotic factors associated with seasonal and geographical differences. No earlier study has evaluated the seasonal dynamics of nutritional composition and nutrient digestibility of *U. lactuca* growing in the Marchica lagoon of Nador, Morocco. The resent study was aimed to investigate the seasonality of nutrient composition and nutrient digestibility of *U. lactuca* from Marchica lagoon.

U. lactuca was collected from Marchica lagoon during the year 2022-2023. Collection of samples started in summer 2022 through autumn 2022, winter 2022-2023 to spring 2023. A total of four samples were collected in each season, dried in shade, and finally dried in greenhouse facility in Nador, Morocco. Then, samples were transported to the animal nutrition laboratory in Turkey. The samples were analyzed for nutritional composition according to methods described previously by the Association of Official Analytical Chemists. Fiber fractions were measured using the methods described by Van Soest et al. in an automatic fiber analyzer (ANKOMA2000 Fiber Analyzer, ANKOM Technology, NY, US). In vitro digestibility of nutrients was measured using ANKOM DaisyII incubator (ANKOM Technology, NY, US) after 48 hours of incubation. In situ digestibility of nutrients was measured in in heifers fitted with rumen cannula. Data were subjected to statistical evaluations using appropriate tests in a computer based statistical software package. The study revealed that the nutritional composition and nutrient digestibiilities differed significantly as a function of season ($P < 0.05$).

This study suggested that the chemical composition, in vitro and in situ rumen digestibility of nutrients is dependent on the harvesting season. Accordingly, adjustments are required while including *U. lactuca* in animal diets.

Acknowledgments

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IDENTIFICATION AND FUNCTION OF ALGAL GROWTH PROMOTING FACTORS IN *ULVA* (CHLOROPHYTA): ADVANCES IN OUR UNDERSTANDING OF CHEMICALLY MEDIATED BACTERIA-INTERACTIONS

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The marine macroalgae *Ulva sp.*, widespread in aquatic habitats, bears considerable ecological relevance and enormous economic potential across numerous industries such as food, feed, pharmaceuticals, cosmetics, and sustainable biofuel generation [1]. For optimal cultivation, *Ulva* requires specific symbiotic bacteria that release algal growth- and morphogenesis-promoting factors (AGMPF's) [2, 3]. The symbionts interact through chemical signalling molecules. The Ulva-associated Roseovarius sp. cells are chemotactically attracted to dimethyl sulfoniopropionate (DMSP) released by the alga [4], and *Ulva* provides glycerol as a carbon source for Roseovarius sp. [4]. In turn, the bacterium supports Ulva's cell division and growth by secreting unknown morphogens. This is followed by the interaction with the second Ulva-associated symbiont Maribacter sp., which releases thallusin and induces Ulva's rhizoid and cell wall formation [5].

This study embarks on a quest to identify novel AGMPF's and unravel their mode of action. Leveraging highresolution mass spectrometry, it opens doors to structure elucidation, subsequent organic synthesis, and the establishment of structure-activity relationships. These findings could potentially improve our understanding of chemically mediated bacteria-algae interactions in *Ulva* (Chlorophyta).

Thallusin, the first identified morphogen in Ulva's chemosphere [5], exhibits remarkably high activity in the picomolar range [6]. The stereochemistry and the three carboxylic acid groups play a pivotal role in the activity of the steroid-like hormone thallusin. The two carboxy groups in the dipicolinic acid facilitate the iron complex unit, ensuring the acquisition of the Fe-thallusin complex through a siderophore-like uptake system [6, 7]. To monitor thallusin in aquaculture and to determine ecologically relevant concentrations in nature, a highly sensitive method for quantifying thallusin has been developed, capable of detecting concentrations close to its half-maximum effective concentration (EC50) [8]. Thallusin has been quantified in various natural systems, often at concentrations multiple times higher than its EC90 value, demonstrating the precision and sensitivity of our method.

In addition to thallusin, the still unknown Roseovarius-AGMPF would complete a fully axenic cultivation of Ulva. With a sterile-filtered supernatant of Roseovarius sp. supplemented with thallusin, it is already feasible to grow bacteria-free *Ulva* cultures. Initial experiments using solid phase extraction and bioassay-guided fractionation indicate that Roseovarius releases multiple potentially synergistically acting AGMPF's. Moreover, recent observations support a long-standing hypothesis [2] that Roseovarius supports the growth of Maribacter. The partially purified and identified AGMPFs, pave the way for a deeper ecological understanding of the symbiotic network of the tripartite community Ulva-Roseovarius-Maribacter. Understanding the mechanism of algal growth-promoting substances not only improves our understanding of *Ulva* ecology and microbial interactions, but it also opens up new biotechnological possibilities for marine bioactives, both in aquaculture and on land.

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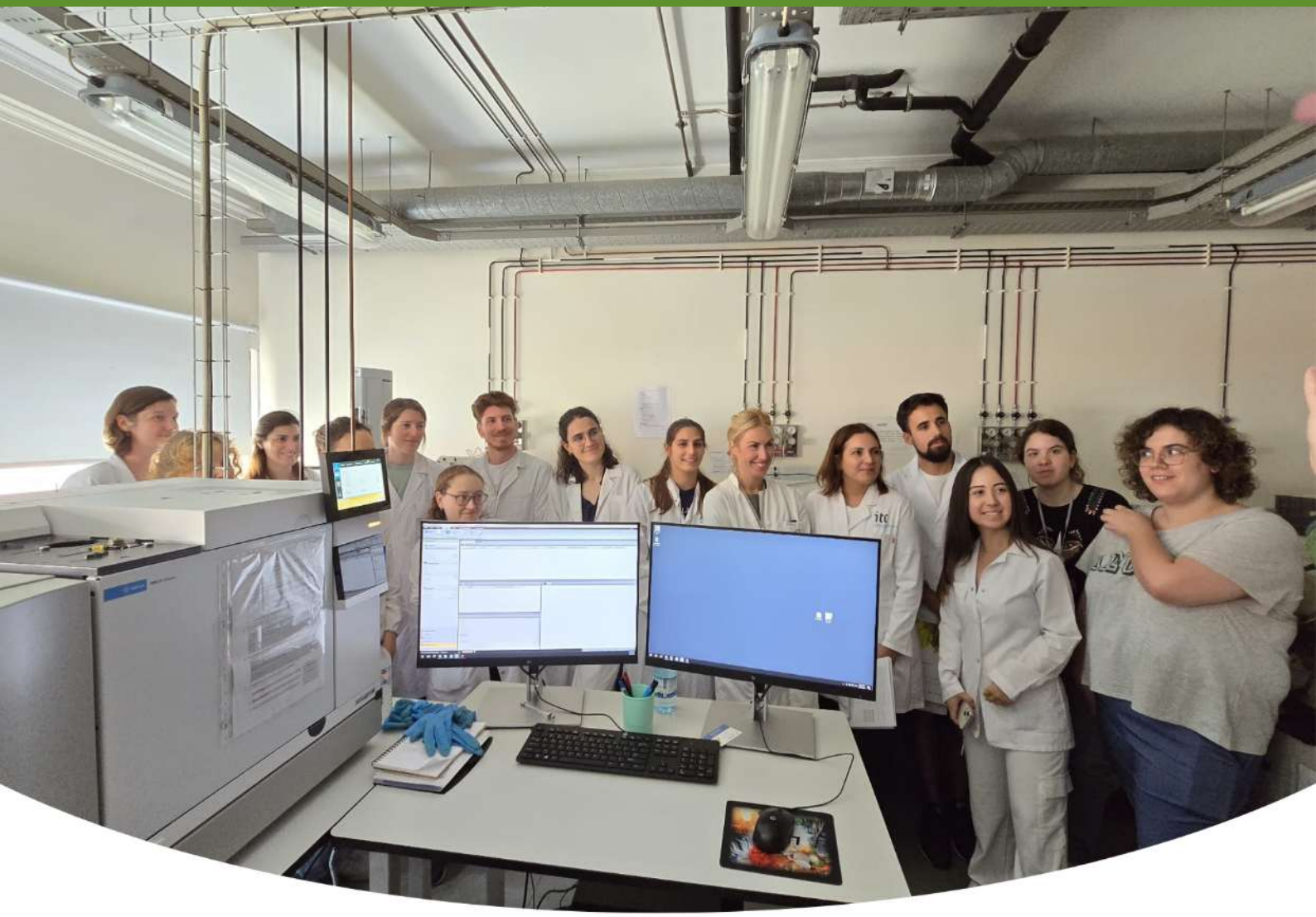
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Bioactives in Ulva

Aveiro, Portugal

ABSTRACTS OF TRAINEES

Ulva
The Future Wheat of the Sea

STRAIN IMPROVEMENT: A KEY-ISSUE IN LAND-BASED CULTIVATION OF *ULVA SP.*

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The European seaweed industry relies mostly on wild stock harvesting, which adds pressure on natural populations and displays seasonal variability reflected in biochemical composition shifts and presented phenotypes. Sustainable cultivation is essential to fulfil the demands of locally sourced seaweed of consistent quality, high-grade, traceable and with predictable biomass yield. Biobanking of genetically diverse strains promotes food security, biosecurity and safeguarding biodiversity conservation under the threat of climate change and anthropogenic pressures.

Since 2012, ALGAplus has been cultivating Atlantic seaweed species in an integrated multitrophic system (IMTA). *Ulva* is the species with the most significant production at the company, leading to a special interest in strain selection for this species. In January 2023, the ALGAplus biobank collection counted with 20 strains of *Ulva sp.*

In the framework of the project Seamark (Horizon Europe), ALGAplus carried out multiple sampling campaigns, increasing the number of *Ulva* strains to 104 in biobank. Samples were collected from the natural populations growing in the surrounding earthen ponds, comprehended in the 14ha property, but also arbitrary individuals from our high performing batch of mixed industrial strains, in cultivation since 2022. Novel strains are being screened, in partnership with NUIGalway, based on genotyping and expressed phenotypes, to detect the highest yielding, value added, resilient strains, to factors such as herbivory and environmental variability. The best performing strains will be validated at pilot scale at the company. In this work, the biobanking and preliminary results of screening will be presented.



Figure 57 Strain improvement: a key-issue in land-based cultivation of Ulva sp.

Acknowledgments

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EXPLORING THE BIOACTIVE POTENTIAL OF ADRIATIC SEAWEED (*ULVA SP.*): GREEN EXTRACTION AND MICROENCAPSULATION TECHNIQUES FOR POLYPHENOLICS AND NATURAL PIGMENTS

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Seaweed *Ulva sp.* as a potential innovative product from the sea till now is not examined and sufficiently exploited [1-4]. The main focus of this research was to establish new ways of utilizing *Ulva* and to show the possibility of applying more advanced, green, and safe technologies in the industry as well as to develop new technologies that will preserve valuable bioactive compounds from *Ulva sp.* The sampling of *Ulva sp.* In Boka Kotorska Bay (Adriatic Sea) was performed from June to July 2023. The dried samples were ground into powders in a laboratory mill and prepared for the extraction process. The goal of the extraction process was to be economically justified and environment friendly, based on the principles of "green extraction". The effects of several extraction parameters (solvent type, time, solid-solvent ratio, extraction method) on the extraction efficiency of solid/liquid extraction (maceration and ultrasound) were examined. To determine the influence of total polyphenols and flavonoid extraction, three levels of green solvent (ethanol-water) (30, 50, and 70%) and two levels of the solid–solvent ratio (1:10 and 1:20) were examined. Based on the results, an ethanol-water mixture (50%, solid solvent ratio 1:20) was chosen as optimal extract conditions. With the optimal extraction conditions, maceration was performed for 1h, 2h, and 18h. The next step was a comparison of maceration-MAC (2h) vs. ultrasound-assisted extraction method-UAE (0.5h), with optimal conditions. The maceration method of 2h was a superior (more powerful) extraction method and was used for the production of a higher amount of extract which was subjected to a microencapsulation process. To preserve bioactive compounds, the optimal extract was microencapsulated. The *Ulva sp.* extract, possessed a high content of total polyphenols, chlorophyll A and B, and carotenoids (3.55 mg GAE/g, 84.53 mg/100g Chl A, 68.41 mg/100g Chl B, 5026.82 mg/100 g), respectively. The novel microencapsulation technologies were used for the preservation of *Ulva sp.* Extracted bio-compounds. *Ulva* liquid extract was spray-dried (Labtex ESDTi spray dryer) and freeze-dried (Beta 1-8 Freeze Dryer), with and without carriers' addition. Microencapsulates were prepared using maltodextrin as a conventional carrier and an innovative one, polydextrose. The obtained powders improved Ulva's stability, limiting the inactivation of the sensitive polyphenolic compounds, achieving their high encapsulation efficiency associated with good bioactive protection. The examined microencapsulates demonstrated good powder properties, exhibited high powder yield, parameters important for further food or pharmaceutical applications. Based on the results, it can be concluded that the microencapsulation techniques and carrier addition had an impact on the protection of *Ulva sp.* bioactive compounds. The *Ulva* microencapsulates demonstrated high antioxidant activities determined by DPPH, RP, and ABTS assays. The thermal analysis proved *Ulva* microencapsulates have good thermal stability. FTIR method indicated that microencapsulation developed effective preservation by physical incorporation, not chemical transformation. All fragments from this valuable sea resource may be used as uncostly bio-products rich in functional compounds, which could have commercial utilization and benefit. To overcome the limitations of the *Ulva* application, through the proposed research we intend to make efforts to demonstrate characteristics of *Ulva* species present along the Adriatic Sea. Outputs from this research will provide a source of valuable research data in the promotion and development of Ulvabased blue-biotech industries.

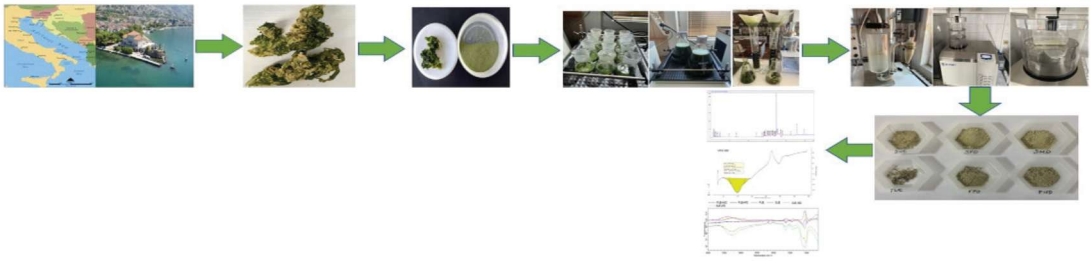


Figure 58 Exploring the bioactive potential of adriatic seaweed (Ulva sp.): green extraction and microencapsulation techniques for polyphenolics and natural pigments

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Acknowledgments

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ASSESSMENT OF *ULVA SP.* RESPONSE TO DIFFERENT ABIOTIC FACTORS UNDER LABORATORY CONDITIONS

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Macroalgae represent a source of high value substances for food, pharmaceuticals, and cosmetic industries. Algae are thus a valuable resource for a greener economy, providing a natural source of a variety of chemicals; they improve the sustainability of industrial processes, thereby contributing to the European Circular Bioeconomy. A variety of abiotic parameters can influence the physiology of macroalgae, such as light, temperature, salinity, nutrient availability, and wave action. Consequently, the composition of algal biomass is affected by these factors, and therefore its commercial use. Within the framework of the MSCA SeaChem Doctoral Network, this PhD project investigates responses of *Ulva* intestinalis under different temperature and salinity regimes. The methods consist of eco-physiological analyses to evaluate changes in photosynthetic activity (oxygen evolution and chlorophyll fluorescence), and biochemical analyses to assess biomass composition (pigments, lipids, and proteins) using HPLC and GC. Further experiments analysing the effects of light and nutrient starvation were conducted in spring 2024, applying the similar analytical approach on *Ulva* compressa, in collaboration with PureAlgae, a startup company based in Denmark. The overall project aim is to determine the optimal growth conditions for the targeted algal species, to obtain the desirable biomass composition. Through identification of the main factors influencing the metabolism of the seaweed, ultimately the accumulation of high value compounds can be enhanced.

Acknowledgements

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MICROBIAL INTERACTIONS IN *ULVA* HOLOBIONT WITH IMPLICATIONS IN AQUACULTURE

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The continuous growth of Aquaculture and its expected intensification could lead to environmental concerns, such as high nutrient effluent discharges, resulting into eutrophication events, or an abusive usage of disinfectants or antibiotics in culture infection treatments, which could contribute to the spread of antimicrobial resistances. An alternative reduce environmental impact of intensive aquaculture is Integrated Multi-Trophic Aquaculture (IMTA), that portrays the co-culture of species belonging to different trophic levels so that the waste from one is the nourishment for the other [1]. IMTA systems using sea lettuce (*Ulva sp.*) associated to fish cultures have been developed, due to the remarkable biofiltration capacity of *Ulva* in the removal of nitrate and phosphate from the environment, high growth rate, vegetative reproduction and high ecophysiological plasticity [2]. Furthermore, *Ulva* may provide a niche for bacteria that can help prevent diseases in the aquaculture systems. Bacteria of the Rhodobacteraceae family are often abundant on the surface of *Ulva* [3], and, among them, genera such as Phaeobacter have been highlighted as potential probiotics for use in aquaculture [4]. Phaeobacter is described as a good colonizer of abiotic surfaces and also to integrate microbiomes of living organisms, including algae species [5]. These bacteria have the ability to produce Tropodithietic acid (TDA), which is an antibiotic capable of preventing the appearance of pathogenic bacteria in aquaculture facilities such as those belonging to the Vibrio genus [6]. In recent years, an approach combining IMTA recirculation system (IMTA-RAS) with the usage of host-associated beneficial bacteria was proposed [7]. This IMTA-RAS comprised the usage of a co-culture of sole with *Ulva* ohnoi inoculated with Phaeobacter sp. 4UAC3, previously isolated from a wild *Ulva* microbiome. *Ulva* colonized with Phaeobacter reduced significantly the mortality of turbot larvae. However, the disappearance of the bacteria from the *Ulva* microbiome was observed in the scaling up of the cultures, and the effect was attributed to light. Del Olmo et al. [8] confirmed that the inoculated Phaeobacter eventually disappeared in *Ulva* cultures with high light intensities, which was not the case in dark conditions. It was also shown that this disappearance was not directly caused by the light, since on non-living surfaces, such as glass or autoclaved Ulva, the inoculated Phaeobacter did not disappear when high light intensity was applied, hypothesizing that the disappearance of Phaeobacter could be caused by changes in the physiology or chemical microenvironment of the *Ulva* holobiont in response to light. To test this hypothesis, an experiment was carried out in 500 mL flasks, where *Ulva* ohnoi was cultured under the following treatments: a) non-inoculated Ulva-Control in light (UCL), b) Ulva-Phaeobacter in light (UPL), c) non-inoculated Ulva-Control in darkness (UCD), and, d) Ulva-Phaeobacter in darkness (UPD). In UPL and UPD, Phaeobacter was inoculated at a concentration of ~106 CFU·mL-1, whereas UCL and UCD were left uninoculated. Light intensity, in UPD and UCL, was adjusted to 120 µmol photon·m-2·s-1 under a regular photoperiod (12h:12h). The experiments lasted 14 days and for each sampling (0, 2, 6 and 14 days), 4 independent replicates were collected of each condition to analyse Ulva's microbiome (CFUs and 16S rRNA gene sequencing) and metabolome (Liquid and gas chromatography mass and spectrometry). After day 2, Phaeobacter disappears completely from the microbiome of *Ulva* cultivated in light conditions but maintained in darkness. Time and the light regime were the main factors affecting both microbial communities of U. ohnoi biofilm and the chemical compounds of the U. ohnoi tissues. We found that under total darkness compared to regular light conditions, there were marked differences in Ulva's metabolome composition, regardless of the presence of Phaeobacter bacteria. This could be due to compounds produced by *Ulva* in light that could be unfavourable to Phaeobacter. Additionally, different carbon-rich compounds were found in *Ulva* under different light conditions, suggesting *Ulva* promotes different

microbial communities in each environment. Targeted analysis showed that TDA was not identified in the controls nor in the UPL treatment at any time, but it was found in the UPD treatment through the four samplings. This knowledge will help to better understand the interactions between *Ulva* and Phaeobacter in order to optimize their co-culture in aquaculture applications.

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ULVA INCLUSION DIET WITH PROBIOTICS SUSTAINABLE FEED FOR THE AQUACULTURE

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Aquaculture is currently the fastest-growing industry in the animal protein production landscape. While being pushed to its limit, high productivity should not compromise the well-being of cultured organisms [1]. In aquaculture, probiotics confer several benefits and play important roles in fish growth and health [2]. In addition, the practical application of probiotics in aquaculture diets could minimize the adverse impacts associated with the use of antibiotics. Recently, we have isolated a novel strain of bacterium Alkalihalobacillus from the gut of an algivorous sea urchin (Tripneustes gratilla elatensis) that was fed on a mono-specific diet of *Ulva sp.* [3]. In vitro studies have confirmed Alkalihalobacillus to be a probiotic bacterium [4]. In a preliminary study, we assessed the Alkalihalobacillus as a probiotic for seabream (Sparus aurata) to feed a commercial diet with different inclusion levels of probiotics to enhance growth, biochemical composition, immune status, and digestibility. Moreover, macroalgae *Ulva* offers an added-value dietary ingredient in formulated diets for fish. Aside from theirbasic nutritional value, they contain antioxidants, pigments, polysaccharides, and bioactive compounds that could benefit farmed fish [5]. However, specific polysaccharides in *Ulva sp.* may act as prebiotics, promoting the growth of beneficial gut bacteria in aquaculture species [6,7]. Future studies will focus on the effects of *Ulva* inclusion in fish diets with probiotic bacteria, which will be a novel approach toward sustainable aquafeed for seabream.

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DIGESTIBILITY, NUTRITIONAL QUALITY AND FUNCTIONAL PROPERTIES OF ALTERNATIVE PROTEIN-RICH EXTRACTS FROM GREEN SEAWEED *ULVA SP.*

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Seaweeds have a significant potential as a sustainable source of alternative proteins of non-animal origin [1-7]. Besides their high protein and dietary fiber contents, seaweeds are garnering attention for their numerous advantages over land-based biomass, including their high mineral and vitamin content, cost-effectiveness and wide acceptance. Despite these benefits, seaweeds are still largely unexplored as food ingredients and their techno-functional and nutritional properties are yet to be investigated. The aim of this work was to characterize the protein digestibility, nutritional quality and functional properties of hybrid protein-polysaccharide extracts obtained from the green seaweed *Ulva sp.* through different extraction protocols and with varying degrees of protein purification. The extraction method was based on a pH-shifting protocol and the application of an ultrasound pre-treatment to disrupt the seaweed cell walls was also evaluated. The gross composition of the extracts was determined, as well as a more exhaustive characterization of the protein and polysaccharide fractions. Subsequently, the digestibility of the whole seaweed and the different extracts was determined by means of in vitro gastrointestinal digestions following the standardized INFOGEST Protocol. Additionally, the extracts' digestibility was compared with the digestibility of traditional plant-based protein sources. Although all the obtained extracts were mainly composed of proteins and polysaccharides, the different steps of the extraction protocol had a significant impact on their composition and functional properties, such as solubility and surface charge. In general, all the extracts showed improved digestibility with respect to the native seaweed, whose digestibility was quite low due to the tough cell walls. However, the presence of polysaccharides in some of the extracts, mainly ulvans, was also determinant on the digestibility, being lower in the extracts with higher amount of polysaccharides. Our results provide a basis for the rational design of strategies to produce nutritious protein-rich ingredients from *Ulva* and evidence the potential of this seaweed as an alternative protein source.

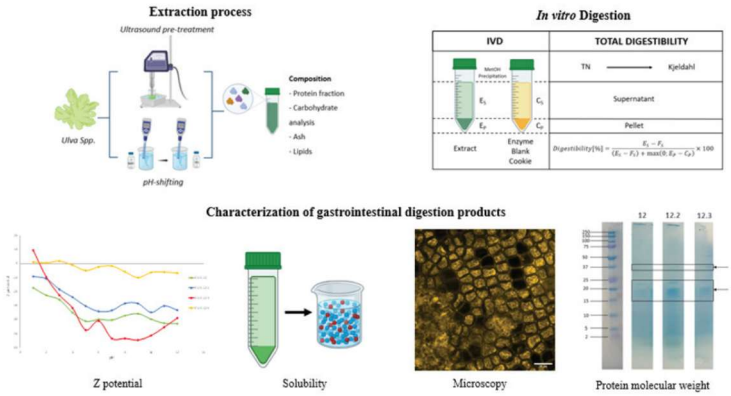


Figure 59 Digestibility, nutritional quality and functional properties of alternative protein-rich extracts from green seaweed Ulva sp.

Acknowledgments

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IDENTIFICATION AND COMPARISON OF PHOTOPIGMENTS BETWEEN *ULVA* LACINULATA AND CLADOPHORA SP. USING HPLC ANALYSIS

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Seaweeds contain different photopigment compositions, which reflect their adaptation to various light environments and ecological niches [1-5]. In this study, we identified and compared the photopigments (chlorophylls and carotenoids) in two green macroalgal strains, *Ulva* lacinulata, and Cladophora sp., cultivated unialgally under identical laboratory conditions, using High-Performance Liquid Chromatography (HPLC). We testified a lot of analytic methods and we concluded to a specific one, which we are going to suggest in this research. Both species shared similar photopigment profiles, with major differences though in the amount of chlorophylls a and b, and carotenoids. The most exciting thing from our results was the identification of fucoxanthin in both species, a photopigment that is not related to the green algae.

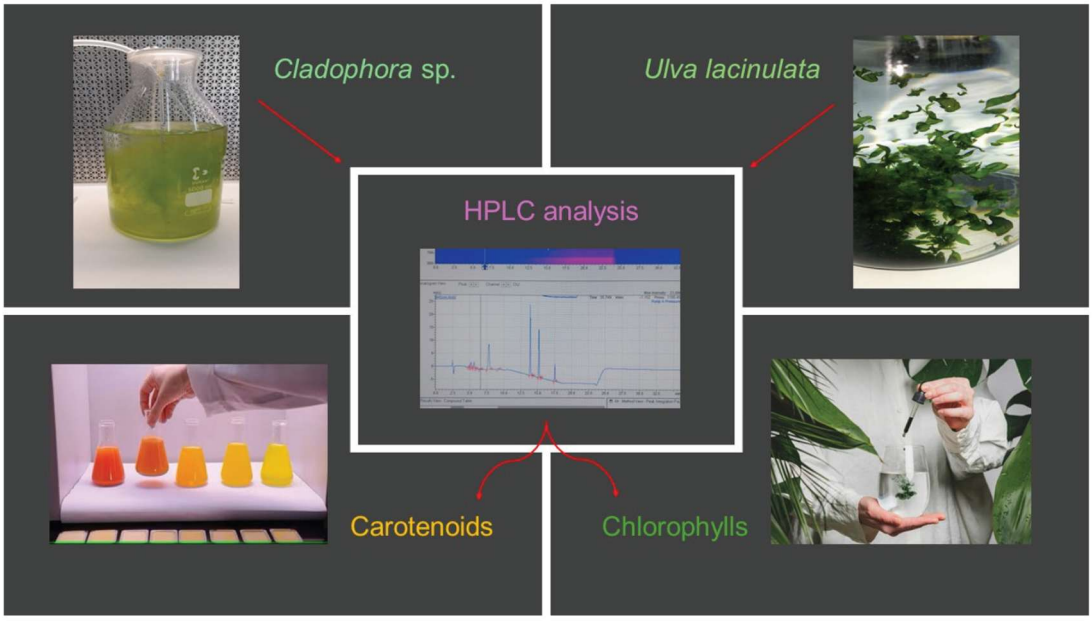


Figure 60 Identification and comparison of photopigments between Ulva lacinulata and cladophora sp. using hplc analysis

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EFFECT OF MACROALGAE EXTRACTS ON SEED GERMINATION AND IN VITRO MICROPROPAGATION OF AQUATIC PLANTS

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Macroalgae, also called seaweed, are multicellular (eukaryotic) organisms [1-5]. Turkey is a country surrounded by seas on three sides. Therefore, there are many algae species in the Mediterranean, Aegean, Black and Marmara Seas. *Ulva* species are especially common in the Aegean Sea. Algae, which has important uses in food, agriculture, pharmacy, medicine and energy fields due to its important contents, has started to be used in in vitro nutrient media and seed germination studies in recent years [1-4]. Therefore, in our study, the effects of *U. lactuca* and Jania rubens extracts on germination of tomato and pepper seeds and on in vitro micropropagation of important aquatic/ aquariums plants Anubias barteri and Micranthemum tweediei (Monte Carlo) were investigated. *Ulva lactuca* and J. rubens species were collected from Izmir Bostancı and Urla seas from June to September. The collected algae were then washed 4 times with tap water and then rinsed with distilled water. After washing, the algae were completely dried in the shade for a week. The dried algae were then roughly ground. 500 g of ground algae was added to 1 liter of sterile distilled water and autoclaved at 121 °C for 30 minutes. The autoclaved samples were filtered through muslin cloth and allowed to cool at room temperature. The filtered solution was stored at +4 °C and used as stock solution. To pre-treat the seeds of tomato and pepper with algae extracts, the seeds were kept in 50 ml of solution containing different amounts of extracts (1%, 2%, 4%, 8% and 16%) at 25 °C in the dark for 16 hours. Seeds used as control were treated with distilled water. Then, two pieces of blotting paper placed in a 90X10 mm petri dish were moistened with 4.75 ml of water and 50 seeds were placed on them. Seeds were germinated in the dark at 20 °C. Seed germination counts were made every other day for 14 days. At the end of the experiment, germination rates and speeds of the seeds were determined. The use of algae extracts especially increased the seed germination speed. MS containing 20 g/l sucrose and 3 mg/l agar was used as the basic in vitro nutrient medium. 10, 20, 40 and 80 ml/l algae extract was added to each of the full, 1/2 and 1/4 strength MS nutrient media [5] and the nutrient media without MS. MS media without algae were used as control. Shoot clumps taken from plants A. barteri and M. tweediei species growing under in vitro conditions were cultured in 100X10 mm glass petri dishes containing MS media with algae extracts. Four clumps of shoots were placed in each petri dish. Shoot cluster diameter, number of shoots, number of roots and root length were counted 4 weeks after the culture initiation in Monte Carlo and 8 weeks after the start of culture in Anubias. Chlorophyll measurements were also carried out in these cultures. Addition of A. barteri and M. tweediei algae extracts, especially to nutrient media containing low amounts of MS, had a positive effect on shoot regeneration and rooting (Figure). Moreover, algae extracts in the nutrient media also increased the chlorophyll content of in vitro growing plantlets. These results we obtained showed that addition of algae extracts to nutrient media at certain concentrations will increase in vitro plant micropropagation.

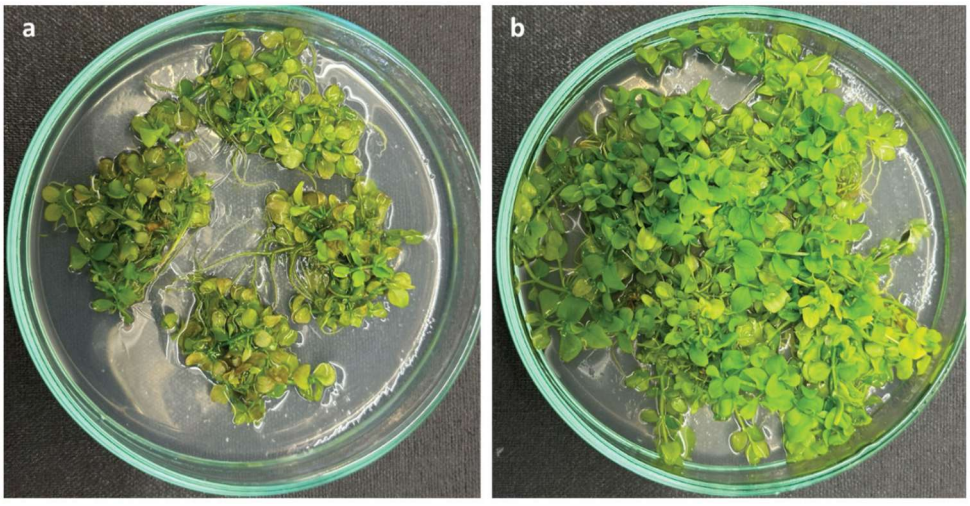


Figure 61 Effect of Jania rubens extract added to in vitro nutrient medium on in vitro micropropagation of Micranthemum tweediei plant. a) Growth in ¼ MS nutrient medium without extract, b) Growth in ¼ MS nutrient medium with 20 ml/l extract.

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ENZYMATIC TREATMENT OF A PHENOLIC-RICH EXTRACT FROM GREEN MACROALGAE *ULVA SP.* FOR THE ENHANCEMENT OF ITS BIOACTIVITY

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Biomass from green marine macroalgae *Ulva sp.* is recognized as a plentiful reservoir of bioactive substances. Specifically, *Ulva* genus species have been demonstrated to metabolize biomolecules with pharmacological potential, including polysaccharides, lipids, proteins and phenols. Alongside, enzymatic biocatalysis is extensively employed for the purposeful biotransformation of natural products to enhance their properties. In this context, a recent work from the Laboratory of Biotechnology at University of Ioannina in Greece demonstrated an increase in the bioactivity of a phenolic-rich extract from green marine macroalgae *Ulva* intestinalis following treatment with two fungal laccases. A comparative investigation was conducted into the antioxidant, enzymeinhibitory, antimicrobial, and cytotoxic activities as well as into the phenolic composition of the enzymatically modified and non-modified extracts. Depending on the laccase utilized, the enzyme-modified extracts displayed improved bioactivities compared to untreated extracts. Specifically, the enzymatically modified extracts exhibited enhanced antimicrobial and enzyme-inhibitory activities along with mild cytotoxicity suggesting that the proposed biocatalytic approach could be employed to produce green macroalgal natural extracts with enhanced biological activities and potential applications in the food, pharmaceutical, and cosmeceutical sectors [1].

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ULVA BIOMASS PRODUCTION IN AN INNOVATIVE BRINE-BASED CULTIVATION SYSTEM: ENHANCING THE NUTRITIONAL QUALITY OF SEAWEED.

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With the exponential growth of the human population and the decline of arable land, food security is a major challenge of this century. In addition to adaptations of traditional agricultural techniques, alternative food sources, such as edible macroalgae, are urgently required to diversify the human food spectrum. In this context, the “food4future” project (https://www.food4future.de/en/home), funded by the German Federal Ministry of Education and Research (BMBF) under the “Agricultural Systems of the Future” program, aims to develop innovative cultivation approaches capable of providing fresh and healthy biomass in urban areas. One creative solution being studied is using marine macroalgae cultivated in brine-based systems. Initial results have shown that regional brine from Brandenburg, Germany, can be utilized to cultivate *Ulva* compressa, even enhancing the nutritional value of this sea vegetable [1]. This approach is promising as it eliminates the need for natural seawater and freshwater usage (required to dilute commercial salt solutions). To enhance the production capacity of innovative systems, the current research focuses on investigating and evaluating the quantity and nutritional value of the produced algal biomass. Apart from different abiotic factors (e.g., light, temperature, or substrates), direct interactions with the associated microbiome influence the development and chemistry of edible macroalgae. For example, the phytohormone-like compound thallusin, produced by the bacterium Maribacter sp., plays a crucial role in the morphology of its hosting macroalga. Thus, it has been identified to affect cell walls and rhizoid formation in *Ulva* compressa [2]. Understanding microbiome data can offer approaches to enhance algae-based nutrient production by engineering its microbiome, particularly in standardized land-based aquaculture systems. In this context, the presented study investigates the cultivability of two different strains of U. compressa: i) a descendant of the isolate of B. Føyn, serving as a model organism for macroalgae, and ii) a more recently isolated strain (by Fricke in 2019). Both strains will be exposed in parallel to the food4future developed brine-based system for at least 3 months. Microbiological composition will be determined at the experimental start and during cultivation. Different cultivation parameters will be monitored (e.g., pH, salinity, minerals), and biomass quantity (e.g., growth rates) and quality (e.g., protein, fat, carbohydrates content, and carotenoid and chlorophyll content) will be determined during the cultivation process. The study will help identify the core microbiome in this innovative system and provide a better understanding of the dynamics in algal biomass production, which is crucial for adapting a suitable harvest strategy.

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GREEN BEAUTY: UTILISATION OF ULVA'S BIOACTIVES FOR ECO-FRIENDLY COSMETIC SOLUTIONS THROUGH ADVANCED ENCAPSULATION AND EXTRACTION METHODS

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In recent years, concerns about the reliability of synthetic substances and their effects on human health have been increasing [1-2]. Alongside the growing public awareness of environmental issues -such as global warming and ecological problems, consumers have started to prefer green, healthy, and chemical-free products [3]. This trend has been reflected in the global market, leading producers to focus on sustainable, natural, and clean ingredients. Concerns about the reliability of synthetic substances used in cosmetics and their effects on human health are increasingly growing. This situation has directed producers towards sustainable, natural, and clean ingredients [4]. Cosmetic raw materials can be obtained from various natural sources such as plants and animals. The use of components obtained from natural sources in cosmetics attracts consumers' interest [5]. Components isolated from marine organisms and algae are also present in the content of many cosmetic products. Nowadays, macroalgae emerge as potential raw material sources due to their novelty and sustainability. It has been found that bioactive ingredients extracted from macroalgae, known as seaweeds, and a range of secondary metabolites have beneficial properties for the skin, such as photoprotective, moisturizing, antioxidant, and anti-inflammatory effects. *Ulva* species belonging to the Ulvaceae family, is a green macroalgae. *Ulva sp.*, a green algae, is a macroalgae family frequently encountered along the Aegean coasts of our country, causing harm to the marine ecosystem, creating odor and visual pollution, and classified as an invasive species when the season comes. Sustainability has become one of the most important issues both globally and in our country. Extraction of bioactive compounds from *Ulva sp.* and encapsulating them by applying a gradual extraction method of the same species will make it possible to develop natural, sustainable, and multifunctional new generation cosmetic raw materials by increasing their stability.

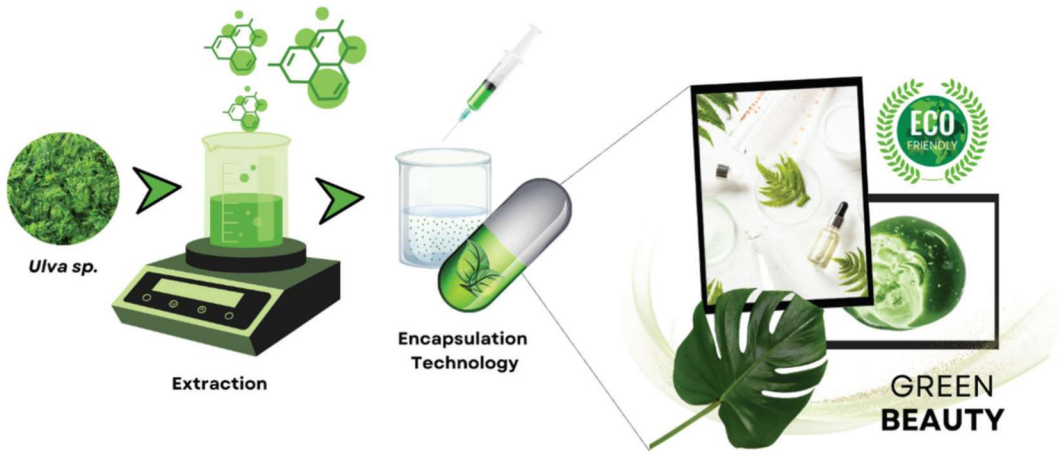


Figure 62 Green beauty: utilisation of ulva's bioactives for eco-friendly cosmetic solutions through advanced encapsulation and extraction methods

By changing the parameters affecting extraction, the extraction method can be optimized by detailed analysis of the bioactivities identified using statistical methods. Turning into particles through encapsulation methods is a highly advantageous feature. Encapsulation is the process of enclosing natural compounds in a protective shell or coating. Encapsulation provides long-term stability to natural compounds sensitive to light and heat. It also enables controlled release of natural compounds, making them useful for drug delivery systems and nutraceutical applications [6]. This way encapsulated bioactive compounds from derived *Ulva sp.* will be valuable raw materials with high added value on their own and can be used for cosmetic purposes.

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TRANSFORMING MACROALGAE INTO VALUABLE PRODUCTS

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Macrocarbon was born out of the Carbon-to-Value Challenge of the German Innovation Agency SPRIN-D in 2023. It is a Canary Islands based start-up developing an integrated supply chain for growing and converting seaweed into sustainable feedstocks for the European chemical industry. Joaquin Ortiz-Cortes will present the solutions that Macrocarbon is developing to achieve large scale, high yield seaweed farming on one side and smaller scale, high value compound farming on the other. The former takes a modular, scalable approach to floating seaweed farming aimed at reliably producing industrial quantities of biomass. The latter focuses on integrated multitrophic aquaculture with local partners, with more controlled conditions allowing for targeted manipulation of environmental conditions.

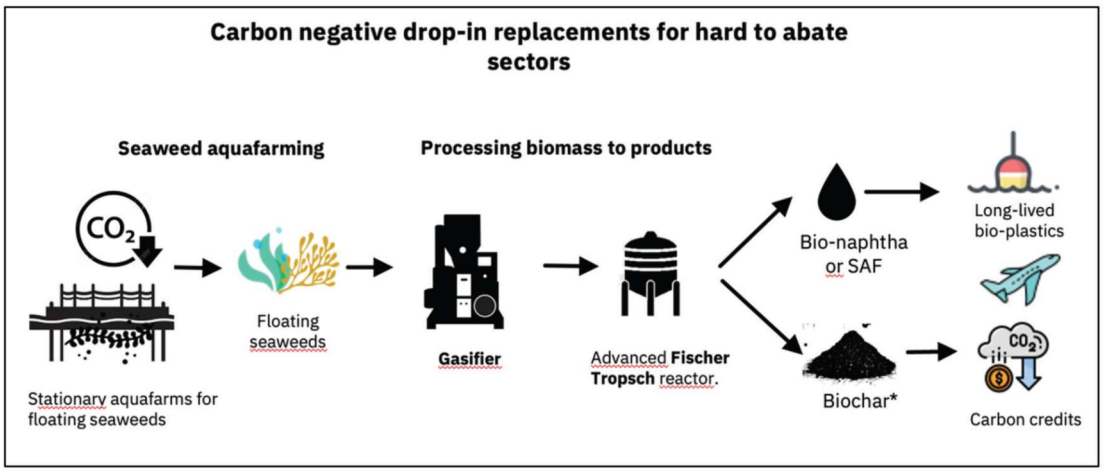


Figure 63 Carbon negative drop-in replacements for hard to abate sectors

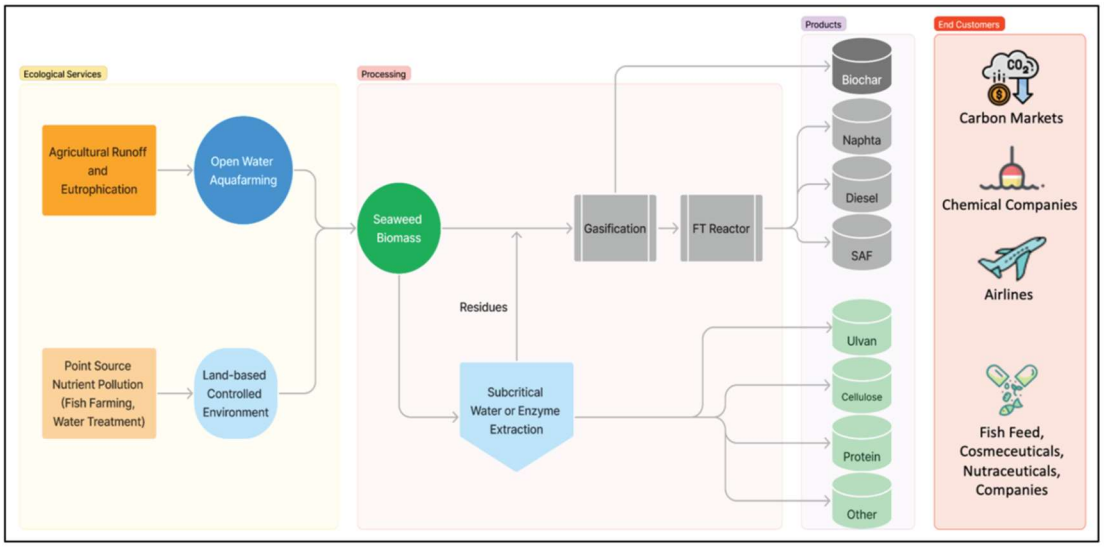


Figure 64 Transforming macroalgae into valuable products

BIOREMEDIATION IN MARINE RAS: TESTING ULVA'S CAPACITY FOR LOWERING TOXIC OZONATION BY-PRODUCTS

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The maintenance of good water quality is crucial for production success in recirculating aquaculture systems (RAS). Ozone (O3) has seen an increase in interest among aquafarmers worldwide as a water treatment tool with a wide array of beneficial effects. Besides its germicidal properties, O3 can enhance solid removal, oxidise toxic nitrogen compounds and degrade a broad spectrum of biogenic and man-made molecules. While its application in freshwater is mostly unproblematic, ozonation of seawater creates by-products termed 'ozone-produced oxidants' (OPO). These can accumulate in a system when process water is recirculated and are detrimental to animal health if species-specific thresholds are exceeded. In this experiment, we tested the seaweed *Ulva sp.* For its OPO-bioremediation capacity in an outdoor IMTA (Integrated Multi-Trophic Aquaculture)-RAS-setup with Gilthead seabream (*Sparus aurata*). Effluent seawater was ozonated and subsequently led through an *Ulva* cultivation unit. OPO-concentrations in the seawater were measured before and after the seaweed unit and the decline was compared to controls without *Ulva*. Additionally, the impacts of OPO on growth, metabolic composition and photosynthetic efficiency of *Ulva* were investigated by comparing OPO-exposed seaweed to controls cultivated without ozonation. 16S rRNA gene sequence analyses were performed to monitor the *Ulva* associated microbiomes in response to ozonation. While seaweed-containing systems exhibited a significantly higher reduction in OPO, ozonation also diminished *Ulva* growth (32%) and caused a darker, fringier, and more rigid morphology. Moreover, ozonation elevated chlorophyll -, total phenolic – and flavonoid contents, and shifted the amino acid composition in *Ulva* towards more glutamine, glutamic acid and serine. With this study we showed that *Ulva* can reduce OPO-concentrations in marine RAS, but with impacts on growth, chemical composition, and morphological properties of algal biomass.

Acknowledgments

The authors would like to thank Dr. Antonio Vieira and Prof. Wolfram Weckwerth for the provision of space, resources, and consultancy.

OPTIMIZATION OF HIGH VALUE COMPOUNDS EXTRACTION FROM ALGAE BIOMASS: SFE AND AUXILIARY EQUIPMENT IN A SEMI-INDUSTRIAL SCALE SCENARIO

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Supercritical fluid extraction (SFE) plays an important role in traditional organic solvent extraction replacement due to its several advantages: higher selectivity and purity, reduced product contamination; minimal organic solvent consumption and lower environmental footprint. In addition, the lack of light and air during the extraction process decreases the highly oxidizable compound degradation. Due to these reasons and among the myriad of methods employed to extract valuable compounds from algae, supercritical fluid extraction (SFE) has emerged as a powerful and efficient technique. SFE has shown significant benefits in high value compound extraction from natural biomass due to its gentle treatment for heat-sensitive materials. Moreover, SFE technique can be developed at different scales: from laboratory scale to pilot plant scale and up to large industrial scale. However, scientific literature has not provided yet an effective approach for this process. Studies based on scale-up are inconclusive [1, 2] because of the interaction among several parameters. The purpose of this work was to highlight the advantages of this technique, design a supercritical extraction system (taking into account factors such as materials, wall thickness, valves, Joule-Thompson phenomena, number and types of separators, among many others) and scale-up the process based on the proposed design. This study provides an overview of the supercritical fluid technique applications on algae biomass, equipment design, technique scale-up and whole process design for the extraction of bioactive compounds. Due to the diversity of algae biomass a process flow diagram was designed, encompassing both processing and post-processing stages. Moreover, various factors such as sample moisture, particle size, matrix effects, etc., were taken into consideration to achieve the extraction objective. The various processing techniques available at the ITC facilities were studied to accomplish an optimal overall process/ an optimization of the whole process.

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SCREENING SEAWEEDS LIPIDS AS SUSTAINABLE SOURCES OF BIOACTIVE AND PREMIUM INGREDIENTS FOR NUTRICOSMETICS

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Lipids and their derivatives play an essential role in maintaining healthy skin since provide a protective barrier that prevents water loss, keeps the skin hydrated, and blocks the entry of external elements [1]. Imbalance of skin lipids have been associated with inflammatory skin diseases (ISD), either to change in metabolism, or due to with oxidative stress, that can induce lipid peroxidation that promote the overproduction of proinflammatory cytokines [2,3]. Polar lipids from seaweeds bearing omega-3 polyunsaturated fatty acids (PUFA) have antioxidant, anti-microbial, and anti-inflammatory properties [4], suggesting that they could be used as new therapeutical agents to prevent or treat ISD [5]. Despite being a highly attractive topic, it remains scarcely explored. Therefore, the research work proposed with the scope of my PhD thesis aims to bioprospecting of polar lipids bearing omega-3 PUFA from different edible seaweeds as a source of bioactive and premium ingredients for cosmetics and cosmeceutical applications as well as for nutricosmetic products. By using high throughput mass spectrometry-based lipidomic approaches as well as in chemico and in vitro models using cells lines, it is expected to highlight the bioactive lipid from green, and *Ulva rigida* and *Codium tomentosum*, and red seaweeds, *Gelidium corneum, Palmaria palmata, Porphyra dioica* for application in cosmetic/cosmeceutics to takle the burden of ISD. Up to this time, we have characterized the lipidome of Gelidium corneum, which is commonly used for agar production and extraction. These processes generate a subsequent biomass residue, which is poorly valorized and this by-product can be a potential source of high-value compounds. The gas chromatography-mass spectrometry analysis performed after transmethylation of the lipid extract revealed a total of 9 fatty acids in both biomass and residue. The arachidonic acid (omega-6 C20:4) and eicosapentaenoic acid (omega-3 C20:5) were more abundant in the biomass, while linoleic acid (omega-6 C18:2) was more abundant 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, with a significant decrease of glycolipids was observed in *G. corneum* residue. Interestingly, the lipidome of this seaweed in rich in several classes of ceramides, a predominant lipid fractions present in skin cells, highlighting its potential as a source of ingredients for cosmetics and cosmeceuticals. Both lipid from *G. corneum* biomass and residue demonstrated antidiabetic activity by inhibiting the activity of α-glucosidase, with a slightly higher effect promoted by the lipids from G. corneum residue, and antioxidant activity by scavenging the 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical cation (ABTS●+) and the 2,2-diphenyl-β- picrylhydrazyl radical (DPPH●), with a slightly higher effect promoted by the lipids from G. corneum biomass. It is expected that the results gathered under this PhD thesis will contribute to the valorization of seaweeds as sustainable sources of high-value premium ingredients, including bioactive lipids for usage in a variety of industries, such as cosmetics, cosmeceuticals, and nutricosmetics.

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Advances in biome research in Ulva

Faro, Portugal

March 18-19, 2025

Ulva

The Future Wheat of the Sea

ORGANIZERS

Dr. Aschwin Engelen, CCMAR, University of Algarve, Faro, Portugal
Lior Gutman The Hebrew University of Jerusalem, IL
Muki Shpigel University of Haifa, IL
Thomas Wichard University of Jena, DE

The COST Action SeaWheat, CCMAR, the University of Faro, the Friedrich Schiller University Jena, and the Hebrew University of Jerusalem joined forces to organize a 2-day Workshop on "*Ulva* microbiome" (Working Group 1), a topic of significant importance in the research field. This collaborative effort, held from 18-19 March 2025 with the CCMAR, University of Algarve graciously hosting the workshop, demonstrated a shared dedication to advancing the understanding of *Ulva* microbiomes.

RATIONAL AND OBJECTIVES

The green seaweed *Ulva* (Chlorophyta) is a valuable source of diverse bioactive compounds with significant potential in health, nutrition, cosmetics, and pharmaceuticals. Its rich content of polysaccharides, proteins, phenolics, vitamins, minerals, fatty acids, and pigments offers a wide range of potential biological activities that can be harnessed for promoting health and well-being. Ulva is under direct and indirect influence of associated micro-organisms. The objectives was to discover the cutting-edge world of Ulva microbiome research at our workshop, *Advances in Biome Research of Ulva*. The dynamic event delved into the intricate relationships between the green seaweed *Ulva* and its microbiomes, exploring their pivotal role in growth, health, and ecological functions. Join us to uncover the latest breakthroughs in microbiome analysis, innovative methodologies, and future applications in sustainable mariculture and biotechnology. Identifying pioneering research shaping our understanding of *Ulva's* microbial communities and discover new frontiers in marine science. This workshop offered an unparalleled opportunity to engage with experts, share insights, and collaborate with researchers, students, and industry leaders driving innovation in this exciting field. The workshop aimed to bring scientists together to uncover the current state of knowledge, identify the knowledge gaps and learn from microbiome research on other model organisms where the direction of future research, as *Ulva* is assumed to play an increasingly important role in various industries. Established and young scientists and enterprise representatives came together to discuss the status quo of the current research on *Ulva* microbiomes.

ORGANIZATION

The workshop was part of Working Group 1's (WG1) planned list of events for the current grant period. The local organizing committee, led by Dr. Aschwin Engelen and Dr. Tania Aires, played a pivotal role in ensuring the workshop's smooth operation. They were supported by Dr. Thomas Wichard (University Jena, DE). The workshop program was discussed and decided upon by experts who constituted the scientific committee: Aschwin Engelen (PT), Thomas Wichard (DE), Lior Guttman (IL), and Muki Shpigel (IL). Aschwin Engelen edited the abstract book (Annex of the report). News and information about the workshop were posted on the SeaWheat COST Action social media channels (such as LinkedIn) and the Action websites.
The local host of the workshop, the CCMAR (Universidade do Algarve), located in Faro, Portugal, is a prominent institution known for its academic excellence, research contributions, and innovative approach to education. CCMAR has become one of Portugal's leading educational and research institutions. CCMAR has hosted several leading projects, studies, and researchers in Ulva microbiome and was a perfect location for the COST Action Workshop on "Bioactives in *Ulva*".

PARTICIPANTS

The COST Action workshop was well received, with 32 registered participants from 11 countries and with a gender balance of roughly 1/3 female and 2/3 male. Combining it with the subsequent Training School in Aveiro at the same location proved excellent. In addition to the COST Action registered participants, further scientists attended the presentations throughout the workshop.

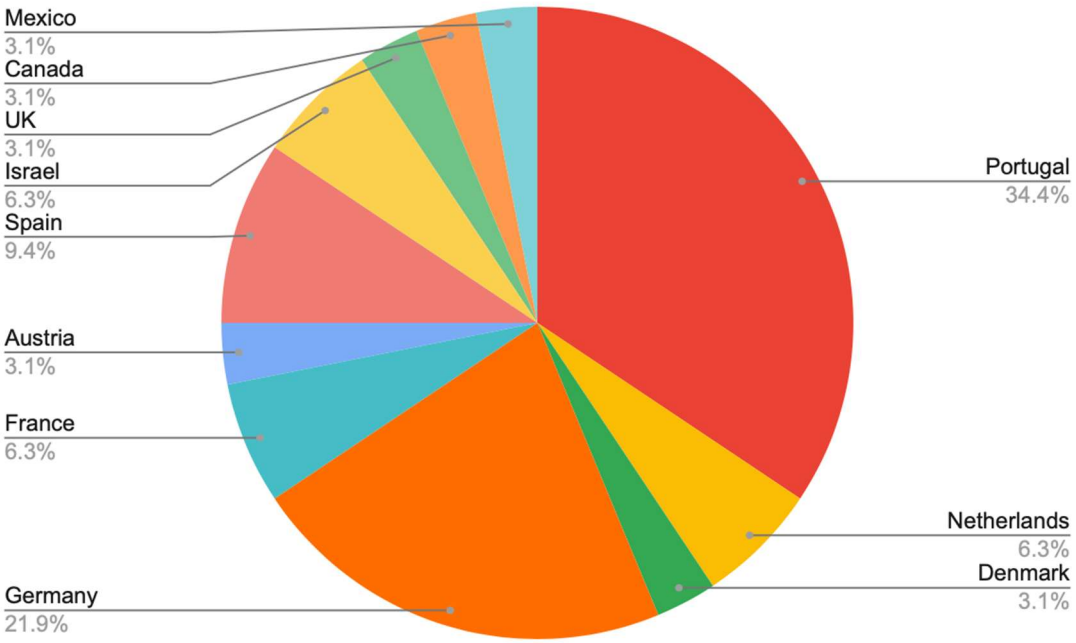


Figure 65 Distribution of the Workshop participants by country.

WORKSHOP DESIGN AND FUNCTIONING

Amongst the participants, current research topics on microbiomes in macroalgae and their applications were presented in five sessions (total of 26 talks, each 10-45 min): (i) "The Biome of Ulva," (ii) "Ecophysiological function and testing of microbial traits," (iii) "Microbiome analysis & dynamics," (iv) "Microbiome engineering & stress", and (v) “Commercial applications of *Ulva* microbiomes”. Each session comprised 3-6 presentations by experts in the field as an overview, including discussions after each presentation.

The breaks between the sessions were used for further discussions and exchanges on other activities of the COST Action participants. Networking was used to establish new projects and existing co-operations were deepened. The status quo of various COST Action activities of the WG1 were discussed: EUlva barcoding and microbiomes, as well as SeaWheat special issues.

Notably, on the morning of the event's second day, the young scientists (Master's students, PhD students) who participated gave flash presentations. These 10-minute presentations again demonstrated the growing interest in microbiome research and provided an exciting overview of the ongoing European research.



Figure 66 Group photo of participants at the end of day 2 of the Seawheat workshop inside the venue, University of Algarve, Faro, March 18-19, 2025.-19, 2025

CONCLUSIONS

This workshop focused on microbiome of *Ulva*, a genus of green algae commonly known as sea lettuce. The event brought together researchers, industry professionals, and students interested in *Ulva* microbiomes. An overview of *Ulva* microbiome research and developments was given.

The organizing team resumed that the workshop promoted research and the cohesion of research, often scattered throughout Europe.

Annex: The report includes the Abstract Book of the Workshop "Advances in Biome Research of *Ulva*"



Advances in biome research in Ulva

Faro, Portugal

PROCEEDINGS

Ulva

The Future Wheat of the Sea

RIDING THE MICROBIAL TIDE: PAST, PRESENT AND FUTURE DIRECTIONS IN MARINE HOST-MICROBE INTERACTIONS

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In this presentation, I will provide a historical overview, examine current challenges, and propose future research directions to advance our understanding of host–microbe interactions in marine organisms. As a case study, I will focus on *Caulerpa*, a genus of green algae found in tropical and subtropical coastal waters[1]. Certain *Caulerpa* species are invasive and toxic, while others are opportunistic and threaten marine flora and fauna. For instance, *C. cylindracea* disrupts seagrass meadows in the Adriatic Sea[2], *C. prolifera* reduces faunal diversity in southern Portugal[3], and *C. filiformis* has sublethal effects on a key herbivore in Australia[4]. With global warming driving the tropicalisation of temperate ecosystems, it is likely only a matter of time before *Caulerpa* species expand into higher latitudes.

Despite its plant-like appearance, *Caulerpa* is a single giant cell containing multiple nuclei, chloroplasts, mitochondria, and symbiotic bacteria—collectively forming a "holobiont" [5,6]. Although little is known about the roles of these bacterial symbionts, they may influence the host's growth and development, facilitate adaptation to environmental stress[7], and contribute to the ecological success of these so-called "killer" algae.

To investigate interactions between *Caulerpa* and its symbionts, we employed a systems biology approach that integrates manipulative experiments, "culturomics," multi-omics, and metabolic modeling[8]. While such a holistic strategy is rarely applied in marine sciences, it is essential to test hypotheses, predict holobiont responses to climate change[9], and evaluate the risk of further invasions. As part of this effort, we sequenced the hologenomes of three *Caulerpa* species (*C. prolifera*, *C. lentillifera*, and *C. racemosa*), analysed the impact of sulfide on both algae and their symbionts using metatranscriptomics, and developed a preliminary genome-scale metabolic model.

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Short biography

Gerard Muyzer is a Professor of Microbial Systems Ecology at the University of Amsterdam, where his research explores the structure, function, and dynamics of microbial communities, emphasizing their roles in biogeochemical cycles and biotechnological applications. He employs a systems biology approach to integrate experimental methods with advanced omics techniques and metabolic modelling. His primary focus is on the microbial sulfur cycle, particularly sulfur bacteria in natural environments (e.g., soda lakes and stratified lakes) and engineered ecosystems, such as full-scale bioreactors designed to remove toxic sulfur compounds from wastewater. Since 2012, he has expanded his investigations to include host-microbe interactions in sponges, seagrasses, and marine algae, particularly the green macroalga *Caulerpa*. For further information, please visit his ORCID profile: https://orcid.org/0000-0002-2422-0732.

STRUCTURE, DIVERSITY, AND FUNCTION OF THE CORAL MICROBIOME

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Coral reefs are important marine ecosystems, supporting immense biodiversity and providing significant ecological and economic benefits. Corals and the reefs they build face unprecedented decline from local and global anthropogenic pressure. Central to the health and resilience of coral reefs is the microbiome, a complex community of microorganisms that inhabit coral tissues, mucus, and the surrounding environment. Microbial communities play a critical role in maintaining coral health, mediating nutrient cycling, and enhancing stress tolerance. Given the importance of the microbiome to host physiology and resilience, current research aims to manipulate the microbiome to provide an alternative path to organismal environmental adaptation. Although the premise of microbiome stewardship and microbiome rescue are demonstrated in principle, the underlying mechanisms or the ability of microbes to form stable novel associations is unknown. The presentation will summarize the current state of knowledge regarding the structure, function, and specificity of the coral microbiome, our efforts regarding functional screening for microbes that ameliorate stress, and elucidation of the underlying mechanisms. Discovery of microbes and microbial mechanisms mediating stress tolerance and our ability to harness them may inform active interventions to protect natural populations and conserve organismal and ecosystem biodiversity.

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Short biography

Dr. Voolstra studies coral reefs combining ecological, environmental, microbial, and molecular approaches. His most recent research particularly advanced knowledge of how the microbiome contributes to coral acclimation/adaptation and the delineation of standardized analytical methods (SymPortal, CBASS short-term acute thermal assays, Coracle, etc.) to fasten the development of approaches to mitigate climate change. He is an advocate of open science, open access, and free data sharing. Dr. Voolstra has published over 300 peer-reviewed research papers, various book chapters, and holds patents related to bioactive lead structures from marine organisms. Dr. Voolstra is the current president of the International Coral Reef Society (ICRS), stressing the urgency of developing and applying science-based solutions to coral reef restoration following a rigorous, evidence-based decision framework. Dr. Voolstra received his PhD at the Institute for Genetics in Cologne, Germany, was a Postdoctoral scholar at the University of California, Merced, before joining KAUST's Red Sea Research Center in Saudi Arabia as faculty, where he served as the Associate Direct from 2016 to 2019. In 2019, Dr. Voolstra moved to the University of Konstanz, Germany where he is Professor (Chair) of Genetics of Adaptation in Aquatic Systems.

THE *ULVA* MICROBIOME: BEYOND BACTERIA

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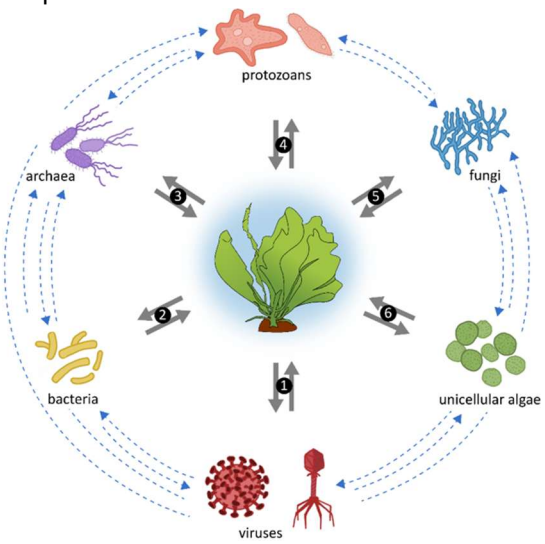
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Seaweeds live together in symbiosis with numerous microorganisms, including bacteria, fungi, and viruses. These microorganisms are essential to the life and functioning of seaweeds. The green seaweed *Ulva* in particular is a model system to study seaweed-bacteria interactions, as they depend on their bacterial symbionts for normal morphological development and growth. Surprisingly little, however, is known about the other symbionts of the *Ulva* microbiome. Using metagenomic sequencing, we characterized viral communities associated with healthy and bleached specimens of *Ulva* for the first time. We identified 20 putative novel and divergent viruses, the majority of which belonged to undescribed viral families. Bleached *Ulva* samples contained particularly high viral read numbers. While reads matching assembled CRESS DNA viruses and picorna-like viruses were nearly absent from the healthy *Ulva* samples (confirmed by qPCR), they were very abundant in the bleached specimens. In addition, we assembled 37 near-complete (>90%) bacteriophage genomes associated with the *Ulva* biofilm, adding another layer of complexity to *Ulva*-bacterial interactions. Phage species often infect a small range of bacterial strains and the identified bacteriophages in our dataset included multiple phages specifically infecting, amongst others, *Alteromonas*, *Loktanella*, *Nonlabens*, *Roseovarius*, and *Sulfitobacter*. The bacteria that are infected by these phages are well-known symbiotic partners of the *Ulva* biofilm and some play a pivotal role in *Ulva* growth and morphogenesis. It is clear that a wealth of seaweed-associated microorganisms and symbiotic relationships remain to be discovered, if we look beyond bacteria.

Graphical abstract:



Short Biography

Luna van der Loos conducted her PhD at Ghent University focusing on the *Ulva* microbiome. After her PhD, Luna continued exploring *Ulva* biodiversity within the European *Ulva* Taxonomy Initiative and worked on *Ulva* genomics. She recently started her tenure track at the Naturalis Biodiversity Center in the Netherlands.

AN EXPERIMENTAL TEST OF THE INFLUENCE OF MICROBIAL MANIPULATION ON SUGAR KELP (*SACCHARINA LATISSIMA*) SUPPORTS THE CORE INFLUENCES HOST FUNCTION HYPOTHESIS

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Microbial symbionts influence many aspects of the biology of kelp and other macroalgae. Manipulating the microbiome through addition of probiotics may be a sustainable way to improve macroalgal productivity, health, and resilience for aquaculture and conservation. The core microbiome hypothesis suggests that the bacteria that are consistently found on a host (the core microbes) are likely to have a disproportionate impact on host biology, making them an attractive target for microbiome manipulation. In this study, we surveyed wild *Saccharina latissima* and their surrounding environment to assess bacterial distributions and identify core bacteria on sugar kelp and experimentally tested how cultured bacterial isolates affect kelp development. We found a significant positive relationship between ecological distribution and impact on gametophyte settlement and sporophyte development in kelp co-culture experiments. These findings are consistent with predictions from the core microbiome influences host function hypothesis and suggest that incorporating knowledge of ecological distribution when designing microbiome manipulation trials will increase success rates.

Short biography
Laura Wegener Parfrey is a biologist studying microbial ecology and evolution. Her lab at the University of British Columbia works to understand how symbiosis with kelp and other macroalgae shape the ecology and evolution of microbes, how these relationships change in changing environmental conditions, aims to use this knowledge to develop microbiome-based strategies to enhance the productivity and sustainability of macroalgal aquaculture. She holds a Ph.D. in organismal and evolutionary biology from the University of Massachusetts and BS in Biology from SUNY at Albany.

ULVA ASSOCIATED FUNGI AND OOMYCETES FROM THE ALGAE PRODUCTION INDUSTRY

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The genus *Ulva*, a green macroalgae commonly known as sea lettuce, has attracted significant attention in the macroalgal production industry due to its ecological and commercial potential. With its rapid growth rates and beneficial nutrient profile, *Ulva* is an ideal candidate for use in various sectors such as food, pharmaceuticals, nutraceuticals, and biofuels. Given its commercial importance, it is crucial to identify potential pathogens that could negatively affect *Ulva* production and result in financial losses. To investigate the fungal and oomycete microbiome associated with *Ulva* species and assess their pathogenic potential, seasonal isolations were carried out in 2024 and January 2025 on biomass provided by the seaweed producer ALGAplus and indirectly by deploying baiting rafts in *Ulva* production tanks and a sedimentation earthen pond. This approach led to the direct isolation of 16 fungal isolates and the indirect isolation of 43 fungal isolates. Sequencing of the ITS region identified several fungal genera, including *Cladosporium*, *Diaporthe*, and *Penicillium*, among others. As for oomycetes, no isolations were obtained from the *Ulva* biomass or production tanks; however, they were isolated from a sedimentation earthen pond where *Ulva* was growing. Infection trials with oomycetes on *Ulva* are currently underway.

Acknowledgments

The authors would like to acknowledge “Pacto da Bioeconomia azul” (Project No. C644915664-00000026) within the WP5 Algae Vertical, funded by Next Generation EU European Fund and the Portuguese Recovery and Resilience Plan (PRR).

Short Biography
Cristiana Maia is a Microbiologist with an interest in oomycetes and fungi associated with seagrasses and seaweeds. She earned her BSc in Microbiology from Escola Superior de Biotecnologia, Universidade Católica Portuguesa and her MSc in Molecular and Microbial Biology from Universidade do Algarve. In the last years she worked with the marine oomycete *Halophytophthora* and more recently started working with fungi associated with algae. Currently she is a PhD student at CCMAR working with oomycetes and identifying their ecological roles and potential biotechnological activities and analysing the fungal and oomycete microbiome from algal species from the algae production industry.

DECODING THE HIDDEN SECRETS OF PLANT MICROBIOMES

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Plant roots harbor an extraordinary diversity of microorganisms, with cell densities often surpassing those of the plant itself. Studies have shown that the plant microbiome plays a key role in enhancing plant tolerance to both abiotic (e.g., drought) and biotic stresses (e.g., disease), while also contributing to nutrition, growth, and development [1]. However, the functions and mechanisms of most plant-associated microorganisms remain poorly understood. Recent advances in 'omics technologies have expanded our knowledge of microbiome diversity and function, shedding light on the complex interactions within plant-associated microbial communities. This presentation will provide new findings on the role of rhizosphere and endosphere bacteria in protecting plants from biotic and abiotic stresses. Additionally, we introduce bacLIFE [2], a novel bioinformatics tool designed for genome annotation, large-scale comparative genomics, and the prediction of lifestyle-associated genes (LAGs) in bacteria. Using bacLIFE, we analyzed 16,846 genomes from the *Burkholderia*/*Paraburkholderia* and *Pseudomonas* genera, identifying hundreds of genes potentially linked to plant pathogenic lifestyles. Site-directed mutagenesis and plant bioassays confirmed that six predicted LAGs are indeed involved in the phytopathogenic lifestyle, including genes encoding a glycosyltransferase, extracellular binding proteins, and homoserine dehydrogenases. An overview will be given on the wealth of genes and functions of the plant microbiome.

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UNLOCKING THE SECRETS OF *ULVA*: HOW MICROBES SHAPE THE GROWTH, MORPHOGENESIS, AND SURVIVAL OF SEA LETTUCE

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Microbial communities play a crucial role in the ecophysiology of *Ulva* (Chlorophyta), influencing its growth, development, stress tolerance, and ecological interactions, including biofilm formation and the settlement of epibionts. While monitoring microbial dynamics provides valuable insights, one of the most challenging research areas remains the functional examination of microbial traits and their impact on *Ulva*.

Ecophysiological Functions of Microbial Traits in Ulva: A key function of surface-associated microbes is their role in *Ulva*'s morphogenesis and growth regulation. The presence of bacteria from the Roseobacteraceae and the genus Maribacter is essential for suitable morphological development, as axenic (bacteria-free) cultures develop into a callus. Specific bacteria release algal growth and morphogenesis-promoting factors, such as thallusin, which induce cell differentiation and cell wall formation and organize thallus development. Without these bacterial signals, *Ulva* remains in an undifferentiated or irregular growth state [1]. Beyond morphogenesis, microbial communities are also crucial for nutrient uptake and metabolism. *Ulva* relies on associated bacteria to enhance nitrogen, carbon cycling, or metal homeostasis. Additionally, microbial partners contribute to stress tolerance, enabling *Ulva* to withstand fluctuating environmental conditions, including variations in salinity, temperature, light, and pH [2, 3]. For example, under temperature changes, specifically stress-adapted bacteria must provide “core functions” such as thallusin production, which is necessary for *Ulva´*s growth under Antarctic conditions at 2°C [3].

Testing of Microbial Traits in Ulva: Notably, a changing microbiome can exhibit consistent traits and maintain functional stability in a dynamic environment. In contrast, a stable microbiome may also exhibit functional shifts in response to biotic or abiotic factors [4]. Therefore, experimental testing of microbial features is required to separate the two situations or complement bacterial activity. Testing microbial traits in *Ulva* involves axenic vs. non-axenic cultures, microbiome sequencing, and stable isotope tracing to analyze microbial influences on nutrient cycling, growth, and stress responses. Additional methods, such as physiological measurements, metabolomics, and bacterial/algal co-cultivation, identify functional interactions and their impact on *Ulva*'s ecophysiology.

This presentation highlights the importance of integrating functional examinations with microbiome analysis to identify trait variations in the (core) microbiome, even when microbiome composition remains unchanged.

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Acknowledgments

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Thomas Wichard is a research group leader and lecturer in Analytical Chemistry at the Institute for Inorganic and Analytical Chemistry of the Friedrich Schiller University Jena (Germany). After being awarded a PhD in Biochemistry (Max Planck Institute for Chemical Ecology), he investigated the metal recruitment of nitrogen fixers at the Princeton Environmental Institute (USA). His team applies various analytical chemistry, chemical ecology, and molecular biology methodologies to understand the basis of eco-physiological processes in bacteria-macroalgae interactions (cross-kingdom interactions).

EVOLUTION OF METABOLIC COMPLEMENTARITY IN ALGAL-BACTERIAL SYMBIOSES

Dittami S.M.[1]*, Pfister C.[2], Got J.[3], Lucas C.[3], Hamon-Giraud P.[3], Blanquart S.[3], Markov G.V.[1], Leblanc C.[1], Stock W.[4], Thomas F.[1], Rousseau C.[1], Meheurst R.[5], Stam M.[5], Siegel A[3].

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Metabolic complementarity is hypothesized to underpin beneficial interactions between symbiotic organisms, allowing the partners to potentially expand their combined metabolic capabilities by offsetting metabolic deficiencies. While genome-based metabolic models offer a promising avenue to predict such complementarity in silico, empirical evidence validating these predictions remains scarce. Here we analyzed 1300 algal-associated bacterial and 40 algal host genomes to investigate the specificity and evolution of metabolic complementarity. We addressed two key questions: 1) Does metabolic complementarity preferentially occur between algae and their direct bacterial associates compared to free-living relatives? and 2) To what extent is complementarity specific to host-bacterial pairs?

The comparative analysis of algal-associated and free-living bacteria did not consistently demonstrate enhanced metabolic complementarity in host-associated strains. However, we identified 38 bacterial taxa exhibiting significantly higher complementarity with their host or closely related algae than with phylogenetically more distant relatives. Metabolic complementarity varied significantly across bacterial classes, with e.g. Bacteroidia displaying high levels of complementarity but low host specificity, while e.g. Gammaproteobacteria demonstrated greater host specificity. The precise metabolic exchanges underpinning these patterns are currently being explored. Our findings provide novel evidence supporting the co-evolution of metabolic complementarity between algal hosts and specific members of their microbiome.

Short Biography

I am a permanent CNRS researcher working on the interactions between brown algae and their associated microbiome. My research subjects include metabolic cooperation between the microbiome and the host, but also role of the adaption of brown algae to environmental stress, or the transmission of the microbiome. To address these questions, I integrate genomic data, (meta-) transcriptomics, metabolite profiling, and genome-based metabolic networks to predict possible beneficial metabolite exchanges between partners. In parallel, I work on the isolation of bacterial partners and establishing co-culture experiments to be able to test any hypotheses generated in vitro. Most of my work is carried out on the species *Ectocarpus subulatus*, *Saccharina latissima*, and *Ascophyllum nodosum*.

LIGHT-REGULATED INTERACTIONS BETWEEN PHAEOBACTER SP. AND ULVA OHNOI(CHLOROPHYTA): EFFECTS ON MICROBIOME DYNAMICS, METABOLOME COMPOSITION, AND TROPODITHIETIC ACID PRODUCTION.

Qui-Minet Z.N.[1]*, Wichard T.[2], Del Olmo G.[1], Pereira M.[1], Holbl H.[2], Ruiz P., Cremades J.[3] and Pintado J.[1]

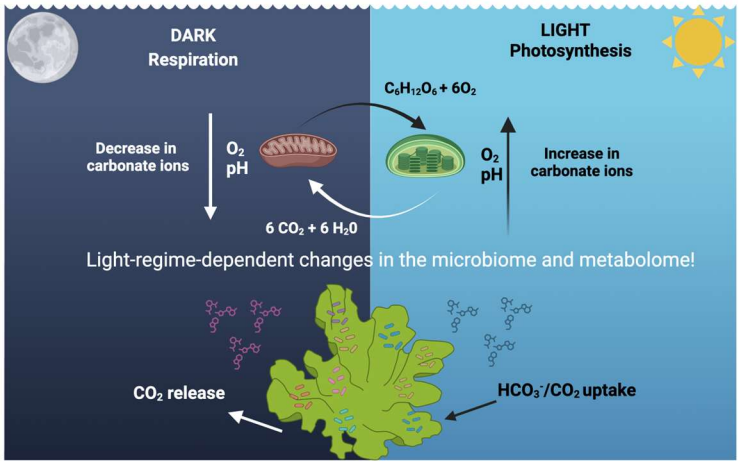
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ULVA spp. are economically important macroalgae with various industrial applications, including as biofiltration agents for fish effluents in integrated multi-trophic aquaculture recirculating systems (IMTA-RAS) [1]. Recent works have proposed inoculating U. OHNOI with the probiotic bacterium PHAEOBACTER sp. strain 4UAC3 to tackle fish pathogens such as VIBRIO spp. in IMTA-RAS [2]. However, the disappearance of PHAEOBACTER sp. 4UAC3 upon inoculation of U. OHNOI under a regular photoperiod presents significant challenges [3]. This study aimed to investigate how different light regimes impact the relationship between the U. OHNOI holobiont and PHAEOBACTER sp., focusing on how the colonization of PHAEOBACTER sp. strain 4UAC3 on U. OHNOI surfaces affects the alga's microbiome and metabolome dynamics. We also sought to validate the presence of tropodithietic acid (TDA), which can act as a probiotic. The study revealed the critical role of light in shaping microbial interactions between PHAEOBACTER sp. and U. OHNOI: The light regime significantly altered the microbial community structure, metabolite production, and physiological responses of both the bacterium and the alga. PHAEOBACTER sp. strain 4UAC3 thrived in darkness, modulating the microbiome and the EXO- and ENDO-metabolomes of U. OHNOI. TDA was only identified under dark conditions and released into the algal chemosphere, while PHAEOBACTER antimicrobial properties were most pronounced in close association with the alga. These findings underline the importance of environmental factors, such as light regime, in driving microbial and molecular dynamics in marine holobionts. In addition, our results have direct implications for the application of U. OHNOIand PHAEOBACTER sp. in aquaculture, providing valuable insights for future research and practical applications in the field.



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Acknowledgments

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Short Biograph

Zujaila N. Qui-Minet completed her PhD at the Station Biologique de Roscoff (Sorbonne University), where she investigated the physiology and ecology of coralline seaweed. She then worked in research and development in the private sector, focusing on plant and microalgae applications. In 2022, she began a postdoctoral position at IIM-CSIC (Spain) in collaboration with the University of Jena (Germany), studying seaweed-probiotic bacteria interactions for aquaculture applications. Since 2023, she has been working at LEMAR – IUEM – UBO (Brittany, France), examining how environmental factors shape the performance and senescence of proliferative seaweeds, and how these processes are associated with shifts in the microbiome and metabolome.

THE CHEMICAL 'LANGUAGE' OF THE ALGAL HOLOBIONT

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Marine algae form intimate relationships with microbes, collectively known as the 'algal holobiont'. These relationships often depend on chemical 'language', yet our understanding of them is limited compared to terrestrial environments. Climate-driven changes like rising temperatures, acidification, hypoxia, and desalination can significantly alter these interactions. My presentation will focus on how algae-bacteria interactions including interactions with pathogenic bacteria can fluctuate in response to stressors such as warmer oceans seaweeds and phytoplankton as model systems. I will also briefly touch upon the concept of microbial 'gardening' and how marine algal volatiles can influence interactions with microbes under temperature and light stress.

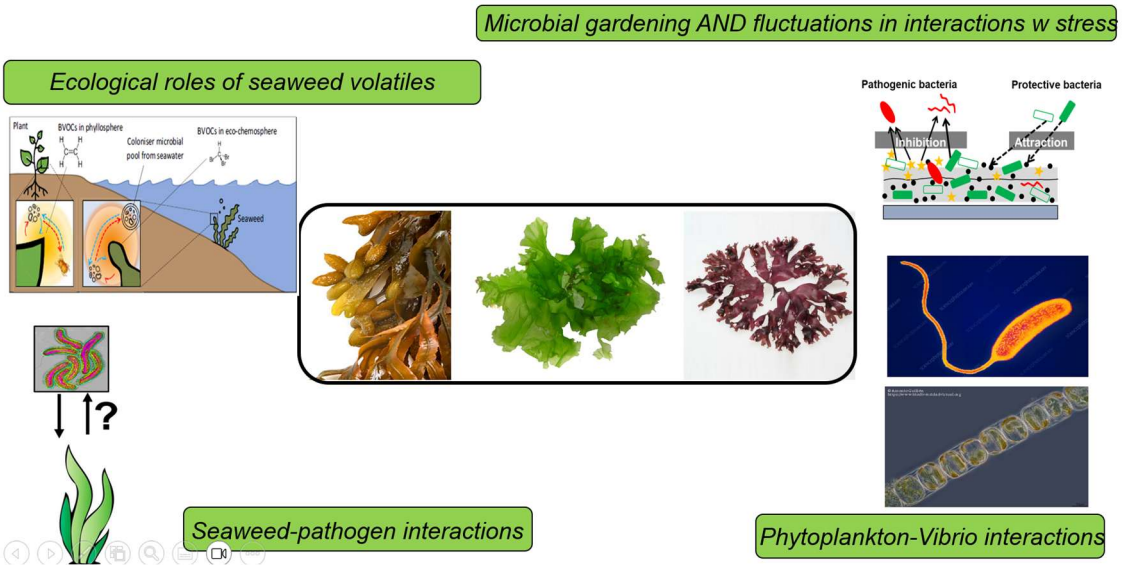


Figure 67 XXX

Short Biography

Dr Saha currently leads the algal chemical ecology group at Plymouth Marine Lab. She has pioneered cross-cutting research on marine info-chemicals, holobionts, trace-gas info-chemistry, climate change and disease ecology and embraces an interdisciplinary approach. She completed her PhD in Algal Chemical Ecology at Helmholtz Centre of Ocean Research (GEOMAR), Germany with a German Academic Exchange fellowship. She sits on the editorial board of multiple international journals and has been awarded the Asian Women of Achievement award in Science category in 2021 and the Paul Somerfield mentorship award in 2024.

DIVERSITY, DISTRIBUTION AND ROLE OF CORAL PROTIST SYMBIONTS

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Corals are threatened by the current climate crisis in the form of ocean acidification and recurrent heat waves. Coral reefs and coralligenous habitats are biodiversity hotspots, provide many ecosystem services for the local communities and protect the shore from the effects of the rising seas. Losing the corals will dramatically impact the earth's biodiversity and the livelihood of coastal communities. The microbial members of the coral holobiont (the coral and its microbiome) strongly influence the host's evolution, physiology, and ecological functions. While some elements of the holobiont have been studied in detail and are well characterized, such as the zooxanthellae (Symbiodiniaceae), a unicellular eukaryotic alga that is essential for the survival of reef-forming corals or specific widespread bacterial symbionts such as Endozoicomonas, the study of other unicellular eukaryotes (protists) forming the holobiont has been neglected. Protists represent one of the primary sources of hidden genomic diversity (genomic dark matter) in most of the microbial habitats, including the coral holobiont. Not knowing this genomic diversity will not allow us to access the potential functions that these protists are playing in corals and how they might impact the resilience of corals to the effects of the climate crisis. Being ignorant of one of the components of the holobiont limits our capacity to fully understand coral biology and subsequently the coral reef and coralligenous ecosystems. My research group has already shown a sneak peek at the protist diversity hidden within the coral holobiont. Our preliminary data shows that we can describe 3 times more diversity of coral protist symbionts using metabarcoding than previously reported. Not only that, our preliminary data suggest that for at least one coral, the Mediterranean red gorgonian *Paramuricea clavata*, the composition of the protist symbionts community can predict how the coral will respond to heat stress. One question arises after these discoveries: could protists be key to understanding coral's resiliency to climate change?

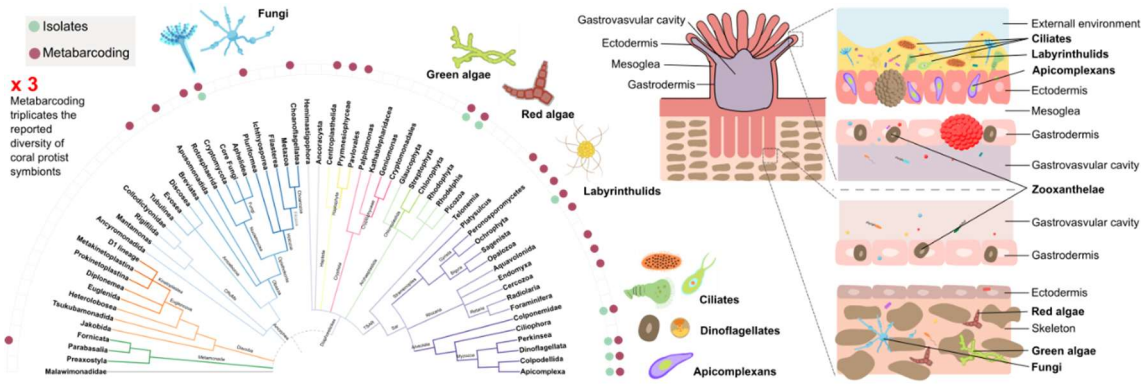


Figure 68 XXX

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Short Biography of the speaker

Dr. Javier del Campo is a microbial ecologist, Group Leader at the Institute of Evolutionary Biology (CSIC-UPF) in Barcelona, and an Adjunct Professor at the Rosenstiel School of Marine, Atmospheric, and Earth Science at the University of Miami. Dr. del Campo's research focuses on microbial symbionts and the effects of global warming on the microbiota of benthic and planktonic marine animals. While climate change significantly impacts free-living marine microbial communities, its effects on symbiotic microbes remain poorly understood. Microbial symbionts, both prokaryotic and eukaryotic, are essential to their host evolution, physiology, and ecological function. His work examines how global warming-driven environmental changes alter the composition and function of symbiotic microbial communities in key marine organisms—including corals, mollusks, and teleost fish—and how these shifts affect host health.

Ulva

The Future Wheat of the Sea

Training Schools



Phenotyping and genotyping of Ulva

Kavala, Greece

April 25-28, 2023

Ulva

The Future Wheat of the Sea

ORGANIZERS

Dr Sotiris Orfanidis, Research Director, INALE (ELGO-DIMITRA)

The COST Action SeaWheat (https://seawheatcost.haifa.ac.il/) organized a 4-days Training School, from April 25-28, 2023, at the Fisheries Research Institute (ELGO-DIMITRA; www.inale.gr), in Nea Peramos, Kavala, Greece.

RATIONAL AND OBJECTIVES

Seaweed mariculture is often considered the most environmentally friendly form of aquaculture. Among the different seaweed genera currently cultivated, *Ulva* is likely one of the most suitable candidates for large biomass production in European mariculture. However, not all *Ulva* species and strains have equal commercially exploitable attributes. A careful selection of the strains to be cultivated should be performed, depending upon the final use of the biomass and the geographical location of the farms. State-of-the-art genotyping and phenotyping methodologies provide the tools for such a selection. Hence, they could support the development of innovative approaches to speed up the genetic improvement of economically important traits, and ultimately their integration into breeding programs, which will make European farms more competitive.

The objective of this training school is to provide the participants with the skills required for genotyping and phenotyping *Ulva* strains. Training will include sampling specimens, DNA extraction for Next Generation Sequencing, PCR amplification, CAPS assays, and computer-based analyses using genetic data for genotyping. For phenotyping, participants will be introduced to chlorophyll-a fluorescence and oxygen release analyses, and become introduced to automated phenotyping platforms.

TRAINERS

Five (5) trainers with their assistants have been participated (alphabetically): Prof. Olivier De Clerck, University of Ghent, Belgium, Prof. Félix Figueroa, University of Malaga, Spain, Dr. Chrysoula Gubili, ELGO-DIMITRA, Greece, Dr. Sotiris Orfanidis, ELGO-DIMITRA, Greece, Dr. Ronan Sulpice, University of NUI Galway, Ireland. The event is also attended by the Action Chair and Scientific representative of the COST Action "SeaWheat", Prof. Muki Shpigel, University of Haifa (Israel).

TRAINEES

From thirty-two (32) early Career Investigators, SMEs, and PhD-students of Network members currently / previously working in seaweed ecology and aquaculture fields applied, fourteen (14) participants have been selected by an internal committee. The trainees, seven males and seven females were masters or postdoctoral students from various countries such as Belgium, Greece, Estonia, United Kingdom, Italy, Spain, Israel, Latvia, Portugal, and Slovenia. All trainees have been funded with a maximum of 980 € (up to 500 € air-ticket; 80 € D.A.-incl. 44 € for the Hotel) grant per participant.

HOST

The meeting has been organized locally by Dr. Sotiris Orfanidis (sorfanid@inale.gr) and members of Benthic Ecology and Technology Laboratory, Fisheries Research Institute (ELGO-DIMITRA): (https://www.google.gr/maps/@40.8559347,24.3156409,84m/data=!3m1!1e3).



Figure 69 Participants of the training school

COURSE DESIGN AND TRAINING MATERIAL

The design of the course was established during three teleconferences between the trainers. It was anticipated that the educational level of the trainees would be variable and that their experience in dealing with seaweed phenotypes and genotypes, interpreting responses, and in analyzing data would be limited and would require strengthening. The training materials, such as protocols and acclimated *Ulva* unialgal cultivated material prepared specifically for the workshop, were based on knowledge and procedures used in advanced courses of Universities and Institutes in Europe. Also, state-of-the-art scientific instruments such as different chlorophyll-a fluorometers, a spectrophotometer with an integrated sphere, and a growth phenotyping platform of "Benthic Ecology and Technology Laboratory" were given to trainees for practice.

Seven training Units were made available to the participants, from 09.00 to 18.30 every day, covering the following topics: Sampling *Ulva* strains from Nea Peramos coasts; Study of *Ulva* material under stereo- and microscope (use of Lugol); Samples sporulation and preparation for analysis in silica gel; Labelling and preparation of herbarium specimens; Genotyping: DNA extraction, PCR amplification, CAPS; Phenotyping: Growth rates estimation using conventional and automated methods; Phenotyping: Chlorophyll-a fluorescence and oxygen evolution cardinal variables of sun vs shade acclimated *Ulva* (P-I curves). Computer-based analyses using genetic data and computer-based analyses using Chlorophyll-a fluorescence (ETRmax, alpha, Ik) and oxygen evolution (Pmax, alpha, Ik) data (Excel) were discussed.

Before the closing, all achievements during TS were presented by representatives of three groups of trainees and discussed with trainers.

CONCLUSION

The Training School in Kavala, Greece, was an enriching experience for all involved trainers, trainees, and hosts. In addition, this Training School helped "SeaWheat" COST Actions to share knowledge and support collaboration.



Cultivation technologies of the land and sea based sytems

Malaga, Spain

June 26-29, 2023

Ulva

The Future Wheat of the Sea

ORGANIZERS

Dr Ricardo Bermejo, IBYDA (University of Malaga)

The COST Action SeaWheat (https://seawheatcost.haifa.ac.il/) and the University of Malaga organized a 4-days Training School on the technical and legal aspects of *Ulva* cultivation (WG2 and WG6), from June 26-29, 2023, at the Experimental Research Station Grice Hutchinson (IBYDA-UMA; https://www.ibyda.es), in Malaga, Spain.

RATIONAL AND OBJECTIVES

The main objective of the SEAWHEAT COST Action is to make a step-change towards a green economy based on *Ulva* mass production and utilization within the European community and beyond. The purpose is the development of *Ulva*-based blue-biotech industries and the utilization of *Ulva* as a model organism in European algaculture. The COST Action aims at facilitating the acceptance of *Ulva* as a new, sustainable, and safe food item in the traditional European diet. The Action will profit from the world-renowned quality standard of European food production. The ultimate goal is to integrate the nutritional value of *Ulva* into the European human diet and animal nutrition.

During this four-day training school, trainers with expertise in reproduction, aquaculture, and regulatory aspects of *Ulva* cultivation introduced the participants to theoretical and practical approaches to *Ulva* farming and consultation of industry stakeholders. The Training School included a site visit and case studies in Almeria (i.e. a factory for biostimulant production from algae, and aquaculture facilities at the University of Almeria and IFAPA).

TRAINERS

The training school involved the participation of seven trainers and four assistants, listed alphabetically:

1. Dr. Ricardo Bermejo from the University of Malaga, Spain
2. Dr. Paula Celis-Plá from the University of Playa Ancha, Chile
3. Prof. Félix Figueroa from the University of Malaga, Spain
4. Dr. Erik-jan Malta from Ctaqua, Spain
5. Dr. Fabio Neves from the University of Santa Catarina, Brazil
6. Dr. Rui Pereira from A4F, Portugal (leader of WG2)
7. Dr. Céline Rebours from Møreforsking AS, Norway (leader of WG6)

Additionally, the event was attended by the Action Chair and Scientific representative of the COST Action "SeaWheat," Prof. Muki Shpigel from the University of Haifa, Israel.

TRAINEES

The call resulted in twenty-eight (28) excellent applications being received. A selection committee consisting of 6 people assessed all applications, selecting 17 applicants. During the assessment process, various factors were taken into consideration, including active involvement in an on-going project with *Ulva*, experience in *Ulva* cultivation (minimum of one year), membership in a Small-Medium Enterprise, gender equality, and applicants from ITC countries.

The selected trainees, eight males and nine females were either masters, PhD students or postdoctoral researchers from eleven different countries: Finland, France, Germany, Ireland, Netherlands, Portugal, Serbia, Spain, Tunisia, Turkey and United Kingdom: 6 trainees from ITC countries; 9 from SMEs (Algabrava, Algues de Roses, Alles Alge, Bluegreen technologies, Green Aqua Vagos, Hydrosphere, Mediterranean Algae, Nemo Seafarms, the Seaweed company).

All trainees have been funded with a maximum of 1340 € (up to 500 € air-ticket; 80 € D.A.) grant per participant. Unfortunately, two trainees did not accept the course invitation.

HOST

The meeting has been organized locally by Dr. Ricardo Bermejo (ricardo.bermejo@uma.es), Prof. Félix López Figueroa (felix_lopez@uma.es) and Dr. Erik Jan Malta (e.malta@ctaqua.es).



Figure 70 Group photo of the participants and trainers of Training School

COURSE DESIGN AND TRAINING MATERIAL

To plan the training school, trainers met on several occasions to design the program and develop the didactic materials. They created a concise practical manual that included various protocols for Pulse Amplitude Modulated fluorometry, micropropagation from spores and protoplasts, as well as determining the content of phenolic compounds, antioxidants, and pigments. At the beginning of the training school, the trainees were provided with this manual, a notebook, a folder containing scientific articles focused on "how to conduct an interview and analyze the data," and a ballpoint pen.

Throughout the training sessions, the trainees had the opportunity to perform tasks such as obtaining protoplasts (Figure 71a), inducing *Ulva* sporulation (Figure 71b and 2c), estimating photosynthetic performance using PAM fluorometry, and determining phenolic, pigment, and antioxidant contents in *Ulva* (Figure 72). Furthermore, the participants developed an interview questionnaire to gather relevant information for the development of a start-up or a small to medium-sized enterprise. They conducted interviews with members of four different companies that were involved in the development of algae-based products or focused on seaweed cultivation (namely A4F, Biorizon, Ctaqua, and a start-up from Brazil) and analyzed the data obtained (Figure 73).

Prior to the conclusion of the training school, all the results obtained during the sessions were analyzed by the trainees and discussed with the trainers.

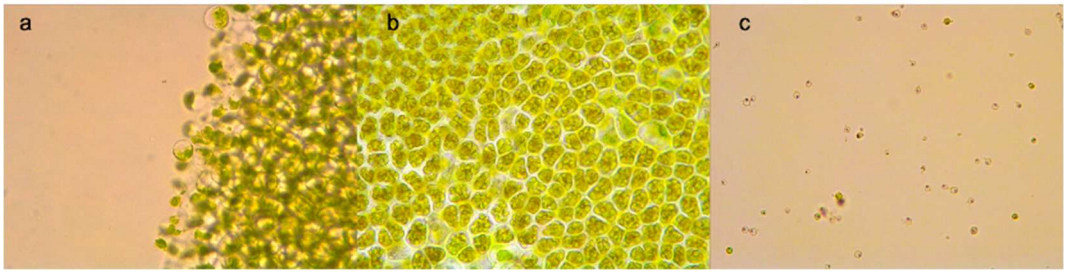


Figure 71 Protoplast release (x10; (a), reproductive Ulva thallus (x10; (b), and detail of spores (x40; (c) under the microscope.



Figure 72 Trainees estimating the phenolic, pigment, and antioxidant contents in dried Ulva samples.



Figure 73 Trainees analyzing the data collected during the interviews.

During the Training School, the trainees had opportunities to visit different aquaculture facilities. They visited the University of Almeria, IFAPA (Andalusian Institute of Agricultural and Fisheries Research and Training), and the Grice Hutchison Experimental Research Station. These visits allowed them to observe the functioning of different land-based culture systems, including raceways, aeration tanks, and thin-layer cascade systems (Fig. 5).



Figure 74 Several participants in the 2nd Seawheat Training School visiting one of the largest raceways in Europe at the University of Almería.

CONCLUSIONS

The Training School in Malaga, Spain, was an enriching experience for all involved trainers, trainees, and hosts. In addition, this Training School helped “SeaWheat” COST Actions to create a network between SMEs and researchers that we are sure will contribute to knowledge transference from academia to the industry. A survey will be developed after the TS2 and be sent to the trainees of TS1 and TS2 in order to use the information to improve the format of the future Training Schools organized in the CA SEAWHEAT project.



Emissions, capture, utilisation: methodologies and challenges for Ulva

Saaremaa, Estonia

May 14-17, 2024

Ulva

The Future Wheat of the Sea

ORGANIZERS

Dr Annette Bruhn, Aarhus University, WG5 leader
Dr Celine Rebours, Møreforsking, WG6 leader
Dr Professor Georg Martin, Estonian Marine Research Institute, local organiser

The COST Action SeaWheat (https://seawheatcost.haifa.ac.il/), the Estonian Marine Institute, Møreforsking and Aarhus University organized a 3½-days Training School on quantifying *Ulva* Ecosystem Services, and the legal and regulatory aspects of *Ulva* cultivation (WG5 and WG6), from May 14-17, 2024, at the Koiguste Marine Field station in Saaremaa, Estonia.

RATIONAL AND OBJECTIVES

The main objective of the SEAWHEAT COST Action is to make a step-change towards a green economy based on *Ulva* mass production and utilization within the European community and beyond. The purpose is the development of *Ulva*-based blue-biotech industries and the utilization of *Ulva* as a model organism in European algaculture. The COST Action aims at facilitating the acceptance of *Ulva* as a new, sustainable, and safe food item in the traditional European diet. The Action will profit from the world-renowned quality standard of European food production, and for acknowledging the ecosystem services provided by *Ulva* aquaculture.
During this 3½-day training school, trainers with expertise in *Ulva* ecophysiology, aquaculture, and regulatory aspects of *Ulva* cultivation introduced the participants to theoretical and practical approaches to quantifying *Ulva* Ecosystem Services with the focus cycling of carbon and nutrients. The Training School included site visits to Estagar and a land-based RAS trout farm where the Estonian Marine Institute has established research facilities for optising the nutrient and carbon uptake by *Ulva* from the fish effluents (part of the Workshop) and social hiking tour to the Koigi Bog.

TRAINERS

The training school involved the participation of seven trainers, listed alphabetically:

1. Dr. Gerli Alberts from the Estonian Marine Institute, Tallinn, Estonia
2. Dr. Annette Bruhn from Aarhus University, Aarhus, Denmark (leader of WG5)
3. Dr. Jack Hall from the Estonian Marine Institute, Tallinn, Estonia
4. Dr. Karel Keesmann from Wageningen University, Netherlands
5. Dr. Georg Martin from the Estonian Marine Institute, Tallinn, Estonia
6. Dr. Liina Pajusalu from the Estonian Marine Institute, Tallinn, Estonia
7. Dr. Céline Rebours from Møreforsking AS, Norway (leader of WG6)

Additionally, the event was attended by the Action Chair and Scientific representative of the COST Action "SeaWheat," Dr. Prof. Muki Shpigel from the University of Haifa, Israel.

TRAINEES

The call resulted in twenty-eight (18) applications being received. A selection committee consisting of 6 people assessed all applications, selecting 16 applicants. During the assessment process, various factors were taken into consideration, including active involvement in an on-going project with *Ulva*, experience in *Ulva* cultivation (minimum of one year), membership in a Small-Medium Enterprise, gender equality, and applicants from ITC countries.
The selected trainees, seven males and seven females were either masters, PhD students, postdoctoral researchers in academia or industry from seven different countries: Denmark, Estonia, Germany, Ireland, Portugal, Spain and New Zealand: 3 trainees from ITC countries; 3 from SMEs (Alles Alge, PureAlgae, Algabrava).
All trainees have been funded with a maximum of 740 € (up to 500 € air-ticket; 80 € D.A.) grant per participant. Unfortunately, two trainees did not accept the course invitation.

HOST

The meeting was organized locally by Dr. Prof. Georg Martin, Jack Hall, Gerli Alberts, Liina Pajusalu and Kristina Tivel.



Figure 75 Group photo of the participants and trainers of Training School at the Koigi Bog.

COURSE DESIGN AND TRAINING MATERIAL

To plan the training school, trainers met on several occasions to design the program and develop the didactic materials. They created a practical and informative manual that included the Training School program, experimental design, various protocols for quantifying *Ulva* photosynthesis via oxygen production using micro optodes, determination of carbon and nitrogen in seaweed tissue as well as in seawater in dissolved organic as well as inorganic form, Pulse Amplitude Modulated fluorometry and Biolog Plates. The material also contained general information about the Baltic Sea, Ecosystem Services and Ulva.

Two weeks in advance of the training school, an on-line meeting was carried out with all students and trainers in order to prepare the trainees for the work at the Training School. A few days in advance of the training school, the trainees were provided with the manual and a folder containing scientific articles.

Throughout the training school the trainees carried out a specific two-day experiment, to analyse the differences in photosynthetic performance, and carbon and nitrogen cycling of two local Ulvas (one from the coast and one from the RAS cultivation system at the Ösel Harvest OÜ) exposed to three different water sources (natural coastal seawater, water from the vicinity of a coastal trout farm and water from the RAS producing trouts at the Ösel Harvest OÜ). The aim of the Training School was for the trainees to become familiar with fundamental methods for quantifying the capture of nutrients from the environment, and the uptake and excretion of carbon by Ulva. The focus was on nitrogen, phosphorus and carbon, and the methods included: 1) Measuring photosynthesis rate through oxygen production; 2) PAM fluorometry to investigate the efficiency of the photosystem II; 3) Quantification of dissolved inorganic nutrients in seawater; 4) Quantification of dissolved inorganic carbon (DIC) in seawater; 5) Quantification of dissolved organic carbon (DOC) in seawater; 6) Quantification of the wet weight, dry weight and dry matter composition of *Ulva* tissue; 7) Quantification of the *Ulva* tissue carbon and nitrogen content; 8) Modelling exercise of *Ulva* growth and nutrient uptake; 9) Evaluation of microbial activity using biologs; 10) Sampling for determination of the *Ulva* microbiome using molecular methods; and 11) sampling for genetic analysis of the two *Ulva* populations used. Regarding 10) and 11), we here contributed to the pan European studies carried out by SeaWheat partners in WP2 on *Ulva* genetics and microbiome.



Figure 76 Cleaning the Ulva material prior to setting up experiments.

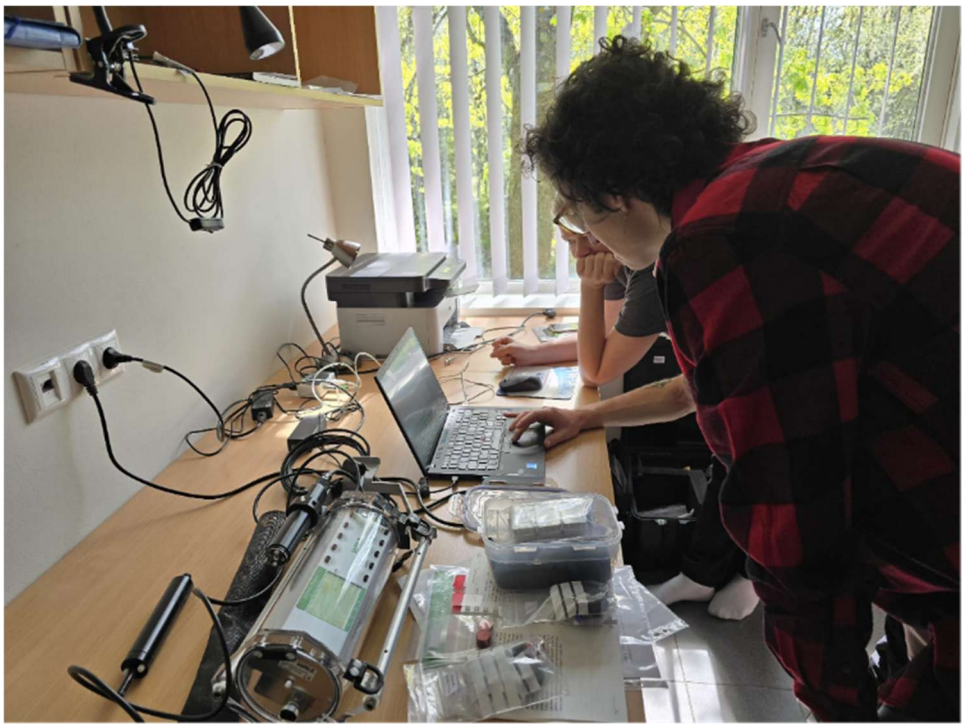


Figure 77 Trainees working on setting up the Diving PAM to measure the photosynthetic performance of Ulva



Figure 78 Trainees busy in the lab setting up experiments.



Figure 79 Trainees calculating and discussing results.



Figure 80 Networking between the trainees at Koiguste Marina who provided the meals during the Training School

CONCLUSIONS

The Training School in Saaremaa, Estonia, was an enriching experience for all involved trainers, trainees, and hosts. In addition, this Training School helped “SeaWheat” COST Actions to create a network between SMEs and researchers that we are sure will contribute to knowledge transference between academia to the industry, and between scientific fields. A survey will be developed after the TS3 and be sent to the trainees of TS3 in order to use the information to improve the format of the future Training Schools organized in the CA SEAWHEAT project.

The results obtained from the experimental work will form the basis for a joint peer-reviewed publication prepared by the trainees interested in following up and gaining even more scientific output from the TS. In this way the contribution to networking and scientific output for the involved young scientists will further improve.



Bioactives in Ulva

Aveiro, Portugal

June 26-28, 2024

Ulva

The Future Wheat of the Sea

ORGANIZERS

Dr. Thomas Wichard, Institute for Inorganic and Analytical Chemistry, Friedrich Schiller University Jena, Germany
Prof. Dr. Rosário Domingues, Departament of Chemistry, University of Aveiro, Portugal, local organizer
Ana Moreira (University of Aveiro, PT),
Margarida Martins (ALGAplus, PT),
Cláudia Nunes (University of Aveiro, PT),

TRAINERS

Ana Moreira (University of Aveiro, PT), Andreia Ferreira (University of Aveiro, PT), Hermann Holbl (University of Jena, DE), Rosario Domingues (University of Aveiro, PT), Thomas Wichard (University of Jena, DE)

The COST Action SeaWheat, the University of Aveiro, and the Friedrich Schiller University Jena organized a 3-day Hands-On-Training School on "Bioactives in *Ulva*" (Working Group 4) in continuation of the previous workshop. This cooperative effort, which took place from June 26–28, 2024, introduced the participants to the fundamental sample preparation approaches in targeted analysis and omics approaches. The Department of Chemistry at the University of Aveiro kindly hosted the training school and provided the laboratories.

RATIONAL AND OBJECTIVES

Ulva, commonly known as sea lettuce, is a genus of green algae found in marine and brackish environments. *Ulva* species are essential for nutritional value, environmental role, and industrial application. In all cases, chemical analysis, including the entire analytical process, is crucial to identify and quantify chemical compounds. The Training School aimed to train the participants on the fundamental principles and their differences in metabolomics, lipidomics, metabolite profiling, and targeted analysis in seminar classes and hands-on practical sessions in the chemistry laboratory.

ORGANIZATION

The training school was part of Working Group 4's (WG4) planned list of events for the current grant period. The local organizing committee, led by Prof. Dr. Rosário Domingues, Dr. Ana Moreira, and Dr. Andreia Ferreira, played a pivotal role in ensuring the training school's successful operation. They were supported by Dr. Thomas Wichard and Hermann Holbl (University Jena, DE).The scientific committee (Rosário Domingues (PT), Ana Moreira (PT), and Thomas Wichard (DE) of the training school selected fifteen trainees from about 25 applicants regarding their defined criteria (such as the quality of the motivation letter, scientific background as well as the applicant's current status). The Protocol Book compiled standardized protocols used within the COST Action CA20107 and taught to participants within the training school. News and information about the training school were posted on the SeaWheat COST Action social media channels (such as LinkedIn) and the Action websites.

PARTICIPANTS

The COST Action training school was well received, so 15 trainees were selected from 7 different countries (including 4 ITC countries). Nine participants were Master's or PhD students, three more advanced researchers, and two employees from the industry. All participants were younger than 40 years old and related to biological sciences (Figure 82).

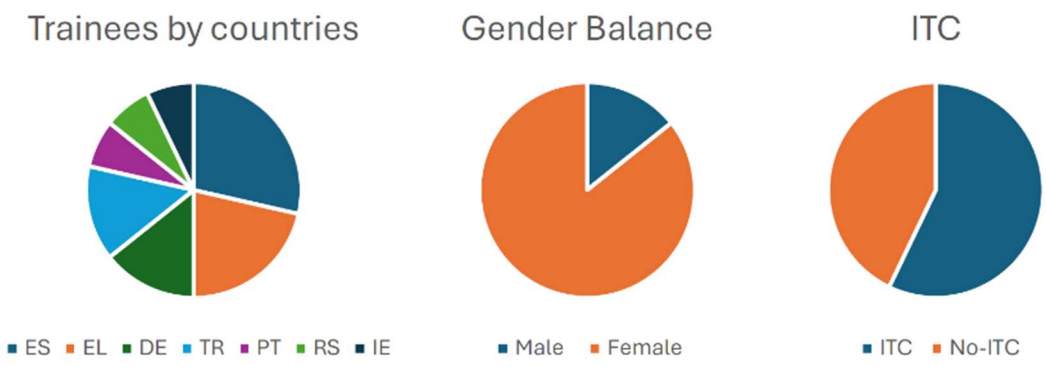


Figure 81 Distribution of the trainees by country, gender, and members of ITC countries



Figure 82 Group photo of 14 Trainees and four trainers of the Seawheat Training School outside the venue, Department of Geosciences, Aveiro, June 26-28, 2024

TRAINING SCHOOL DESIGN AND FUNCTIONING

By participating in the bioactives training school at the University of Aveiro, researchers and students gained valuable insights and skills, contributing to advancements in bioactive compounds and their applications. The curriculum was split between instructional seminars in the mornings and hands-on laboratory practice in the afternoons. In addition, the participants had their first introduction to data analysis. An introduction to theory and practice was provided in the following subject areas.

- Lipidomics workflow and lipid extraction of dried macroalgae (Ulva), including an introduction to the in-house mass-spectrometric platform.
  (by Prof. Rosário Domingues and Dr. Ana Moreira)
- Glycomics workflow and workflow of neutral sugar analysis (by GC-FID).
  (by Prof. Dr. Manuel A. Coimbra and Dr. Andreia Ferreira)
- Metabolomics workflow, extraction, and subsequent targeted analysis of thallusin.
  (by Dr. Thomas Wichard and Herman Holbl)

A trip to the aquaculture company (AlgaPlus) enriched the Training School. The company's industrial cultivation was visited, and algae material was taken for chemical analysis in the laboratory the next day.

CONCLUSIONS

This training school focused on approaches to analyzing bioactive compounds (e.g., lipids, sugars, thallusin) in the holobiont Ulva, a genus of green algae commonly known as sea lettuce. The event supported, in particular, newcomers in chemical analysis with a strong background in (marine) biology. The training school taught skills from the algal collection (at the local company AlgalPlus) to sample preparation and extraction of chemicals ready for mass spectrometric analysis. The class applied protocols in lipidomics, sugar analysis, and a specific approach for thallusin quantification, which was developed within the COST Action CA20106 during the first grant period.

The organizing team resumed that the Training School promoted approaches to the next generation of scientists and enhanced their technical skills and interests in modern analytical techniques. These techniques are very relevant to entering or continuing in the research field of bioactives in macroalgae. We believe that gaining knowledge and experience in analytical chemistry can open up career opportunities in academia and industry. Notably, the Training School participants are ready for their individual sample preparation at their home institution and for more efficient networking with chemistry laboratories within the COST Action.



Ulva cultivation systems: design, construction &optimization

Kavala, Greece

April 1-4, 2025

Ulva

The Future Wheat of the Sea

ORGANIZERS

Dr Sotiris Orfanidis, Research Director, INALE (ELGO-DIMITRA)

The COST Action SeaWheat (https://seawheatcost.haifa.ac.il/) organized a 4-days Training School, from April 1-4, 2025, at the Fisheries Research Institute (ELGO-DIMITRA; www.inale.gr), in Nea Peramos, Kavala, Greece.

RATIONAL AND OBJECTIVES

Seaweed mariculture is often considered the most environmentally friendly form of aquaculture. Seaweeds do not require land, freshwater, or fertilizers, which significantly reduces their environmental footprint compared to land-based agriculture or other forms of aquaculture. Among the different seaweed genera currently cultivated, *Ulva* is likely one of the most suitable candidates for large biomass production in European mariculture.

The objective of "*Ulva* cultivation methods" training school (TS) was to offer a valuable opportunity to gain practical skills and knowledge in this emerging field. For example, to receive practical training in various cultivation systems, including tank cultivation, open-water farming, and integrated multi-trophic aquaculture; to learn about seeding, harvesting, and processing techniques; to gain insights into the latest advancements and best practices; to connect with other participants, including students, researchers, and entrepreneurs interested in *Ulva* cultivation; and finally to build valuable relationships for future collaborations.

TRAINERS

Eight (8) trainers have been participated (alphabetically): Prof. Laurie C. Hofmann, Bremerhaven University, Germany, Dr. Stefan Kraan, Chief Scientific Officer at Oceana Organic Products Ltd, Ireland, Dr. Erik Malta, CTAQUA, Spain, Dr. Sotiris Orfanidis, ELGO-DIMITRA, Greece, Dr. Céline Rebours, Møreforsking AS, Norway, Prof. Muki Shpigel, University of Haifa, Israel, Dr. Yossi Tal, SEA-KURA, Israel, Dr. Ioannis Tzovenis, Microphykos, Greece.

TRAINEES

From sixty-four (64) early Career Investigators, SMEs, and PhD-students of Network members currently / previously working in seaweed ecology and aquaculture fields applied, seventeen (17) participants have been selected by an internal committee. The trainees, six males and eleven females, were masters or postdoctoral students from various countries such as Germany, Denmark, Greece, UK, Spain, Italy, Israel, The Netherlands, Portugal, Slovenia, and Finland. All trainees have been funded with a maximum of 980 € grant per participant.

HOST

The meeting has been organized locally by Dr. Sotiris Orfanidis (sorfanid@inale.gr) and members of Benthic Ecology and Technology Laboratory, Fisheries Research Institute (ELGO-DIMITRA): (https://www.google.gr/maps/@40.8559347,24.3156409,84m/data=!3m1!1e3).



Figure 83 Group photo of Training School

COURSE DESIGN AND TRAINING MATERIAL

The course was designed during two teleconferences between the trainers. It was anticipated that the educational level of the trainees would be variable and that their experience in dealing with *Ulva* cultivation, interpreting responses, and in analyzing data would be limited and would require strengthening. The training materials, such as protocols and *Ulva* mass-cultivated biomass specifically prepared for the workshop, were based on knowledge and procedures used in advanced courses of Universities and Institutes in Europe. Also, state-of-the-art scientific instruments such as different chlorophyll-a fluorometers (Dual-PAM, DIVING PAM), a spectrophotometer with an integrated sphere of “Benthic Ecology and Technology Laboratory” were given to trainees for practice.

Nine training Units were made available to the participants, from 09.00 to 20.00 every day, covering the following topics: Nutrient uptake efficiency of *Ulva* at different stocking densities; Determination of *Ulva* chlorophylls and carotenoids by HPLC analysis (demonstration); Study of *Ulva* morphology and photosynthesis at different stocking densities; Description and construction of a cage for *Ulva* cultivation; Study of *Ulva* photosynthesis and growth cultivated at different salinities; Determination of *Ulva* fatty acids by GC (demonstration); Regulations and policies for the Ulva production; Products development, sales and marketing of *Ulva*; Environmental Economic Accounts, EU legislation and EU Aquaculture Assistance Mechanism. Computer-based analyses using genetic data and computer-based analyses using Chlorophyll-a fluorescence (ETRmax, alpha, Ik) and oxygen evolution (Pmax, alpha, Ik) data (Excel) were discussed.

Before the closing, all achievements during TS were presented by representatives of three groups of trainees and discussed with trainers.

CONCLUSION

The Training School in Kavala, Greece, was an enriching experience for all involved trainers, trainees, and hosts. In addition, this Training School helped “SeaWheat” COST Actions to share knowledge and support collaboration.

Ulva

The Future Wheat of the Sea

Short Term Scientific Missions



Isabel Cardoso

Optimizing the protoplast isolation from different Ulva strains

01/05/2022 to 31/07/2022

Ulva
The Future Wheat of the Sea

Start and end date: 01/05/2022 to 31/07/2022

Isabel Cardoso, PhD student
Supervisors: Prof. Dr. Bela H. Buck[1], Dr. Laurie Hofmann[1], Dr. Liam Morrison[2], Dr. Ricardo Bremejo[2]

[1] Alfred Wegener Institute (AWI), [2] National University of Ireland Galway

DESCRIPTION OF THE WORK CARRIED OUT DURING THE STSM

Objectives of the STSM and timeline (Figure 84 and Figure 85):

1. Test two pre-treatments for the optimization of the protocol for protoplasts isolation in Ulva
2. Isolate protoplasts from 3 strains of *Ulva* lacinulata (from Portugal, Greece, and Ireland) and 2 strains of U. flexuosa (from Greece and Ireland)
3. Determine the maximum protoplasts yield and protoplasts survival rate for each strain
4. Determine the germination success rates of protoplasts.

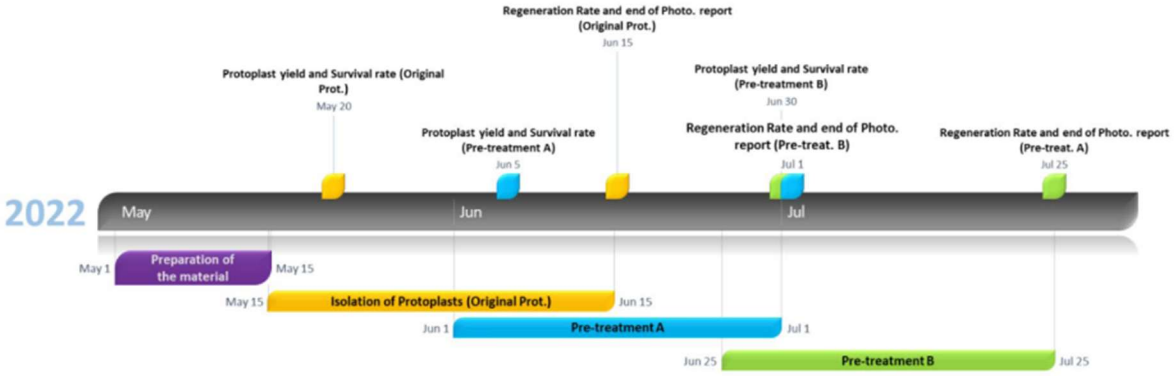


Figure 84 Gantt Chart presenting the workplan for the duration of the Project

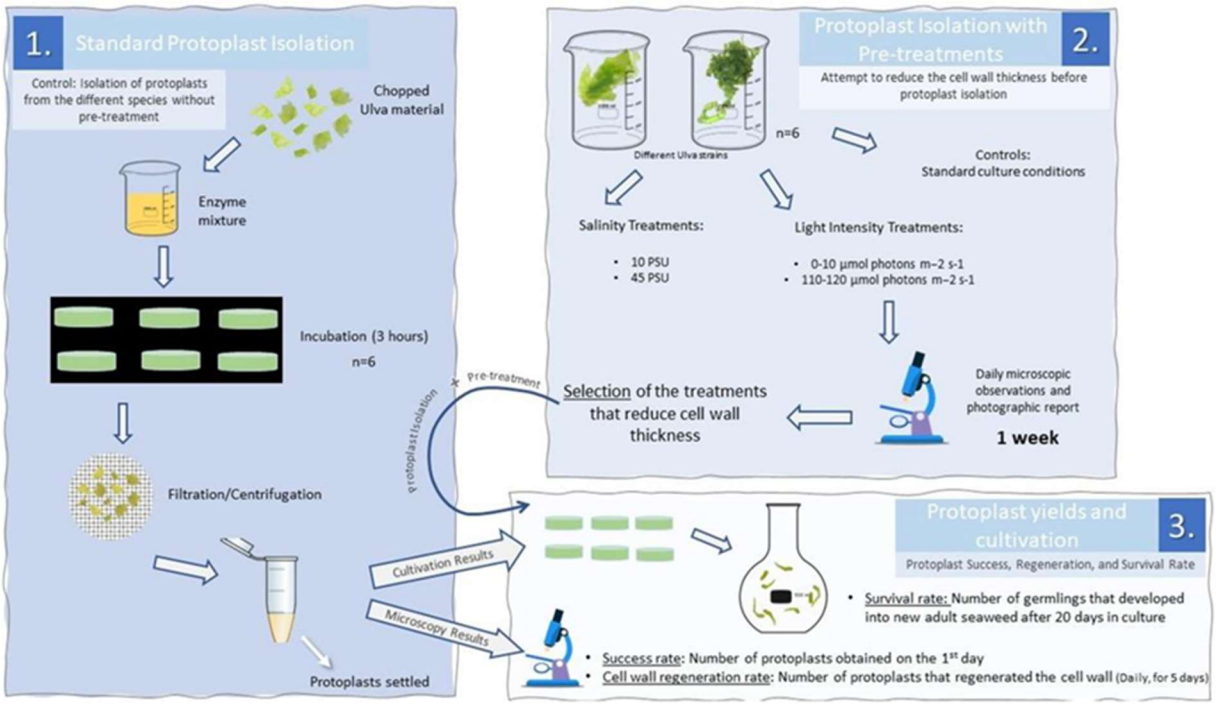


Figure 85 Experimental design developed for the STSM

Objectives achieved during the STSM:

1) two pre-treatments were tested for their impact on the thickness of the cell wall and their potential to facilitate protoplast isolation,

2) a new method for measuring the cell wall thickness was tested,
3) several protocols on protoplast isolation (based on the existing literature) were tested,
4) protoplasts were isolated from U. lacinulata without a pre-treatment,
5) protoplasts were isolated from both species when a pre-treatment was added to the original protocol.

Changes in the number of strains:

- only two strains were tested (one blade species and one filamentous species), both collected in Ireland. These changes had to do with concerns about the Nagoya Protocol and the transportation of the strains that were being cultivated in Germany (Portuguese and Greek).
- different species: since the material needed to be collected from the wild, both species are still to be identified using molecular techniques. Microscopy observations indicated that the filamentous species used was likely U. compressa.

Changes in the original timeline:

- collection and acclimation of the seaweed: the original plan was to start the work with pre-cultivated material, however, there was a need to collect new wild material that still required acclimation.
- pre-treatments tested at the beginning of the project: This is justified by the need to check if the pre-treatments would cause an effect on the seaweed, in particular if it would reduce the thickness of the cell wall (assumption: if the cell wall gets thinner with the pre-treatments, than the isolation success of protoplasts would be improved).
- 5-day experiments were conducted for each species and each pre-treatment and the cell wall thickness was measured.
- It is worth noting that there was no clear protocol or literature available on how to prepare *Ulva* samples for measurement of the cell wall. Therefore, some time was spent exploring different microscopy/histology and sectioning methods (including sectioning the samples with a cryostat) before deciding on a final approach for measuring the samples.
- Isolation of the protoplasts: several attempts were made before achieving the goal and for that reason, there was no time to determine the germination success rate of the protoplasts obtained. Only the protoplast yield and the initial survival rate can be reported*.
- COVID-19: I tested positive for Covid-19 and couldn't work for one week (12-20th, June).

Other work performed:

- setting new cultures in the lab,
- teaching research assistants to cultivate Ulva,
- inducing reproduction on *Ulva* to guarantee biomass.

DESCRIPTION OF THE STSM MAIN ACHIEVEMENTS AND PLANNED FOLLOW-UP ACTIVITIES

From the work carried out during the STSM, the outcomes are the following:

- Success in finding a working protocol for protoplast isolation for *Ulva lacinulata* (Figure 3),
- Development of a new technique for determination of the cell wall thickness (Figure 86),
- Success in comparing a standard protocol for protoplast isolation against the proposed "optimized" protocol with the added pre-treatment for both species.

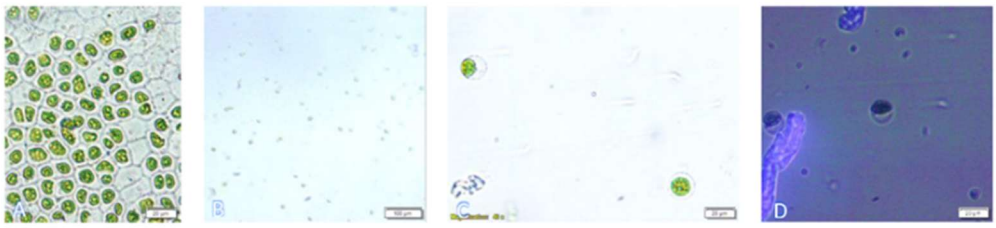


Figure 86 Microscopic observations of protoplast formation: changes in the thalli before protoplast detachment (A). Protoplasts after detachment from the thalli (B and C). Protoplasts dyed with calcofluor-white to confirm the absence of cell wall (cell wall would appear in fluorescent blue) (D).

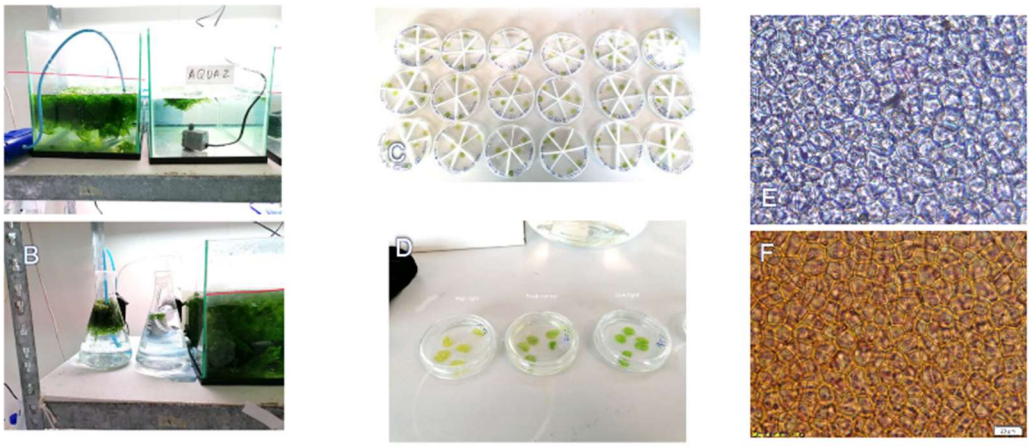


Figure 87 From initial cultures to microscope observations: cultures of U. lacinulata and U. compressa (A and B). Experimental set-up to test the effects of the pre-treatments (Salinity and Light) on cell wall thickness (C). Visible changes on the thalli appearance after exposed for 5 days to the light pre-treatment (D). Microscopy images of the seaweed thalli with cell well highlighted (E and F).

Concerning the scientific objectives of the COST Action, this project contributed to:

- ▪ WG1 objectives (task 1.3) – "The seasonal and environmental factors that impact on *Ulva* reproduction, growth, biochemical composition, and productivity (including nutrients, light, temperature, etc.) will be identified"
- by evaluating the impact that light and salinity have on the cell wall thickness of two *Ulva* species over 5 days.
- ▪ WG2 objectives (task 2.2) - "Reproduction control will be established; seeding and preservation techniques will be improved and optimized" and Task 2.3 "The potential impediments (biological, technological, and economical) for *Ulva* biomass production (including IMTA) will be evaluated and minimized"
- by adapting existing protocols for protoplasts isolation in two species of *Ulva* and testing the reproducibility of these protocols.
- by showing that low light and high salinity do not reduce the cell wall thickness of *Ulva* after 3-5 days, and therefore did not have a significant impact on protoplast isolation success rate.

Future publication on the following topic:

- The impact of light and salinity pre-treatments on protoplast isolation in *Ulva* strains

Considering the knowledge gained and shared by the two institutes during the STSM, we aim to progress the project further and optimize the protocol for protoplast isolation for more *Ulva* species and strains.

Future follow-up collaborations:

- Optimization of the protoplast isolation protocol based on the knowledge acquired during the STSM.
- Isolation and cultivation of protoplasts from *Ulva* strains from Portugal, Greece, and Germany (U. lacinulata, U. linza, U. flexuosa, U. fenestrata).
- Participation as an instructor in a Seawheat COST Action workshop on *Ulva* protoplast isolation.



Gulcin Sevim

Isolation of bioactive compounds from xenic and axenic Ulva cultures applied in various bioassay-guided test systems

02/05/2022 to 10/06/2022

Ulva
The Future Wheat of the Sea

Start and end date: 02/05/2022 to 10/06/2022

Thomas Wichard, University of Jena, Germany

DESCRIPTION OF THE WORK CARRIED OUT DURING THE STSM

1. Studies in Thomas Wichard Laboratory, University of Jena, Germany

The activities carried out during the STSM process can be examined under the following headings combining methods in analytical chemistry (1) with bioassays (2)

2) **Growing axenic and xenic *Ulva mutabilis* individuals and initiating heat stress treatments** xenic and axenic *U. mutabilis* gametophytes were obtained parthenogenetically from zooids derived from unpaired gametes or sporophytes under standard conditions in the laboratory of Thomas Wichard. *Ulva mutabilis* individuals were grown in UCM at 18°C under 60-120 µmol photon m-2 s-2 (50% GroLux, 50% daylight fluorescent tubes; München, Germany). *U. mutabilis* individuals grown in large boxes (>1 L) for experiments were transferred into air and light-permeable flasks containing fresh UCM. The experiment was carried out as 3 independent repetitions. For each experimental group, 5 flasks containing 25 individuals were used. The stress conditions initially determined as 5, 18, 25 and 28 °C for heat stress treatments were changed to 2 °C for cold stress, 18 °C for optimal growth and 28 °C for high temperature stress. The thallus lengths of xenic cultures and diameters of axenic cultures were recorded at day 0 and day 14 to determine the physiological differences that may be caused by heat stress.
3) **Harvesting algae at the end of the treatments** On the 14th day of heat stress application, xenic individuals whose thallus lengths were measured and axenic *U. mutabilis* individuals whose diameters were measured were washed with micropure water, frozen directly in liquid nitrogen, and then stored at -80°C until the extraction stage.
4) **Extraction for LC-MS analysis.** For metabolomic analyses of axenic or xenic cultures, ~25 mg of sample from the harvested plants was put into the Eppendorf tube. After 2 metal beads were placed inside, the samples were ground by shaking. 1 ml of solution solvent (methanol/chloroform/ethanol, precooled at -20) and 5 µl of 40 mM 13C1-sorbitol as internal standard was added to the samples. Samples incubate in an ultrasonic bath for 10 minutes and centrifuge at 30000 g for 15 minutes at 4°C. The supernatant (from ~1 ml extract) was transferred to 1.5 ml glass vials and evaporated overnight in a desiccator. Samples were re-dissolved in 100 µl methanol for running. A quality control (QC) sample is also prepared containing 5 µl of each sample. Samples can be measured directly in LC-MS.
5) **Metabolomic Analysis**. Ultra high-performance liquid chromatography (UHPLC) coupled with high-resolution MS (HRMS) for untargeted metabolites was performed using an UltiMate HPG- 3400 RS binary pump (Thermo Scientific, Bremen, Germany) and WPS-3000 autosampler (Thermo Scientific) loop. Mass spectrometer: Mass spectra were recorded with QExactive plus orbitrap MS (Thermo Scientific, Schwerte, DE). Electrospray ionization was conducted in both negative (spray voltage=3300 V) and positive (spray voltage=3000 V) ionization modes. UHPLC: Ultra high-performance liquid chromatography (UHPLC) coupled with high-resolution MS (HRMS) for untargeted metabolites was performed using an UltiMate HPG-3400 RS binary pump (Thermo Scientific, Bremen, Germany) and WPS-3000 autosampler (Thermo Scientific).
6) **Data Analysis.** Datas were analyzed using Metaboanalyst. Some of the results obtained are as follows (Figure1). The projection shows a clear significant distinction between control *U. mutabilis* extracts (red dots) and cold stress treated (green dots) and heat stress treated (blue dots) *U. mutabilis* extracts (highlighted by superimposed 95% confidence ellipses).

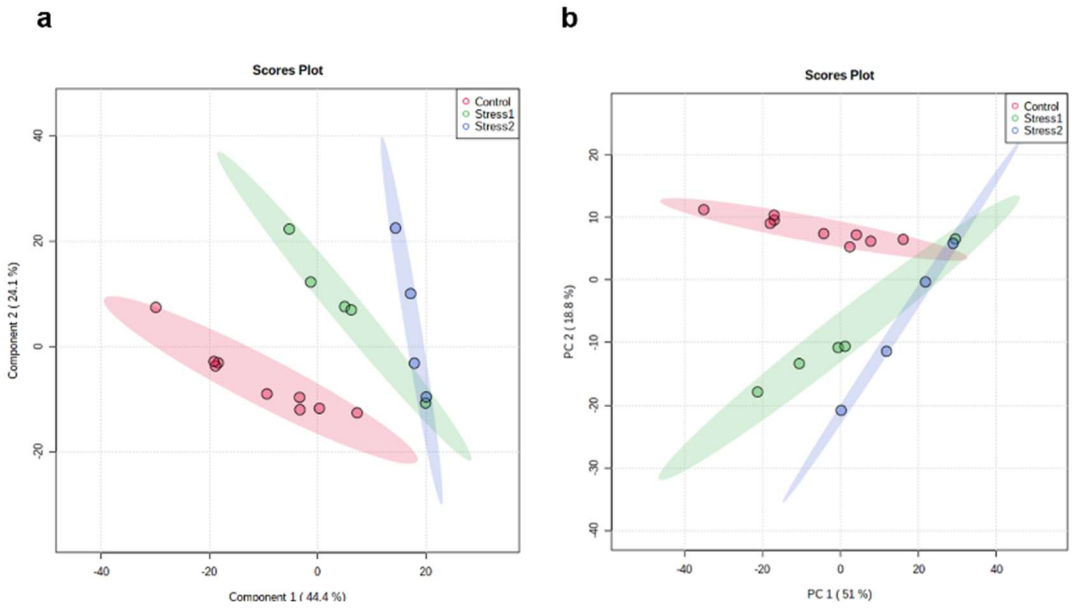


Figure 88 a- Principal component analysis (PCA) and b-Partial least squares discriminant analysis (PLS-DA) scores plot for metabolomic differences of a control group and heat stress treatment groups (stress1 (2°C) , stress2 (28°C)).

2. Studies in Dilek UNAL Laboratory, University of Bilecik, Turkey

Some of the samples prepared for LC-MS analysis were brought to Turkey for targeted bioassay analysis. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay of the total antioxidant capacity of the samples was performed in Dilek UNAL laboratory. In this assay, a molecule or antioxidant with weak A-H bonding will react with a stable free radical DPPH• (2,2-diphenyl-1-picrylhydrazyl, λmax = 517 nm), causing discolouration of the molecule. According to the results, high-temperature stress decreased the antioxidant capacity by 37.5% compared to the control group, while cold temperature stress increased it by 125.1% (Figure 89).

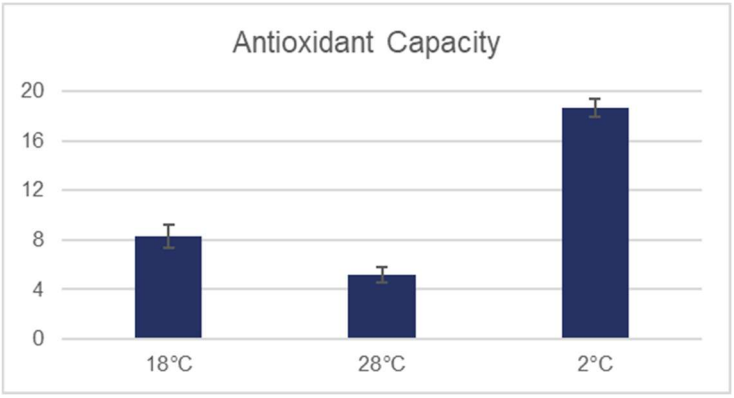


Figure 89 The effects of low and high-temperature stress on the antioxidant capacity of U. mutabilis

A microbial activity test was carried out to investigate the effect of *Ulva sp.* extract on probiotic bacteria, which is one of our goals. The prebiotic effects of *U. mutabilis* extract was tested with Lactobacillus plantarum 299V2 (Figure 3) and Lactobacillus paracasei B-1922 (Figure 4). According to the results, it was observed that the extracts of *U. mutabilis*, with or without stress, have not any significant effect. Related studies are still in progress.

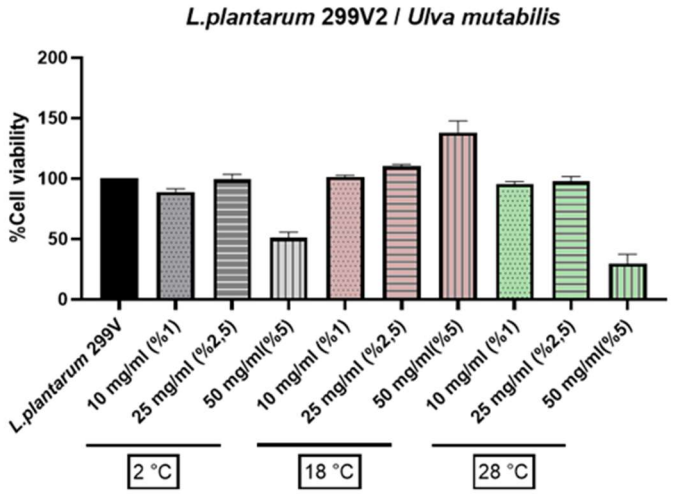


Figure 90 The prebiotic effects of U. mutabilis extracts on L. plantarum 299V cell viability

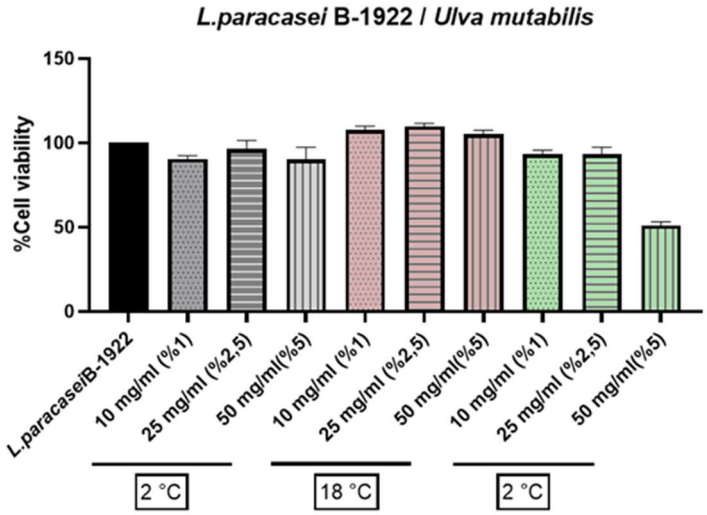


Figure 91 The prebiotic effects of U. mutabilis extracts on L. paracasei B-1922 cell viability

DESCRIPTION OF THE STSM MAIN ACHIEVEMENTS AND PLANNED FOLLOW-UP ACTIVITIES

Within the scope of STSM, the Jena University, Germany step of the work titled 'Isolation of bioactive compounds from xenic and axenic *Ulva* cultures applied in various bioassay guided test systems' was successfully completed. In addition, bioassay studies have started in Turkey and according to the result to be obtained, the metabolomic analysis performed in Germany will be expanded and the data will be studied more. It is still a question mark from which metabolites the differences between the groups arise specifically.

In summary:

- Temperature changes influence the metabolite profiling of *Ulva´*s thalli (tripartite community: Ulva-Roseovarius-Maribacter).
- Low temperatures increase the amounts of antioxidants in *Ulva´*s tissue.
- The change of metabolite profile due to the temperature shift experiment did not influence the viability of probiotic bacteria.



Sarah Schembri

Biochemical characterization of Ulva biomass: looking for bioactive compounds, particularly healthy lipids for food and feed applications

09/06/2022 to 06/07/2022

Ulva
The Future Wheat of the Sea

Start and end date: 09/06/2022 to 06/07/2022

Sarah Schembri, Applied Phycology Lab, Department of Biology, University of Malta
Rosario Domingues, Lipidomics Laboratory, Department of Chemistry, University of Aveiro
Gabrielle Zammit, Applied Phycology Lab, Department of Biology, University of Malta

DESCRIPTION OF THE WORK CARRIED OUT DURING THE STSM

This STSM was carried out at the Lipidomics Laboratory at the University of Aveiro. The lyophilised biomass of *Ulva sp.* from the Maltese Islands, that were provided by the Phycology Laboratory at the University of Malta, were extracted and analysed for their lipid content. The procedure was initially optimised using one replicate for each sample. Lipidomic analysis was then performed for five replicates for each *Ulva sp.* sample.

Lipids were extracted from freeze-dried and homogenised samples using the Bligh and Dyer method, where dichloromethane and methanol were used as extraction solvents. Fatty acid analysis was then carried out by gas chromatography-mass spectrometry (GC-MS). Fatty acid methyl esters were prepared from the lipid extracts of each replicate and analysed by GC-MS (Agilent Technologies). Identification of the fatty acids was performed using Agilent MassHunter Software, where the retention times, MS spectra of fatty acid analysis based on comparison of MS spectra with chemical databases as well as MS spectra of standards were considered (Lopes et al., 2019). For the identification of the total lipidome, the total lipid extract was analysed directly by LC-MS (Thermo Scientific) and full scan LC-MS spectra, and LC-MS/MS spectra in positive and negative ion modes were acquired for each replicate. LC-MS/MS analysis for the identification of molecular species of lipids was performed using the bioinformatics tool MS-DIAL, which was based on matching with exact mass, retention time and the acquired MS/MS data that was compared with an associated database. The identification of the lipid species provided by the software was validated and confirmed with the manual analysis of the MS/MS spectra. Manual analysis involved assigning the molecular ions observed in LCMS spectra, retention time and interpretation of LC-MS/MS spectra, based on the detection of typical fragmentation patterns of each lipid class, to confirm the identification of the molecular lipid species as well as the fatty acyl chain composition. MZmine software was used for the processing of LC-MS/MS data for the integration of the peak and quantification (Lopes et al., 2019; Rey et al., 2022).

DESCRIPTION OF THE STSM MAIN ACHIEVEMENTS AND PLANNED FOLLOW-UP ACTIVITIES

The STSM was successful in achieving the expected goal and outcome to create new scientific knowledge about the lipid composition of *Ulva sp.* from the Maltese Islands, which was never studied previously. The mass spectrometry-based approach employed during this STSM allowed the extraction and identification of several molecular lipid species and the fatty acid composition of three *Ulva sp.* From the Maltese Islands.

Fatty acids of *Ulva sp.* included saturated fatty acids such as 14:0, 16:0, 18:0 and 22:0; monounsaturated fatty acids such as 16:1 and 18:1 and polyunsaturated fatty acids such as 16:2, 16:4, 18:2, 18:3, 18:4, 20:4, 20:5, 22:4 and 22:5.

The lipid profile evaluated allowed for the identification of glycolipids, betaine lipids, phospholipids, sphingolipids and neutral lipids in *Ulva sp.* The glycolipids identified in *Ulva sp.* included the sulfoquinovosyl diacylglycerol (SQDG) and the neutral glycolipid digalactosyl diacylglycerol (DGDG) and monogalactosyl diacylglycerol (MGDG). Betaine lipids identified in *Ulva sp.* included the diacylglyceroltrimethyl homoserine (DGTS), monoacylglyceroltrimethyl homoserine (MGTS) and lysodiacylglyceroltrimethyl homoserine (LDGTS). Phospholipid classes identified in *Ulva sp.* Included phospatidylglycerol (PG), phosphatidylinositol (PI), phophatidylethanolamine (PE) and phosphatidylcholine (PC). Sphingolipids identified in *Ulva sp.* included ceramides and neutral lipids included diacylglycerols (DG) and triacylglycerols (TG).

The STSM also provided new skills, knowledge and training on the use of modern mass spectrometry based techniques for the analysis of lipids from marine algae to a young researcher from the Maltese Islands. This transfer of skills from working with professionals in the field of lipidomics has helped in strengthening the professional network for future collaborations between members of the COST Action CA20106 from two participating countries, namely chemists from Prof Rosario Domingues' team at the University of Aveiro, Portugal and phycologists from Dr Gabrielle Zammit's team at the University of Malta, Malta.

At least two publications resulting from the STSM are expected following complete data analysis, which will contribute to the objective described in the STSM to identify the phospholipids and glycolipids in the *Ulva sp.* biomass and the potential to be used as an alternative food source and animal feed:

- one publication relating to the interpretation of fatty acid data, as well as the antioxidant properties from *Ulva sp.* samples from the Maltese Islands;
- one publication on lipidomics and characterization of the lipid profile from LC-MS and MS/MS data of the *Ulva sp.* samples analysed during the STSM and evaluation of their biological activities.

The STSM partially contributed to the aims of Working Groups of this COST action including the use of *Ulva* in aquaculture and *Ulva* as food and feed and bioactive chemicals. The publications will directly contribute to the Action deliverables WG 3: *Ulva* as food and feed and WG 4: Bioactive products. Considerations will be made about the applications and nutritional value of *Ulva* for human consumption and animal feed, as well as lipids extracted from *Ulva* biomass to be utilised as healthy food supplements, and the potential of *Ulva* extracts and isolation of bioactive molecules.

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Rey, F., Melo, T., Lopes, D., Couto, D., Marques, F., & Domingues, M.R. (2022). Applications of lipidomics in marine organisms: Progresses, challenges and future perspectives. Molecular Omics.



Viviana Pasquini

Intensive biomass production of Ulva in tanks, training and experimental trials

29/08/2022 to 22/09/2022

Ulva

The Future Wheat of the Sea

Start and end date: 29/08/2022 to 22/09/2022

Viviana Pasquini University of Cagliari
Prof. Juan Luis Gómez-Pinchetti Banco Español de Algas (BEA) – University of Las Palmas de Gran Canaria (ULPGC),

DESCRIPTION OF THE WORK CARRIED OUT DURING THE STSM

During the training stage, I acquired knowledge about the intensive *Ulva* tank cultivation in an outdoor pilot-scale. *Ulva lactuca* biomass was collected and scaled-up in order to increase biomass production. The growth parameter indicated that the *Ulva* productivity can be highly variable according to the growth conditions, and the maximum increase in biomass was observed in the 750-L tanks with an increase of the 450% during the period, starting from an inoculation of 1 g/L and harvesting about 5g/L. With subsamples of *Ulva* biomass, firstly, the wet weight-dry weight (WW/DW) ratio for different *Ulva* batch was determined. The results indicated that the DW is between 20 – 18% of the WW (DW/FW = 0.19). *Ulva* cultivation can be carried out both for purposes of production and as biofilter, in all cases, the evaluation of the uptake efficiency of nutrients (NUE) and nutrient uptake rate (NUR) is fundamental for *Ulva* production and were calculated as reported by Vega et al. (2020). NUE values ranged between 75 and 60 with higher values in tanks (750L) with a higher surface (1.8 m2) in comparison with small capacity tanks (90L; 0.2 m2), indicating that the reduction (%) of this nutrient concentration in the effluent was probably dependent on higher light availability. Similarly, the NUR was between 0.375 and 0.749 µmol N-NH4 + L-1 h-1, with differences between tanks assayed. These results were probably related to the health status of the culture. However, according to the final use of *Ulva* biomass (discharge clean waters to the environment or increase the biomass), the NUR is the parameter to maximize or to control according to our objectives. *Ulva* produced in the facility was analyzed and characterized in terms of total phenols (Floroglucinol equivalent: mg DWg-1), photosynthetic pigments (chlorophyll-a; chlorophyll-b; total carotenoids), and antioxidant activity (DPPH standard method, % inibition). Prior the analyses, *Ulva* extracts were obtained from fresh biomass fine grinded with a mortar and three different extractions were performed using: Methanol (90%); Distilled water; Ethanol 50%. Analyses were conducted in triplicate for each tank using subsamples of *Ulva* biomass following the standard protocols provided by the laboratory.

The results showed that the best extraction for the analyses of the total phenol is the methanol (90%), whereas distilled water and ethanol (50%) provide similar results. Total phenol in *Ulva* were affected by the health condition of the biomass, no matter the solvent. In details, considering the methanol extraction, it showed higher value (600.29 mg g-1± 33.04SD) in high quality biomass compared to lower quality *Ulva* (489.97mg g-1±30.77SD).

Photosynthetic pigments content was analysed only in methanol extract. The results show that also the chloroplastic pigments were affected by the health state of the biomass, showing higher values in good-shape Ulva. The antioxidant activity was carried out using the methanol and distilled water extracts, however, the methanol ones showed good results with an inhibition between 26 and 28%.

Similarly, chlorophyll fluorescence determination in vivo using the AQUAPEN (Fv/Fm; Fm–F0) provide information about the health status of the culture. Samples were dark- adapted for 30 min prior to fluorescence measurements for the determination of the optimal quantum yield (Fv/Fm).

DESCRIPTION OF THE STSM MAIN ACHIEVEMENTS AND PLANNED FOLLOW-UP ACTIVITIES.

During the STSM project I acquired the starting point to implement the production of *Ulva* biomass in land-based facility in Sardinia (University of Cagliari) where it can be used for the formulation of experimental feed for aquaculture (e.g. sea urchins), allowing an independent *Ulva* production from the local lagoon. The main point of producing *Ulva* in tanks is to avoid and/or limit the contamination and obtain high-quality *Ulva* for reared species.

The laboratory activities I carried out in my STSM included the preparation of *Ulva* extract using different solvents: 1) methanol, 2) distilled water, 3) ethanol 50%. The extracts were analysed in terms of total phenol, chloroplast pigments and antioxidant activity, indispensable analyses for the definition of the quality of Ulva. I also used standard tools useful in the cultivation of *Ulva* including the determination of the nitrogen uptake efficiency (NUE) and the nitrogen uptake rate (NUR), both necessary to optimize the production in the facility.

New approaches were investigated with the AQUAPEN fluorimeter to evaluate the metabolic condition of *Ulva* biomass in-vivo.

The results of the experiment produced data on *Ulva* growth and biochemical composition in intensive pilot-scale facility. Such information will be useful in order to adapt the cultivation condition to the final use of *Ulva* (i.e. biomass production, source of biomaterials, food, feed, bioactive compounds).

Contribution to the Action

This STSM project contributed to the WG2 and WG3 of the COST Action and in particular with the following tasks and deliverables:

- WG2: Task 2.3 The potential impediments (biological, technological, and economical) for *Ulva* biomass production (including IMTA); Task 2.2 Reproduction control will be established; seeding and preservation techniques; Deliverables D2.1.
- WG3: Task 3.1 The applications and nutritional values of *Ulva sp.* in humans and animal nutrition, including food processing and Deliverables D3.1.

Future Plans

This STSM gave me the opportunity to transfer my knowledge on the *Ulva* intensive production acquired from the Spanish Bank of Algae (BEA) to the University of Cagliari and to create a profitable collaboration with Dr. Juan Luis Gomez Pinchetti and BEA-ULPGC. This collaboration represents the first step to produce high-quality *Ulva* in Sardinia at the University of Cagliari, where it will be used for the preparation of diets for aquaculture. Finally, the activity carried out in this STSM, and the results will be disseminated in international conferences and congress.



Justus Hardegen

Removal capability and stress response in Ulva aquacultures towards phenolic endocrine disruptors

13/03/2023 to 14/04/2023

Ulva
The Future Wheat of the Sea

Start and end date: 13/03/2023 to 14/04/2023

Justus Hardegen[1]
Supervisors: Dr. Thomas Wichard[1], Prof. Alexander Golberg[2]

[1]Institute for Inorganic and Analytical Chemistry, Friedrich-Schiller University Jena

[2]Department of Environmental Studies Department of Geophysics, Tel Aviv University[2]

DESCRIPTION OF THE WORK CARRIED OUT DURING THE STSM.

In Israel, I visited the Israel Oceanographic and Limnological Research Institute in Haifa, where the *Ulva* aquacultures of the research group are located to sample *Ulva rigida* (Fig. 1a,b). Moreover, I set up a 12 x 40 L culture system in Tel Aviv (Fig. 1c and 1d). The experimental design included three controls and three treatments with bisphenol A, deuterated bisphenol A, and ethinylestradiol performed in triplicates. The completely labelled isotopologue of bisphenol A will aid in detecting transformation/degradation products by isotope pattern analysis utilizing the DeltaMS software tool (Baumeister et al. 2018).

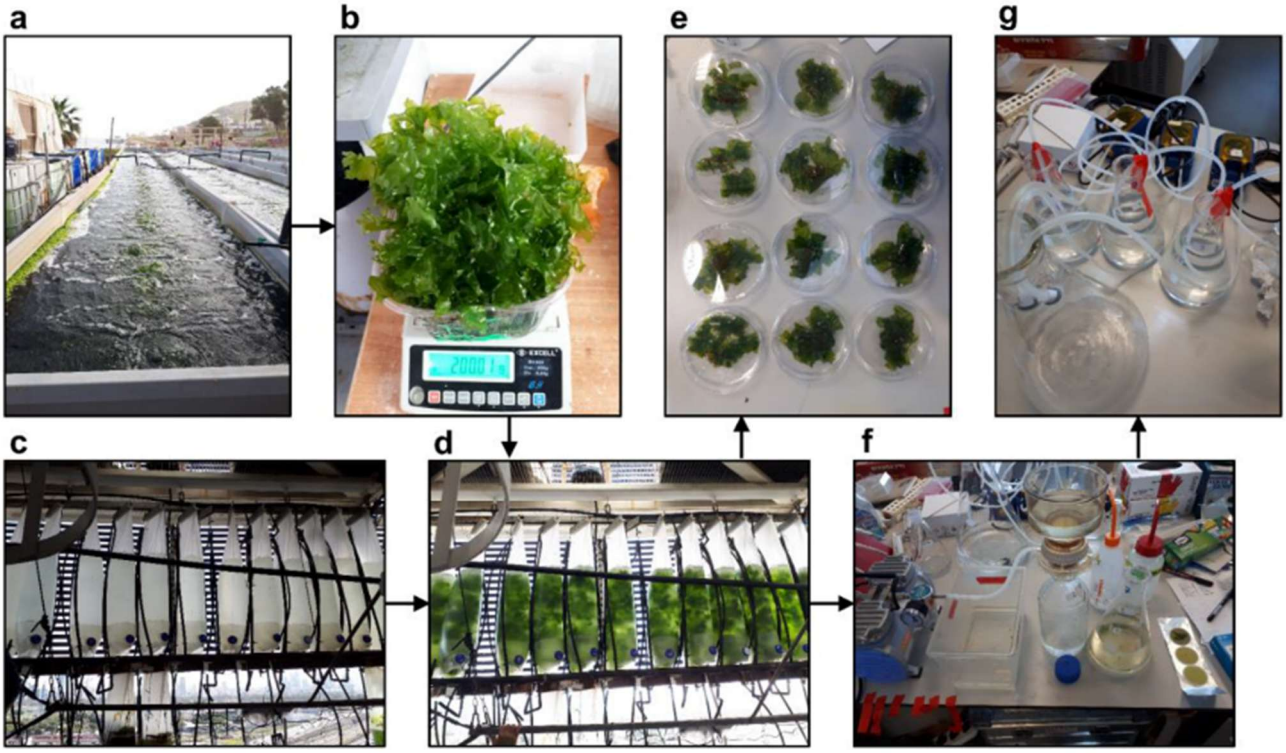


Figure 92 Work carried out during the STSM. Aquacultures in Haifa (a), harvested Ulva rigida (b), sleeve system with artificial seawater (c) and with Ulva (d), tissue samples (e), filtration of water samples (f), and solid phase extraction of water samples (g).

Water and algal tissue samples (Figure 92e) were taken hourly and daily to quantify the removal of micropollutants and uptake/sorption kinetics. To reveal the impact of the tested compounds on the *Ulva* and its holobionts, samples were collected to monitor changes in metabolomics and microbiome. I sampled elevated amounts of water (1 L) and algal tissue (> 5 g FW) at four-time points (before and 1 d, 3 d, and 6 days after the addition of the micropollutants). These samples will be used to identify and characterize transformation and degradation products along with microbiome composition changes. After filtration (Figure 92f), samples for metabolomics were extracted by solid phase extraction on-site in Israel (Figure 92g). The metadata of the sleeve experiment was recorded (Figure 93), and the sampling times were noted (Figure 94).
The primary purpose, sample production and sampling, was thus accomplished within the framework of STSM. The samples will now be studied further in our laboratory in Jena (Germany), in collaboration with Prof. Dr. Alex Golberg.

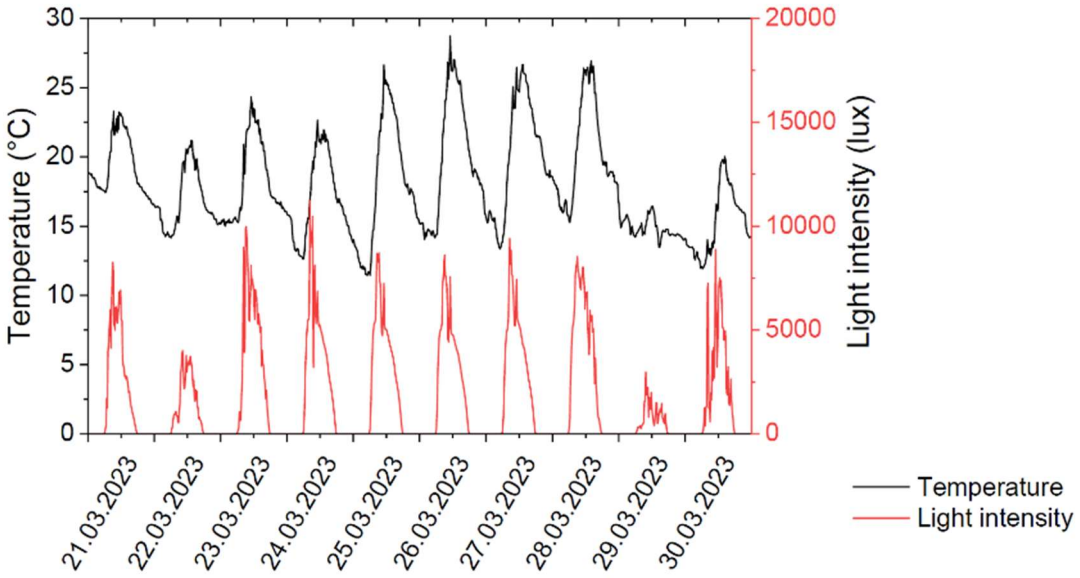


Figure 93 Metadata of experiment 1.

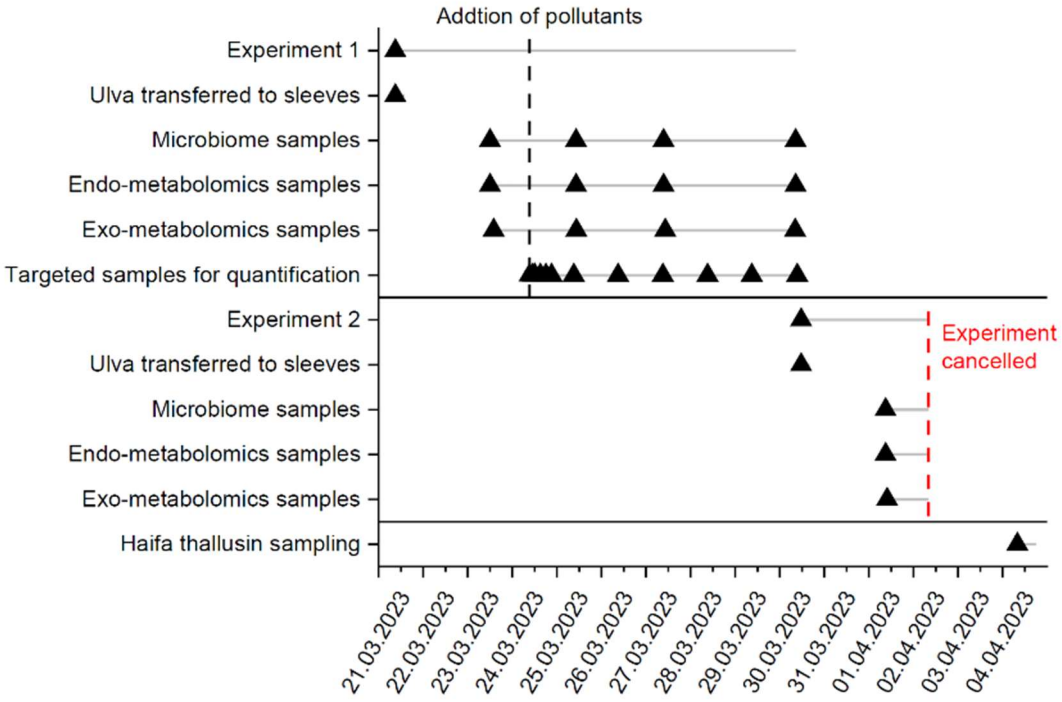


Figure 94 Experimental overview and sampling points.

The experiment plan (Figure 94) was finalized on-site and covered everything planned besides the quantification of thallusin in the core experiment. Due to a family emergency, I had to abort the STSM early and could only spend 22 of 31 planned days in Israel. However, I finished the most important experiment and the sampling in Haifa.

DESCRIPTION OF THE STSM MAIN ACHIEVEMENTS AND PLANNED FOLLOW-UP ACTIVITIES

The experiment design was based on my recent publication in the Special Issue of the COST Action *Ulva* conference in Cádiz, Spain (Hardegen et al., 2023). The design and scaling of aquacultures to an economically relevant scale proved effective.

Due to the split setup of the main experiment, all samples for three treatments were taken successfully during the time of the STSM in Prof. Golberg's laboratory. This complete sampling is the prerequisite for achieving the deliverables presented in the proposal for the combined laboratory tank (Israel) and lab-based experiment. As

planned, these samples are now being processed and evaluated as part of the cost action in collaboration with Prof. Golberg so that the results are made available for the various work packages of the COST Action as explained below.

I plotted the metadata of the basic parameters of light and temperature during the experiment (Fig. 2), as well as the experimental overview and sampling times (Fig. 3), to demonstrate the success of the experiment conducted in Prof. Golberg's laboratory. These results demonstrate that the experiment was carried out satisfactorily. The samples were collected at the agreed-upon intervals and will be examined in the coming months.

The research project meets several objectives and deliverables of the COST Action CA 20106. We anticipate three major outputs after the upcoming data analysis, which directly contribute to the research goals of several working groups (WG) of the COST Action.

(1) Understanding the removal kinetics of phenolic endocrine disruptors by *Ulva* and identifying break-down products enables us to evaluate Ulva's use as a biofilter in bioremediation systems (WG 5: ecosystem services: deliverable D 4.1.a). In particular, it is necessary for a risk assessment when *Ulva* is employed as a biofilter in conjunction with a feed and food source (WG 3: *Ulva* as food and feed: deliverable D 3.1.b).

(2) Endo-metabolome measurements will determine extractable substances under stress caused by MPs (WG 4: Bioactive products: deliverable D 4.1.a). Stress response and microbiome changes will shed light on *Ulva* microbiome interactions to anthropogenic environmental influences (WG 1: *Ulva* biology, deliverable D 1.1.b and 1.1.c).

(3) In up-scaled cultures, measurements of the algal growth and morphogenesis boosting factor thallusin will reveal whether this morphogen becomes limiting under larger scale conditions. Results of this survey might suggest if thallusin can be applied as a booster for algal biomass production (WG 2: *Ulva* in aquaculture: deliverable D 2.1.c). The thallusin determination also contributes to the survey of bioactives in *Ulva* cultres of WG4 (D 4.2).

2. In summary:

The cultivation techniques in Tel Aviv allowed using of higher biomass required for extraction and estimation of MP-removal in larger-scale aquacultures. Thus, the trials conducted in Tel Aviv have significant advantages over similar tests in smaller cultures that we may undertake in Jena (Germany):

- Large quantities of water enabled the extraction of enough substances to detect degradation products and understand degradation mechanisms.
- Sampling had no effect on the experimental results, even when only a few litres or grams of alga were gathered.

Despite the project's premature discontinuation, I could finish the most important part of the project and acquire satisfactory samples for my planned publication. Based on my preliminary results, Wichard's research group and Professor Alexander Golberg will organize further projects and fundraising in 2023.

Literature

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Dr. Umair Ahsan

Seasonal changes in the nutritional and quality profile of Ulva sp. in Nador region

22/03/2023 to 20/06/2023

Ulva

The Future Wheat of the Sea

Start and end date: 22/03/2023 to 20/06/2023

Dr. Umair Ahsan – Burdur Mehmet Akif Ersoy University, Turkey
Host : The Seaweed Company B.V., Schiedam, The Netherlands (Morocco)
Host contact : Amine Kharoub | amine.kharoub@theseaweedcompany.com

DESCRIPTION OF THE WORK CARRIED OUT DURING THE STSM

The purpose of this STSM was to to investigate the seasonal variation in the nutritional and quality profile of *Ulva sp.* occurring in Marchica lagoon in Nador, Morocco. During the STSM, different locations were carefully identified on the map to ensure the sustenance of sampling during different seasons and to include different species of Ulva.

The purpose of this STSM was to investigate the seasonal variation in the nutritional and quality profile of *Ulva sp.* occurring in Marchica lagoon in Nador, Morocco. During the STSM, different locations were carefully identified on the map to ensure the sustenance of sampling during different seasons and to include different species of Ulva. To further identify the sampling sites, an on-site survey was conducted with the employees of The Seaweed Company Red Turtle SARL, a subsidiary of The Seaweed Company B.V., the Netherlands. Following the on-site survey, the sites identified for the sampling to study the seasonal variation in nutritional and quality profile of *Ulva sp.* were: 1) The Seaweed Company Red Turtle – Cooperative harvesting site in Nador, Morocco (as coordinates 35°07'39"N 2°52'14"W); 2) Salinas, Nador, Morocco (as coordinates 35°08'55.4"N 2°54'08.6"W); 3) Beni Ensar, Morocco (as coordinates 35°15'18.3"N 2°55'31.0"W); and Arekmane, Nador, Morocco (as coordinates 35°09'35.7"N 2°48'12.0"W). First sampling within the framework of this STSM was conducted in April 2023. After the first sampling in April, the location of Arekmane facing the doors opening towards the Mediterranean ocean was discontinued due to the insufficient quantity of *Ulva* to continue the study in this location. The second round of sample collection was conducted in May 2023 and the third round of sample collection took place in June 2023. In the third round of sample collection, a new location namely Corniche, Nador, Morocco (as coordinates 35°10'00"N 2°54'39"W), was identified with the help of an academician at the Multidisciplinary Faculty of Nador, University of Mohammed Premier. Approximately, 500 g of *Ulva sp.* fresh weight was collected from each identified location and transferred to the Nador facility of The Seaweed Company Red Turtle. Besides Ulva, samples of water and sediment were collected to correlate the water and sediment quality to that of *Ulva sp.* In addition, the samples were also collected for genotyping and microbiome analysis of *Ulva sp.* in June. The samples for nutritional and quality analyses of *Ulva* were dried under dark conditions followed by further drying in the green house. The drying in the green house dried the sample up to a maximum of 10% moisture. The samples for genotyping and microbiome analysis were immersed in silica gel and Longmire buffer. These samples have been dispatched to the concerned laboratories for genotyping and microbiome (SeaWheat COST Action working group volunteers).The dried Ulva, water, and sediment samples were carefully labelled and transported to the grantee's laboratory at Burdur Mehmet Akif Ersoy University, Turkey. The nutritional composition of *Ulva sp.* collected from Marchica lagoon of Nador during the STSM has been shared within the COST Action and to the WG3 leader Sylvia Strauss. Some results reported to the SeaWheat COST Action as part of the deliverables are presented below:

Table 2 Mineral and trace element composition of water and sediment in different parts of Marchica lagoon, Nador, Morocco

Location	Sample	Date	Zn (ppb)	Pb (ppb)	Cd (ppb)	Cu (ppb)	P (ppm)	Ca (ppm)	Na (ppm)	Co (ppm	As (ppb)	Hg (ppb)
Beni Ensar	Water	13.Jun.23	-	-	-	7,511	0	1675,5	51540	-	-	-
Salinas	Water	13.Jun.23	0,25	-	-	11,09	4,029	7390,5	70995	-	-	-
Beni Ensar	Water	13.Apr.23	3,337	-	0,054	9,572	2,481	1756,5	53505	-	-	-
Salinas	Water	13.Apr.23	-	-	0,087	11,61	18,45	4999,5	139590	-	-	-
Beni Ensar	Sediment	13.Jun.23	48,72	9,014	0,551	17,302	346	33500	15952	3,752	-	-
Beni Ensar	Sediment	13.Apr.23	49,94	26,62	0,054	18,784	241,8	105040	10570	3,3	-	-
Salinas	Sediment	13.Apr.23	103,12	5,332	-	34,48	443,6	28500	20740	8,814	-	-

Table 3 Nutrient composition and in vitro dry matter digestibility of different Ulva sp. in Marchica lagoon, Nador, Morocco[1,2]

Location	Date	Species	Process	CP	EE	Ash	CF	aNDFom	ADFom	ADL	12 h IVTD	24 h IVTD
Salinas	13.Apr.23	*U. lactuca*	As-is	14,56	0,47	43,97	10,03	21,80	7,27	2,08	35,19	39,89
Salinas	8.May.23	*U. lactuca*	As-is	14,72	0,46	45,01	5,81	26,86	8,87	3,31	39,12	41,15
Salinas	13.Jun.23	*U. lactuca*	As-is	8,36	0,41	47,02	12,90	18,04	7,22	2,58	36,99	39,04
Beni Ensar	13.Apr.23	U. rigida / lactuca	As-is	3,33	0,29	50,41	8,27	42,76	13,49	4,55	35,32	42,40
Beni Ensar	13.Jun.23	U. rigida / lactuca	As-is	5,88	0,27	45,06	9,92	28,42	7,05	1,54	35,14	37,86
Beni Ensar	13.Jun.23	U. rigida / lactuca	Washed	5,42	0,29	21,99	16,66	41,65	5,19	1,34	37,93	38,56
Corniche	21.Jun.23	U. compressa / linza	As-is	13,44	0,37	28,77	12,87	35,97	15,00	3,77	34,07	37,64

[1]All the data are presented in %, on dry matter basis.

[2]CP: crude protein; EE: ether extract (crude fat); CF: crude fiber; aNDFom: ash-free neutral detergent fiber after amylase treatment; ADFom: ash-free acid detergent fiber; ADL: acid detergent lignin; IVTD: in vitro true dry matter digestibility in sheep after 12 or 24 h incubation in ruminal fluid

Table 4 Mineral and trace element composition of different Ulva sp. in Marchica lagoon, Nador, Morocco[1]

Location	Date	Species	Process	Zn	Pb	Cd	Cu	P	Ca	Na	Co	As	Hg
Salinas	13.Apr.23	*U. lactuca*	As-is	-	-	-	-	4550,77	17867,31	159173,08	3,478	-	-
Salinas	8.May.23	*U. lactuca*	As-is	-	-	0,043	-	3703,27	11411,54	97903,85	1,302	-	-
Salinas	13.Jun.23	*U. lactuca*	As-is	-	-	0,117	-	2625,6	21456	108960	1,414	-	-
Beni Ensar	13.Apr.23	U. rigida / lactuca	As-is	-	-	-	-	2032,22	24072,22	186166,67	0,316	-	-
Beni Ensar	13.Jun.23	U. rigida / lactuca	As-is	-	-	0,094	-	2142,35	29252,94	81529,41	1,172	-	-
Beni Ensar	13.Jun.23	U. rigida / lactuca	Washed	-	-	0,178	-	108,65	17846,15	6565,39	0,118	-	-
Corniche	21.Jun.23	U. compressa / linza	v	-	-	0,185	-	1073,53	4302,94	53450,98	0,294	-	-

[1]All the data are presented in mg/kg, on dry matter basis.

To further identify the sampling sites, an on-site survey was conducted with the employees of The Seaweed Company Red Turtle SARL, a subsidiary of The Seaweed Company B.V., the Netherlands. Following the on-site survey, the sites identified for the sampling to study the seasonal variation in nutritional and quality profile of *Ulva sp.* were: 1) The Seaweed Company Red Turtle – Cooperative harvesting site in Nador, Morocco (as coordinates 35°07'39"N 2°52'14"W); 2) Salinas, Nador, Morocco (as coordinates 35°08'55.4"N 2°54'08.6"W); 3) Beni Ensar, Morocco (as coordinates 35°15'18.3"N 2°55'31.0"W); and Arekmane, Nador, Morocco (as coordinates 35°09'35.7"N 2°48'12.0"W). First sampling within the framework of this STSM was conducted in April 2023. After the first sampling in April, the location of Arekmane facing the doors opening towards the Mediterranean ocean was discontinued due to the insufficient quantity of *Ulva* to continue the study in this location. The second round of sample collection was conducted in May 2023 and the third round of sample collection took place in June 2023. In the third round of sample collection, a new location namely Corniche, Nador, Morocco (as coordinates 35°10'00"N 2°54'39"W), was identified with the help of an academician at the

Multidisciplinary Faculty of Nador, University of Mohammed Premier. Approximately, 500 g of *Ulva sp.* fresh weight was collected from each identified location and transferred to the Nador facility of The Seaweed Company Red Turtle. Besides Ulva, samples of water and sediment were collected to correlate the water and sediment quality to that of *Ulva sp.* In addition, the samples were also collected for genotyping and microbiome analysis of *Ulva sp.* in June. The samples for nutritional and quality analyses of *Ulva* were dried under dark conditions followed by further drying in the green house. The drying in the green house dried the sample up to a maximum of 10% moisture. The samples for genotyping and microbiome analysis were immersed in silica gel and Longmire buffer and stored at appropriate temperatures. The dried Ulva, water, and sediment samples were carefully labelled and transported to the grantee's laboratory at Burdur Mehmet Akif Ersoy University, Turkey. The nutritional and quality analyses of *Ulva sp.* collected from Marchica lagoon of Nador continues and will be shared within the COST Action once the *Ulva sp.* are identified by genotyping.

DESCRIPTION OF THE STSM MAIN ACHIEVEMENTS AND PLANNED FOLLOW-UP ACTIVITIES

Main achievements

- This STSM has helped the grantee to learn more about the biology, harvesting, handling, drying, and processing of *Ulva* under commercial settings since the background of the grantee is animal nutrition. It has been a great learning experience for the grantee to identify different forms of Ulva, and practical learning of algal blooms and green tides.
- The STSM has contributed to conduct the study in order to understand the seasonal changes in the nutritional and quality profile of *Ulva sp.* occurring in the Marchica lagoon of Nador, Morocco.
- The STSM has contributed to joint sampling initiative of Working Group 1 and Working Group 3 for genotyping, microbiome, and nutritional analyses of the SeaWheat COST Action 20106.
- This STSM has allowed to coordinate the research activity between the institutions involved and fostered the networking opportunity for the grantee for personal and professional development. In addition, it has contributed to the development of intercultural skills of the grantee.

Planned follow-up activities and future collaboration

- As already known that this STSM was conducted in an ongoing study involving the seasonal changes in the nutritional and quality profile of *Ulva sp.* occurring in Marchica lagoon of Nador, Morocco, samples will be collected in the upcoming seasons and analyzed at grantee's laboratory. Therefore, the results will be submitted at the end of the study.
- The samples of *Ulva sp.* processed for genotyping and microbiome analysis will be transported to the respective laboratories of the contributors.
- The data and results generated from the analyses will be incorporated into the database of the SeaWheat COST Action.
- The results of this study will be disseminated to the primary and secondary audience via appropriate platforms.
- An article in a suitable journal is expected upon the completion of the study.
- Further studies in collaboration with the host organization will be planned to evaluate the use of *Ulva* and other seaweeds in animal diets using in situ and in vivo studies. This will help build further collaboration and networking and contribute to the advancement of knowledge of *Ulva* and support the use of *Ulva* in food and feed due to aligned objectives.



Caterina Pezzola

Southern vs Northern Europe: the impact of climate and genetics on performance and compound composition of land-cultivated Ulva

01/06/2023 to 15/07/2023

Ulva
The Future Wheat of the Sea

Start and end date: 01/06/2023 to 15/07/2023

Grantee: Caterina Pezzola
Host Institution: Spanish Bank of Algae, University of Las Palmas (Spain)

DESCRIPTION OF THE WORK CARRIED OUT DURING THE STSM

Characterizing the full range of variations among commercial *Ulva sp.* species is key for the optimization of farming practices and boosting the *Ulva* aquaculture sector in Europe. Delineating *Ulva* genetic variability across Europe and discerning species-specific phenotypical and commercial traits are among the biggest tasks embarked on by the scientific community and the seaweed industry. The achievement of such knowledge features among the main objectives of the SeaWheat COST Action and was thus the focus of this Short-Term Scientific Mission (STSM).

The project was carried out at the Spanish Bank of Algae (BEA) in Gran Canaria (Spain) and aimed to investigate performance (productivity rates = g DW m2 d-1) and compound composition (lipids and fatty acids) variations between two morphotypes of *Ulva sp.* found growing together in an outdoor tank. The two *Ulva* morphologies consisted of a laminar, blade-like type and a curly-morphology type. The biomass in the tank was previously identified as *Ulva rigida*, a species characterized by a thin, sheet-like thallus. The curly morphology in the tank could be explained either by the presence of a different *Ulva* species or by a spontaneous modification of the U. rigida laminar thallus. To characterize differences beyond each type's taxonomic position, weekly growth measurements were carried out for 5 consecutive weeks, and lipids and fatty acids composition analyses were performed at the end of the STSM. Despite spontaneous sporulation events, which affected the two types at different times during the experiment, the two batches displayed promising productivity rates, with the laminar type outperforming the curly one (26 g DW m2 d-1 vs 16 g DW m2 d-1, respectively). The range of productivity displayed by both batches is in line with previously reported production figures and deemed promising for the scaling-up of *Ulva* commercial production. The dry lipids content was approximately 2.5% in both batches. Total saturated fatty acids (SFAs) were the major FA component in both batches (~ 40%), while the omega-3 acid α-linolenic acid (ALA) was the second most abundant component. SFAs and ALA bear highly valuable properties for human health (i.e., heart and cardiovascular functioning) and provide both *Ulva* types with high commercial value within the nutraceutical, pharmaceutical, and medical industries. Molecular identification of the curly type is still ongoing.

Within the scope of WP1 of the SeaWheat COST Action, this project provided new knowledge on the basic biology of different *Ulva* morphotypes from the Canary Islands and the North-East Atlantic. Moreover, in line with the objectives of WP2 of the COST Action, this STSM provided information regarding the use of endemic *Ulva* stains for large-scale, commercial production of *Ulva* biomass in the Canary Islands and delivered valuable information regarding the potential use of these Canary strains for high-value market applications.

The original plan of the STSM comprised determining the role of climate and genetics in affecting Ulva's growth yields and compound composition in two different European regions, the Netherlands and the Canary Islands. Due to impediments and delays with biomass production in the months of May and June at my home institution, which hindered the collection of the necessary data, adjustments were made to the original plan, and the STSM became solely focused on the Canary Islands.

The aim of this Short-Term Scientific Mission thus became to investigate productivity rates and compound composition variations between two different *Ulva* (sub)species, characterized by different phenotypic traits, growing at the Spanish Bank of Algae (BEA) in Gran Canaria (Spain).

Two *Ulva* morphologies, a laminar (L) and a curly (R) type, were found growing together in an outdoor 750 L tank at BEA. The biomass maintained in this tank was previously identified as *Ulva rigida*, a species characterized by a sheet-like thallus. The curly morphology was deemed to be either a subspecies of U. rigida or a different species. By comparing the productivity rates (g DW/m2/d-1), and compound composition (total lipids, fatty acids) of the two morphotypes, we aimed at assessing whether phenotypic traits were linked to variations in performance and composition. The two morphotypes were separately cultivated for 5 weeks under similar environmental conditions. Weekly measurements were carried out for the assessment of growth and

productivity rates. The influence of light and temperature on the weekly FW growth of the two batches was statistically tested through univariate linear regression analysis but results revealed no significant effect on growth. However, growth and productivity rates were strongly affected by spontaneous sporulation events.
During the first 3 weeks, batch R outperformed batch L (R > 14 g DW m2 d-1; L < 10 g DW m2 d-1), as the latter was affected by sporulation and blade degradation, which eventually led to complete biomass loss in Week 3. Consequently, batch L was replaced with another batch of "pure" laminar biomass, already identified as U. rigida. During the last 2 weeks, the new batch L showed the highest growth rates (> 25 g DW m2 d-1) of the entire experimental period, while batch R started to sporulate and was lost by the end of Week 4. Overall, the high-end range of productivity from both batches (> 15 g DW m2 d-1) was in line with previously reported production figures deemed promising for the scaling-up of *Ulva* commercial production.
Biochemical analyses revealed similar lipid and FA compositions for the two morphotypes. Dry lipids content was approximately 2.5% in both batches. Total saturated fatty acids (SFAs) were the major FA component in both batches (~ 40%), while the omega-3 acid α-linolenic acid (ALA) was the second most abundant component. Both SFAs and ALA bear highly valuable properties for human health (i.e., heart and cardiovascular functioning) and provide both *Ulva* types with high commercial value.
The documented productivity rates and valuable compound composition uncovered the high potential of both morphotypes for scaled-up production in the Canary Islands.

DESCRIPTION OF THE STSM MAIN ACHIEVEMENTS AND PLANNED FOLLOW-UP ACTIVITIES

Characterizing the full range of variations among commercial *Ulva sp.* species (i.e., growth rates, bioactive compound composition, etc.) is key for the optimization of farming practices and for boosting the *Ulva* aquaculture sector in Europe. Characterizing *Ulva* genetic variability across Europe and discerning species-specific phenotypical and commercial traits are among the biggest tasks embarked on by the scientific community and the seaweed industry. The achievement of such knowledge features among the main objectives of the SeaWheat COST Action and was thus the focus of this Short-Term Scientific Mission (STSM).
Lamentably, the initial objective of this STSM, which entailed assessing the combined effect of climate and genetics on the growth and compound composition of *Ulva sp.*, could not be met. The original plan had to be changed due to the impossibility (given by technical issues with the growing tanks) to collect the necessary data at my home institution (Hanzehogeschool Groningen) in the Netherlands. However, upon arrival at the host institution (Spanish Bank of Algae) another possibility arose for me to investigate the role of genetics on the performance and composition of local *Ulva* species. The query that was raised provided me with the opportunity to assess differences between/among species (depending on the results of molecular analysis, yet to be delivered) and to gain important information for the development of the sector on the Canary Islands.
Within the scope of WP1 of the SeaWheat COST Action, this project provided new knowledge on the basic biology and taxonomy of Ulva, with the aim of identifying the best-performing strain for large-scale production of *Ulva* biomass and for commercial exploitation. Moreover, in line with the objectives of WP2 of the COST Action, this STSM delivered valuable information regarding the use of these Canary Islands strains for high-valued applications (e.g., pharmaceuticals, medicines, etc.) by investigating their lipids and fatty acids composition. Ultimately, the output of this STSM can support decision-making processes for a growing seaweed aquaculture industry in the Canary Islands, where the weather can sustain year-round and large-scale production of *Ulva* biomass.
Ultimately, the delivery of genetic results, longer-term monitoring, and a better understanding of sporulation-triggering factors can provide more accurate indications on which morphotype to choose for scaling up cultivation practices.
Finally, the comparison between the two morphotypes must be validated by molecular analysis that can reveal whether the different phenotypic traits were determined by genetic variability (different species) or if the two morphotypes were subspecies of the same taxonomic species (*Ulva rigida*). Regrettably, the analysis did not deliver results in time for them to be included in this report. Nonetheless, analyses are undergoing at BEA and their results will be part of the output of this STSM, as part of an extended collaboration. Additionally, the expected production data from the Netherlands will be collected in the coming weeks and compared with the results obtained at BEA. This will allow me to meet the original objective of the STSM, although outside of the official scope of this report.



Dr. Nada Ćujić Nikolić

Microencapsulation of Ulva sp. from the Adriatic Sea, as the promising ingredient in foods and pharmaceuticals

20/08/2023 to 02/09/2023

Ulva

The Future Wheat of the Sea

Start and end date: 20/08/2023 to 02/09/2023

Dr. Nada Ćujić Nikolić, Institute for Medicinal Plants Research Dr. Josif Pančić
Host institution: Institute of Marine Biology, Kotor, Montenegro
Supervisor: Dr. Dragana Drakulović

DESCRIPTION OF THE WORK CARRIED OUT DURING THE STSM

The sampling of *Ulva sp.* in Boka Kotorska Bay (Adriatic Sea) has been performed by researchers from Institute of Marine Biology in July 2023, and after that identification, and drying process. The *Ulva sp.* sampling and drying strategy was defined from the both Institutions. The samples were dried for two days in the shade of the sun (away from direct sunlight). The dried samples were collected prior to STSM. At the beginning of STSM, dried samples were grounded into powders in laboratory mill, then separated by sieve 0.75 (Yugoslavian Pharmacopeia) and dry residues were determined (10.62%) and prepared for extraction process. The goal of the extraction process was to be economically justified and environment friendly, based on the principles of "green extraction". The effects of several extraction parameters (solvent type, time, solid-solvent ratio, extraction method) on the extraction efficiency of solid/liquid extraction (maceration and ultrasound) were examined. Preliminary screening by maceration was performed on orbital shaker (180 rpm, 25 ◦C, for 1 h). To determine the influence of total polyphenols (TP) and flavonoids extraction (TF) (UV-VIS, spectrophotometrically), three levels of green solvent (ethanol–water) (30, 50 and 70%) and two levels of the solid–solvent ratio (1:10 and 1:20) were examined. With the optimal extraction conditions, maceration was performed for 1h, 2h and 18 h. Extraction process more than 2h had not influence on extraction yield. Next step was comparison of maceration-MAC (2 h) vs. ultrasound assisted extraction method-UAE (0.5 h), with optimal conditions. Increasing the temperature has not been effective during extraction process. Analysis was done in duplicate.



Figure 95 Figure 1. a) Dried Ulva lactuca b) Grinded Ulva sp. c) Maceration of Ulva sp.

Maceration of 2h was used for the production of a higher amount of extract, 4 L of 50% ethanol-water extract. Subsequently, ethanol was removed by rotary evaporator (vacuum, at 50◦C to preserve the sensitive compounds), and dealcoholized liquid *Ulva sp.* extract (LUE) was subjected to microencapsulation process. Dry residue of LUE was determined (4.79%), and alcohol content (5.14%, w/w).



Figure 96 Maceration, extract preparation, evaporation

Ulva sp. liquid extract (LUE) was spray-dried (Labtex ESDTi spray dryer, inlet (135 ± 5oC) and outlet (60 ± 5oC) temperatures) and freeze-dried (Beta 1-8 Freeze Dryer, -60oC with 0.011 mbar pressures, 24h and 0.0012 mbar for 1 hour), with and without carriers' addition. Microencapsulates were prepared using maltodextrin as conventional carrier-MD (50%, w/w) and innovative one, polydextrose-PD (50%, w/w)). Biopolymers were independently dissolved in LUE prior to microencapsulation process. The three obtained spray-dried powders, spray-dried *Ulva sp.* extract (SUE), and (SUE+MD and SUE+PD) and freeze-dried powders (FUE, FUE+MD and FUE+PD) were kept in high-density glass bottles in a desiccator at room temperature before next set of analysis.



Figure 97 Spray drying and freeze-drying method

After spray drying and lyophilization process, the obtained microencapsulates were analysed for the technological powder properties (powder yield, moisture content, encapsulation efficiency, bulk and tapped densities, Carr index-CI and Hausner ratio-HR, pH of dissolved powders, rehydration time) and FTIR analysis. The concentration of the total polyphenols, carotenoids, chlorophyll A and B in obtained particles were determined using UV/VIS. The individual compounds were analyzed by HPLC method.

DESCRIPTION OF THE STSM MAIN ACHIEVEMENTS AND PLANNED FOLLOW-UP ACTIVITIES

The optimization of the extraction process was performed as preliminary screening based on the results of TP and TF. Table 5 shows the contents of TP and TF. Based on the results, ethanol-water mixture (50%, solid solvent ration 1:20) was chosen as optimal extract conditions. Optimal extract, rich with bioactive compounds was produced with the principles of green chemistry, using ethanol-water as solvent, without use of temperature and produced by traditional extraction method, maceration (Table 6 TP content of extracts obtained by Maceration (2h) vs. Ultrasound assited extraction (0.5h). Maceration method of 2h was superior (more powerful) extraction method and was used for the production of a higher amount of extract.

Table 5. TP and TF content during optimization of extraction process by maceration

Extraction conditions	TP (mg GAE/g)	TF (mg Catehin/g)
30% EtOH, 1:10	0.3567 ± 0.0166^{d}	0.0972 ± 0.0020^{b}
30% EtOH, 1:20	0.5702 ± 0.0250^{b}	0.0194 ± 0.0009^{d}
50% EtOH, 1:10	0.3101 ± 0.0095^{e}	0.0910 ± 0.0033^{c}
50% EtOH, 1:20	0.6161 ± 0.0096^{a}	0.1918 ± 0.0038^{a}
70% EtOH, 1:10	$0.3551 \pm 0.0138d^{e}$	0.0863 ± 0.0010^{c}
70% EtOH, 1:20	0.4698 ± 0.0198^{c}	0.0229 ± 0.0003^{d}

Table 6 TP content of extracts obtained by Maceration (2h) vs. Ultrasound assited extraction (0.5h)

Extraction methods	TP (mg GAE/g)
MAC 50%EtOH, 1:20	0.6161 ± 0.0208^{a}
MAC 70%EtOH, 1:20	0.4698 ± 0.0149^{b}
UAE 70%EtOH, 1:20	0.4733 ± 0.0107^{b}
UAE 50%EtOH, 1:20	0.3899 ± 0.0118^{c}

Dealcoholized liquid *Ulva sp.* extract (LUE), possessed high content of total polyphenols, chlorophyll A and B, and carotenoids (3.55 mg GAE/ g, 84.53 mg/100g Chl A, 68.41 mg/100g Chl B, 5026.82 mg /100 g), respectively. The novel microencapsulation technologies were used for preservation of *Ulva sp.* extracted bio-compounds. Obtained powders improved Ulva's stability, limiting inactivation of the sensitive polyphenolic compounds, achieving their high encapsulation efficiency.

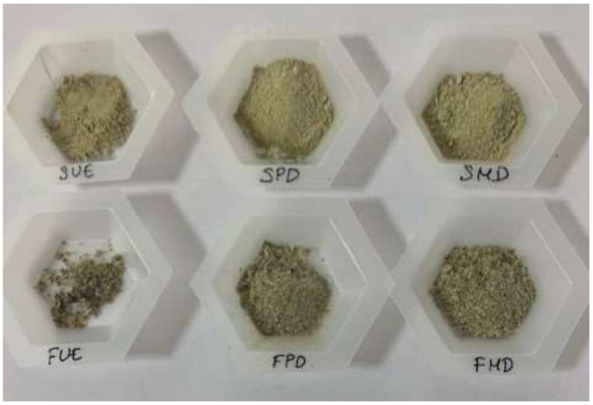


Figure 98 Obtained spray and freeze-dried powders of Ulva sp. Extract

Table 7 Technological properties of spray-dried and freeze-dried micro-powders

	Yield (%)	Moisture (%)	Bulk den.	Tapped den.	CI	HR	Rehyd. time (s)
SUE	27.23 ± 0.27^{d}	9.83 ± 0.19^{b}	0.27 ± 0.01^{b}	0.29 ± 0.01^{bc}	5.88 ± 0.35^{e}	1.06 ± 0.02^{d}	271.44 ± 8.81^{c}
SUE+PD	58.45 ± 1.25^{c}	7.67 ± 0.10^{c}	0.27 ± 0.02^{b}	0.31 ± 0.02^{b}	12.50 ± 0.44^{d}	1.14 ± 0.07^{cd}	470.96 ± 30.96^{a}
SUE+MD	58.55 ± 0.94^{c}	7.48 ± 0.26^{c}	0.31 ± 0.02^{a}	0.38 ± 0.03^{a}	20.00 ± 0.60^{f}	1.25 ± 0.03^{bc}	324.25 ± 3.32^{b}
FUE	93.76 ± 2.04^{a}	13.58 ± 0.68^{a}	0.07 ± 0.00^{e}	0.12 ± 0.00^{e}	36.84 ± 0.66^{a}	1.58 ± 0.10^{a}	217.29 ± 2.66^{d}
FUE+PD	74.02 ± 1.11^{b}	9.15 ± 0.37^{b}	0.13 ± 0.00^{d}	0.18 ± 0.00^{d}	26.32 ± 1.61^{b}	1.36 ± 0.07^{b}	153.83 ± 4.16^{e}
FUE+MD	97.72 ± 3.52^{a}	9.35 ± 0.61^{b}	0.21 ± 0.01^{c}	0.26 ± 0.01^{c}	22.22 ± 0.68^{c}	1.29 ± 0.07^{bc}	133.32 ± 6.30^{e}

Table 8 Chemical properties of obtained spray-dried and freeze-dried micro-powders

	EE %	TP (mg GAE/g)	Chl A (mg/100g)	Chl B (mg/100g)	Carot (mg/100g)
SUE	86.50 ± 3.45^{a}	3.07 ± 0.05^{a}	60.45 ± 2.72^{b}	42.06 ± 1.33^{c}	3369.09 ± 75.79^{c}
SUE+PD	70.91 ± 0.72^{c}	2.51 ± 0.04^{c}	52.45 ± 2.22^{c}	52.92 ± 2.05^{b}	4321.18 ± 172.24^{b}
SUE+MD	53.81 ± 2.20^{d}	1.91 ± 0.03^{d}	33.62 ± 1.21^{d}	23.24 ± 0.57^{e}	1981.53 ± 91.58^{e}
FUE	75.59 ± 1.11^{bc}	2.68 ± 0.17^{bc}	47.86 ± 1.84c	34.22 ± 0.63^{d}	2693.61 ± 127.65^{d}
FUE+PD	79.27 ± 3.14^{b}	2.81 ± 0.08^{b}	52.03 ± 2.49c	40.88 ± 1.38^{c}	3366.34 ± 161.84^{c}
FUE+MD	58.71 ± 2.57^{d}	2.08 ± 0.03^{d}	25.88 ± 0.31	17.63 ± 0.82^{f}	1418.79 ± 25.41^{f}
LUE			84.53 ± 3.24a	68.41 ± 1.66^{a}	5026.82 ± 118.72^{a}

Obtained powders beside high content of microencapsulated compounds, exhibited high encapsulation efficiency, associated with good bioactives protection. Based on the results, it can be concluded that the microencapsulation techniques and carrier addition had an impact on the protection of *Ulva sp.* bioactive compounds.

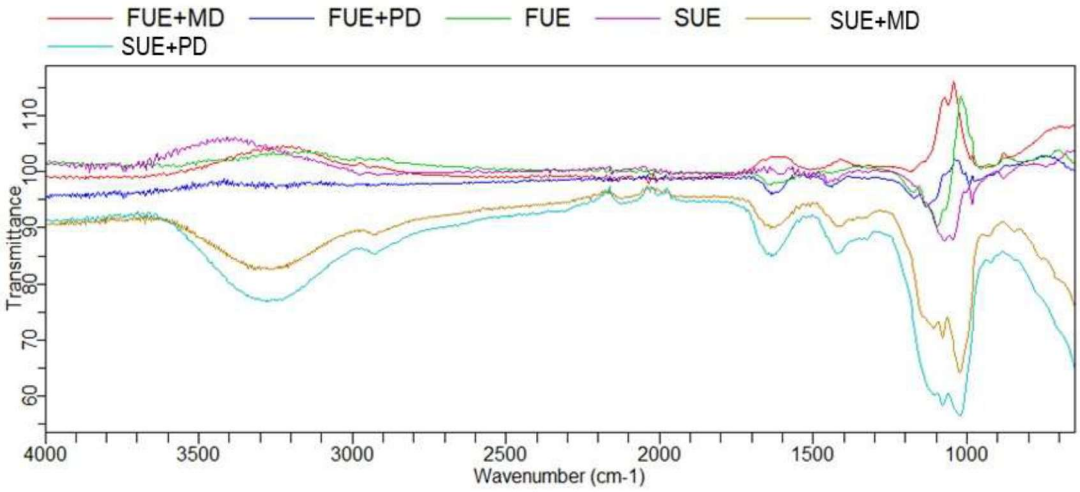


Figure 99 FTIR spectra of spray-dried and freeze-dried micro-powders

FTIR analysis provided information about microencapsulated guest molecules (*Ulva sp.* extract) in selected biopolymers. The spectra exhibited high absorption band, around 3250 cm−1, originated from O–H (hydroxyl) groups associated with preservation of phenolic compounds. The spectra contained typically strong absorption bands of carbohydrates in the region of 1000 cm−1, related to C-H and C-O aromatic ring stretching, signifying the aromatic compound existence originating from phenolics in the extract. FTIR method indicated that microencapsulation developed effective preservation by physical incorporation, not chemical transformation.

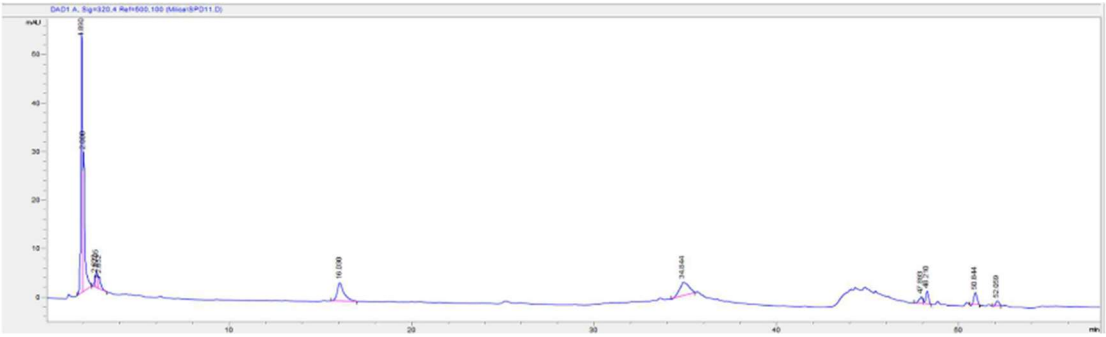


Figure 100 HPLC diagram based on the method by Pappou et al. 2021.

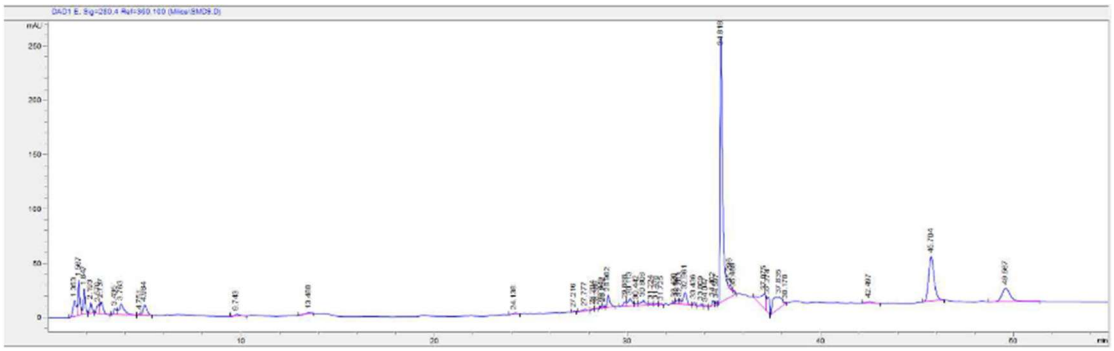


Figure 101 HPLC diagram based on the modified method

HPLC method was performed on Agilent 1260 RR HPLC instrument, as it was described by Pappou et al. 2021 (https://doi.org/10.3390/app12042117), but the peaks cannot be clearly identified as gallic acid, caffeic acid, and catechin which were used for the identification of the chromatograph's phenolics peaks (Figure 100). After that, method was modified by Rashad et al. 2021 (https://doi.org/10.1007/s13399-021-01516-8), detected peaks are presented in Figure 101. Since there was not enough time at the end of STMS to completely modified and optimize the HPLC method, experiments with individual compounds analysis will follow.

The stability of produced powders will be examined after the one month of storage in a stability chamber, under accelerated conditions (40 °C, 75% RH).

Ulva sp. as a potential innovative agent from the sea till now are not examined and sufficiently exploited. The main focus of this STSM was to establish new ways of utilizing *Ulva sp.* as potential food and pharmaceutical by-products, and to show the possibility of applying more advanced, green, and safe technologies in the industry, as well to develop the new technological lines that will preserve valuable bioactive compounds from *Ulva sp.* Through the STSM experiments it was improved stability and bioavailability of *Ulva* species present along the Adriatic Sea using two advanced microencapsulation techniques. *Ulva sp.* as sea by-products was converted into the more valuable and more applicable bioactive products with possible commercial applications as food, feed and pharmaceuticals. On the other hand, interaction of two research groups, members of CA20106, and exchange of practical and theoretical knowledge will be very useful for both sides and for their future scientific work. Through exchange visits, both institutions can strengthen existing networks and foster collaboration. Outputs from the STSM will provide a source of valuable research data of the Cost Action in the promotion and development of Ulva-based blue-biotech industries and the use of *Ulva* as a model organism. The collected data are suitable for dissemination of the results via publications in highly ranked scientific journals, international scientific conferences and symposia, invited lectures which can consequently increase the visibility of the obtained results associated with CA20106. Additionally, the producers (food and pharmaceutical industry) who are interested to these sea by-products will be informed about the STSM results and the potential possibilities of its valorisation.



Kristoffer Larsen-Ledet

Species Identification of Green Tide Ulva Species for Specialised Mass Cul-tivation in Recirculated Aquaculture Systems (RAS)

26/08/2023 to 10/09/2023

Ulva
The Future Wheat of the Sea

Start and end date: 26/08/2023 to 10/09/2023

Grantee: Kristoffer Larsen-Ledet1
Supervisors: Annette Bruhn[1] & Olivier De Clerck[2]

[1]Department of Ecoscience, Section of Marine Ecology, Aarhus University, Denmark.

[2]Phycology Research Group, Ghent University, Belgium.

DESCRIPTION OF THE WORK CARRIED OUT DURING STSM

Aquaculture is the fastest growing food production sector in the world (FAO 2020). Land-based Recirculated Aquaculture Systems (RAS) allow for production sys-tems with control of nutrient emissions (Ahmed and Turchini 2021). Integrated Multitrophic RAS (IMRAS) integrating cultivation of seaweeds with RAS produc-tion has the potential to reduce nutrient emissions. *Ulva* species (Ulvophycea, Chlorophyta) are optimal for use in IMRAS due to their fast growth and efficient nutrient uptake (Bruhn et al. 2011; Ben-Ari et al. 2014; Sebök and Hanelt 2023). Correct species identification of tar-get *Ulva* species is key to commercialization of RAS production of *Ulva* to secure replicability and stability of production. This STSM aimed to use molecular methods of species identification to identify 18 individ-ual *Ulva* specimens while training the grantee in mo-lecular methods and bioinformatics.

The sampled *Ulva* specimens were collected in Danish coastal areas spanning different salini-ties (Aarhus Harbour, Fornæs, Klintholm, Skive Fjord and Stege, Figure 1), and from a land-based *Ulva* cultivation SME (Pure Algae Denmark APS). In Ghent, DNA was successfully extracted from 15 out of the 18 *Ulva* specimens and PCR was conducted using primers for the nucleoid ITS region and tufA and rbcL plastid genes. Additional work included varying meth-ods of quality control of the PCR-product. Barcoding was carried out by an external partner (Mikrogen Diagnostics) After barcoding the initial genetic library was obtained. The *Ulva* specimens were identified based on curated genetic databases provided by Olivier de Clerck. The collected *Ulva* specimens were identified as U. fenestrata (6 specimens), U. lacinulata (3 specimens), U. linza (3 specimens) and U. compressa (3 specimens).

The results obtained during this STSM will be used for future ecological studies of *Ulva* species distribution and studies of application and bioremediation of *Ulva* cultivation.

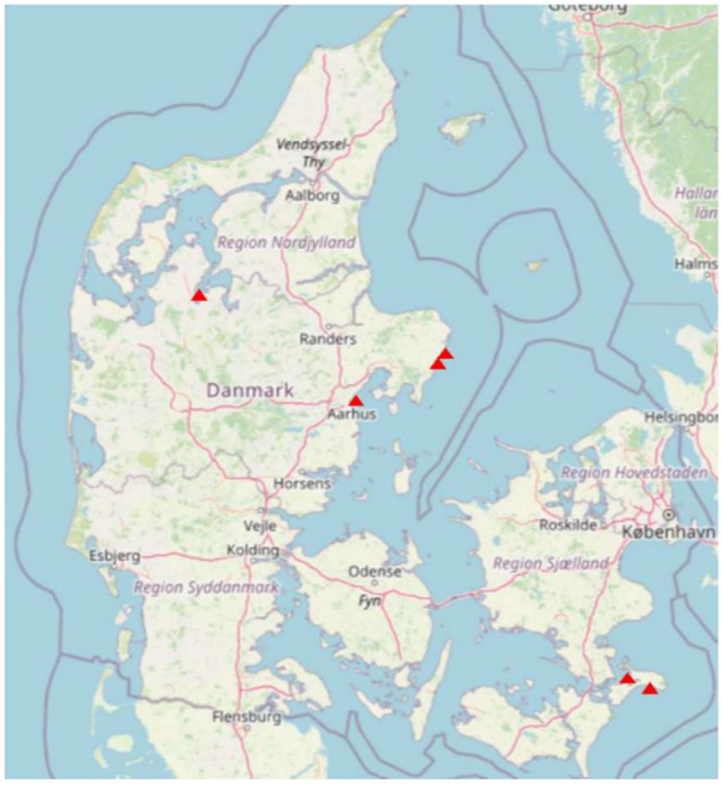


Figure 102 Sampling locations (source: OpenStreetMap contributors)

Main goals and aims:

This Short Term Scientific Mission (STSM) focussed on identifying different *Ulva* which are to be used in cultivation studies at Aarhus University in cooperation with a SeaWheat SME partner, Pure Algae Den-mark. Due to high morphological plasticity of *Ulva*, species determination based on morphology is insuf-ficient, and molecular methods to conduct species identification are highly relevant (Tran et al. 2022). This STSM aimed to use PCR based methods to identify 10 *Ulva* species while training the grantee in molecular methods and bioinformatics.

Summary of activities:

Prior to the STSM a total of six different *Ulva* strains were sampled with a total of 18 individual algae. Five of the sampled *Ulva* strains were collected in Danish coastal areas spanning different salinities (Aarhus Harbour, Fornæs, Klintholm, Skive Fjord and Stege), and one of the sampled *Ulva* strains was an already cultivated *Ulva* strain (Pure Algae Denmark). The samples were dried and transported to Ghent, Belgium for the molecular analysis.

In Ghent, DNA was successfully extracted from the different *Ulva* samples. The extracted DNA was subject to PCR protocols using the nucleoid ITS region and tufA and rbcL plastid genes as genetic mark-ers. Following steps included varying methods of quality control of the PCR-product before barcoding and quantification could take place. Barcoding took place at an extern partner (Mikrogen Diagnostics).

After barcoding the initial genetic library was obtained. This library is to be used to correctly identify the sampled *Ulva* strains based on curated genetic databases provided by Olivier de Clerck. The grantee obtained teaching in processing and analysis methods while in Ghent and was supervised by Olivier de Clerck's team.

Deviations from original plan:

It was not possible to sample the desired number of *Ulva* strains prior to the STSM. This resulted in 6 out of the desired 10 *Ulva* strains to be analysed during the STSM, however 18 individuals were still investigated.

Post PCR-analysis and bioinformatics have not been fully completed as of the report hand-in date (10/9/2023). It is estimated that these analyses will be finalised before 13/9/2023.

References

Ahmed N, Turchini GM (2021) Recirculating aquaculture systems (RAS): Environmental solution and climate change adaptation. Journal of Cleaner Production 297:126604

Ben-Ari T, Neori A, Ben-Ezra D, Shauli L, Odintsov V, Shpigel M (2014) Management of *Ulva lactuca* as a biofilter of mariculture effluents in IMTA system. Aquaculture 434:493-498.

Bruhn A, Dahl J, Nielsen HB, Nikolaisen LS, Rasmussen MB, Markager S, Olesen B, Arias C, Jensen PD (2011) Bioenergy potential of *Ulva lactuca*: growth yield, methane production and combustion. Bioresource Technology, vol 102.

FAO (2020) The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome

Sebök S, Hanelt D (2023) Cultivation of the brackish-water macroalga *Ulva lactuca* in wastewater from land-based fish and shrimp aquacultures in Germany. Aquaculture 571:739463

DESCRIPTION OF THE STSM MAIN ACHIEVEMENTS AND PLANNED FOLLOW-UP ACTIVITIES

Achievements:

This STSM allowed for the grantee to learn several molecular procedures which are vital to modern species identification of *Ulva*. The results of the conducted species identification are to be used in the grantee's future PhD research and is a foundation for future work with the sampled *Ulva* strains.

Future projects associated with the results of this STSM include cultivation trials for recirculating aqua-culture systems (RAS) and offshore systems. The cultivation trials will further increase the knowledge of *Ulva* biology (WG1) and application prospects within aquaculture for both biomass and ecosystem ser-vices (WG2 and WG5) associated with CA20106.

Furthermore, the results of this STSM can be used by other CA20106 partners to map species distribution and genetics of *Ulva* species across Europe (WG1 and WG5).

Lastly, the STSM allowed for exchange of knowledge between two CA20106 partner institutions – Aarhus University and Ghent University, as well as an associated SME - encouraging future cooperation inside the cost-action network.

Already planned future collaborations:

Future work areas of interest include genetic mapping of *Ulva* genomes. Here the grantee will participate in full genome sequencing of target *Ulva* species and will gain insight into more molecular methods used at state-of-the-art laboratories.

Expected future publications:

1. De Clerck O et al. Genetic variance and distribution of European *Ulva* species.
2. Larsen-Ledet K et al. Selection of fast growing *Ulva* strains for cultivation and nutrient uptake in RAS.
3. Larsen-Ledet K et al. Optimised cultivation of the two specific *Ulva* strains under relevant RAS conditions securing optimal yield, biomass quality and emission capture.
4. Larsen -Ledet K et al. Up-scaling ecosystem services of cultivating selected *Ulva* strains in large-scale RAS.
5. Larsen-Ledet K et al, Marine cultivation of the selected *Ulva* strains – productivity and ecosystem services.

Contribution of STSM to the SeaWheat Objectives:

1. Contribute to generating and strengthening the SeaWheat network and capacity building (Aarhus and Ghent universities, PureAlgae (SME))
2. Generate and share new knowledge in relation to SeaWheat Core Activities in WG2 and WG5, producing new knowledge on *Ulva* strains and applicability in large-scale specialized IMRAS cultivation.
3. Early Career Investigator (ECI) will produce and disseminate COST action results via publications, COST channels and other relevant science-industrial fora (including at the Nordic Seaweed conference, Denmark October 2023).
4. Significant contribution to ECI career development in the cross field between science and industry (Aarhus and Ghent universities, PureAlgae (SME)) introducing to new opportunities in academia and seaweed industry sector.
5. Sharing of information – outcome of STSM will be shared via open access publications
6. Outcomes of the STSM will form the basis for future commercial applications of *Ulva* in both cultivation technology (PureAlgae) as well as nutraceuticals (diabetes) and food via SeaWheat COST action network and the project SeaFree

References:

Tran L-AT, Vieira C, Steinhagen S, Maggs CA, Hiraoka M, Shimada S, Van Nguyen T, De Clerck O, Leliaert F (2022) An appraisal of *Ulva* (Ulvophyceae, Chlorophyta) taxonomy. J Appl Phycol.



Dr. İbrahim Seyda Uras

Secondary Metabolite Detection from Ulva Derived Fungi

30/08/2023 to 09/09/2023

Ulva
The Future Wheat of the Sea

Start and end date: 30/08/2023 to 09/09/2023

Dr. İbrahim Seyda Uras, Agri Ibrahim Cecen University, Faculty of Phar
Host institution: University of the Basque Country, San Sebastian, Spain
Supervisor: Prof. Jalel Labidi

DESCRIPTION OF THE WORK CARRIED OUT DURING THE STSM

The extracts brought for STSM were prepared for analysis. For NMR analysis, approximately 10 mg of each sample was taken and sent for analysis. 1H and 13C NMR analyzes will be performed on the samples. The samples were sent to the relevant laboratory and queued to complete the analysis. FT-IR analysis results of all samples were completed using the Perkin Elmer Spectrum Two FT-IR Spectrometer device. The grantee learned the working principle of this device and how to use it. For Gel permeation chromatography (GPC) analysis, approximately 25 mg of all samples were taken into 10 ml volumetric flasks and 5 ml of N,N-Dimethylformamide (Sigma) was added to each one. The samples were mixed on a magnetic stirrer for 24 hours. Grantee learned the use of the gel permeation chromatography device and performed his analyzes on the GPC device of the Jasco brand. Samples of all extracts were prepared for LC-MS analysis and directed to the unit where the analysis would be performed. During STSM, the fellow gained information about the host scientist's research group and established new relationships. He also learned different methods that can be used for the analysis of secondary metabolites of Ulva-derived fungi.

DESCRIPTION OF THE STSM MAIN ACHIEVEMENTS AND PLANNED FOLLOW-UP ACTIVITIES

The grantee's scientific knowledge has advanced within the scope of the studies carried out within the scope of STSM. With the FTIR analysis of the examined extracts, information was obtained about what type of bonded chromophore groups they contained and there was an opportunity to compare whether they contained similar groups with each other. Thanks to the GPC analysis, information was obtained about the molecular weights, molecular weight distributions and the proportional data of the compounds contained in the extract.

When these data are combined with other analyses, it will be possible to elucidate the structures of the secondary metabolites contained in the extracts.

Within the scope of the analyses, *Ulva* extracts with different type of solvents, extracts of *Ulva* derived fungi and extracts of other marine organisms were examined, and the results of the extracts of *Ulva* and *Ulva* derived fungi are findings that directly serve the objectives of the COST action.

It is aimed to prepare more than one publication based on the data obtained by bringing together the findings of the completed and to be completed analyzes.

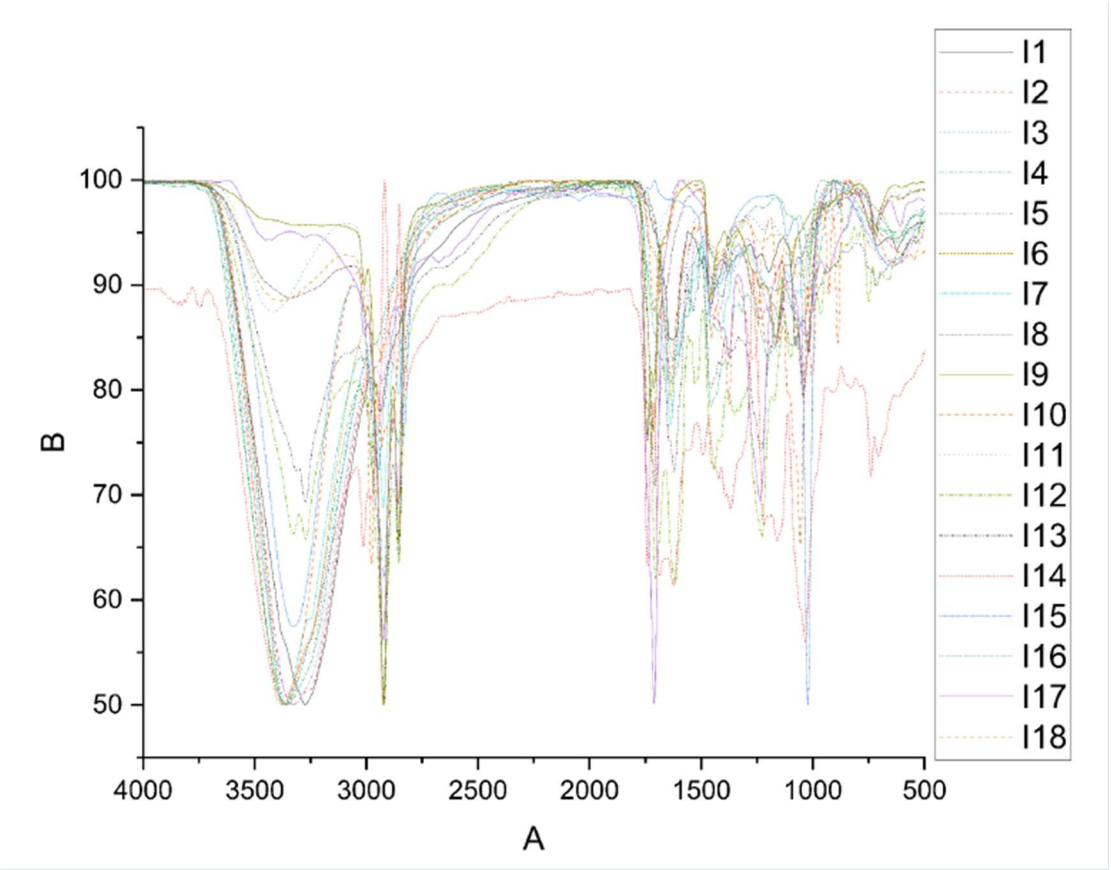
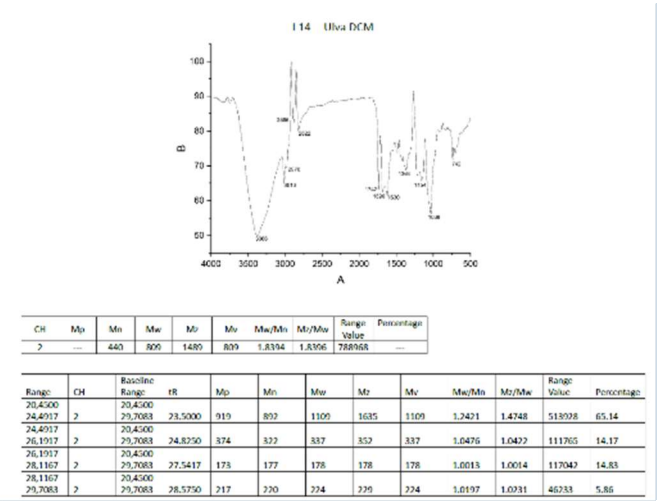
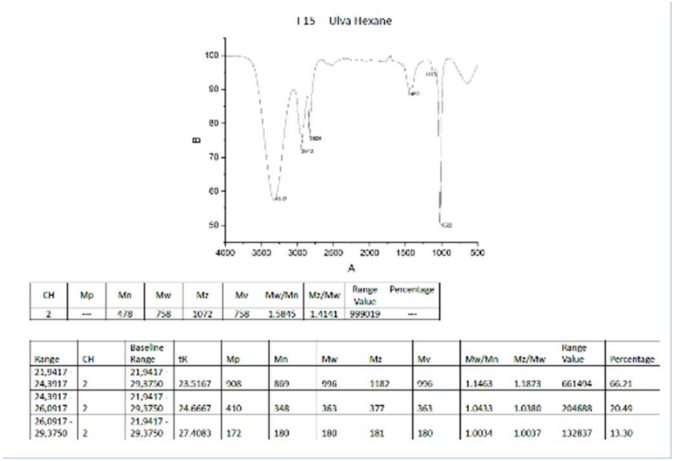


Figure 103 FT-IR analysis of 18 samples.



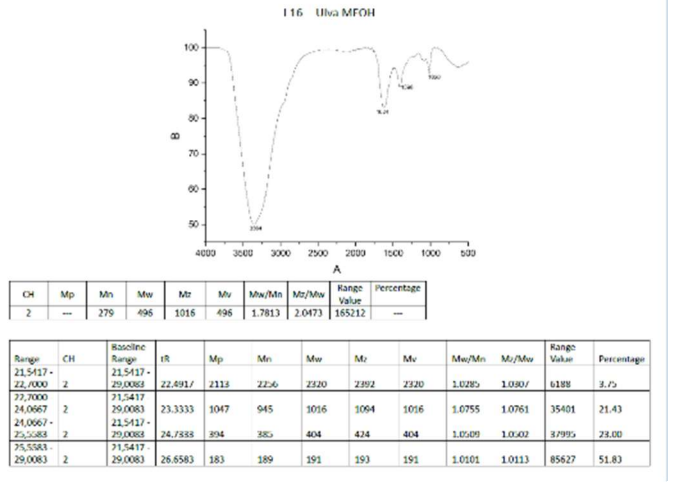
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2	---	440	809	1480	809	1.8394	1.8306	788968	---

Range	CH	Baseline Range	tR	Mp	Mn	Mw	Mz	Mv	Mw/Mn	Mz/Mw	Range Value	Percentage
20,4500 24,4917	2	20,4500 29,7083	23,5000	919	892	1109	1635	1109	1.2421	1.4748	518008	65.14
24,4917 26,1917	2	20,4500 29,7083	24,8250	374	322	337	352	337	1.0476	1.0422	111765	14.17
26,1917 28,1167	2	20,4500 29,7083	27,5417	178	177	178	178	178	1.0013	1.0014	117042	14.83
28,1167 29,7083	2	20,4500 29,7083	28,5750	217	220	224	226	224	1.0197	1.0031	46233	5.86



CH	Mp	Mn	Mw	Mz	Mv	Mw/Mn	Mz/Mw	Range Value	Percentage
2	---	478	758	1072	758	1.5845	1.4141	999019	---

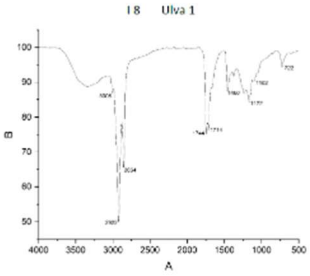
Range	CH	Baseline Range	tR	Mp	Mn	Mw	Mz	Mv	Mw/Mn	Mz/Mw	Range Value	Percentage
21,9417 - 24,3917	2	21,9417 - 29,3750	23.5167	908	869	996	1182	996	1.1463	1.1873	661494	66.21
24,3917 - 26,0917	2	21,9417 - 29,3750	24.6667	410	348	363	377	363	1.0433	1.0380	204688	20.49
26,0917 - 29,3750	2	21,9417 - 29,3750	27.4083	172	180	180	181	180	1.0034	1.0037	132837	13.30



CH	Mp	Mn	Mw	Mz	Mv	Mw/Mn	Mz/Mw	Range Value	Percentage
2	---	279	496	1016	496	1.7813	2.0473	165212	---

Range	CH	Baseline Range	tR	Mp	Mn	Mw	Mz	Mv	Mw/Mn	Mz/Mw	Range Value	Percentage
21,5417 - 22,7000	2	21,5417 - 29,0083	22.4917	2318	2256	2320	2392	2320	1.0285	1.0307	6188	3.75
22,7000 - 24,0667	2	21,5417 - 29,0083	23.3333	1047	945	1016	1094	1016	1.0755	1.0761	35401	21.43
24,0667 - 25,5583	2	21,5417 - 29,0083	24.7833	394	385	404	424	404	1.0509	1.0502	37995	23.00
25,5583 - 29,0083	2	21,5417 - 29,0083	26.6583	183	189	191	193	191	1.0101	1.0113	85627	51.83

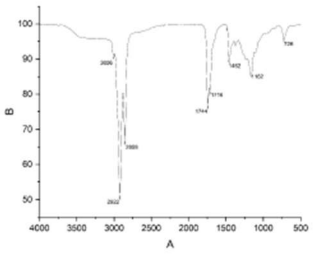
Figure 104 GPC and FT-IR analysis of Ulva extracts.



CH	Mp	Mn	Mw	Mz	Mv	Mw/Mn	Mz/Mw	Range Value	Percentage
2	---	326	617	1070	617	1.8915	1.7337	761047	

Range	CH	Baseline Range	tR	Mp	Mn	Mw	Mz	Mv	Mw/Mn	Mz/Mw	Range Value	Percentage
21,1417 - 24,4500	2	21,1417 - 29,3750	23.2417	1127	924	1072	1273	1072	1.1597	1.1878	350951	46.11
24,4500 - 26,3000	2	21,1417 - 29,3750	25.3167	288	283	301	320	301	1.0633	1.0643	162760	21.39
26,3000 - 29,3750	2	21,1417 - 29,3750	27.1917	172	180	180	182	180	1.0048	1.0061	247331	32.50

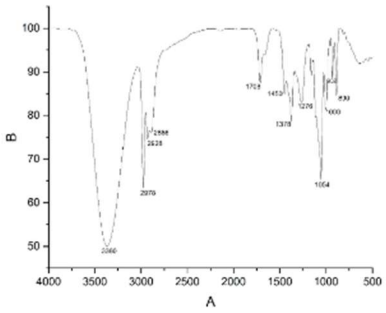
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CH	Mp	Mn	Mw	Mz	Mv	Mw/Mn	Mz/Mw	Range Value	Percentage
2	---	377	706	1087	706	1.8733	1.5397	742798	---

Range	CH	Baseline Range	tR	Mp	Mn	Mw	Mz	Mv	Mw/Mn	Mz/Mw	Range Value	Percentage
21,0083 - 24,5083	2	21,0083 - 29,4417	23.2667	1104	911	1039	1209	1039	1.1397	1.1645	440800	59.34
24,5083 - 25,9167	2	21,0083 - 29,4417	24.8250	374	324	337	350	337	1.0395	1.0365	76369	10.28
25,9167 - 29,4417	2	21,0083 - 29,4417	27.2667	172	180	181	183	181	1.0058	1.0070	225628	30.38

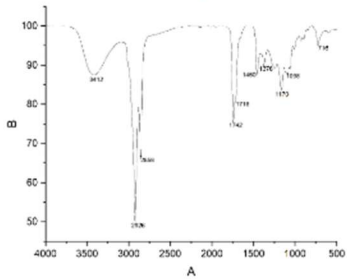
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CH	Mp	Mn	Mw	Mz	Mv	Mw/Mn	Mz/Mw	Range Value	Percentage
2	---	207	355	1097	355	1.7125	3.0867	250457	---

Range	CH	Baseline Range	tR	Mp	Mn	Mw	Mz	Mv	Mw/Mn	Mz/Mw	Range Value	Percentage
21,7750 - 25,4583	2	21,7750 - 29,5083	22.4083	2273	812	1301	1808	1301	1.6027	1.3895	38420	15.34
25,4583 - 29,5083	2	21,7750 - 29,5083	26.8000	179	183	184	185	184	1.0061	1.0072	212037	84.66

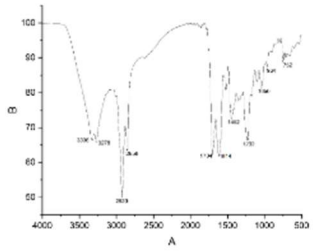
I 11 Ulva 9



CH	Mp	Mn	Mw	Mz	Mv	Mw/Mn	Mz/Mw	Range Value	Percentage
2	---	232	473	1141	473	1.8256	2.6982	416564	---

Range	CH	Baseline Range	tR	Mp	Mn	Mw	Mz	Mv	Mw/Mn	Mz/Mw	Range Value	Percentage
21,5417 - 22,7333	2	21,5417 - 29,5417	22.3917	2306	2409	2392	2483	2392	1.0360	1.0381	20297	4.87
22,7333 - 24,5250	2	21,5417 - 29,5417	23.2083	1157	892	998	1099	998	1.1186	1.1018	60853	14.61
24,5250 - 29,5417	2	21,5417 - 29,5417	26.2250	202	195	200	207	200	1.0241	1.0356	335414	80.52

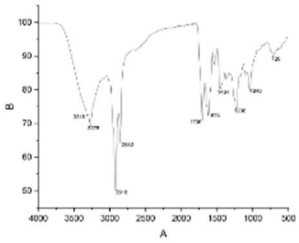
I 12 Ulva 15



CH	Mp	Mn	Mw	Mz	Mv	Mw/Mn	Mz/Mw	Range Value	Percentage
2	---	265	395	721	395	1.4894	1.8257	807884	---

Range	CH	Baseline Range	tR	Mp	Mn	Mw	Mz	Mv	Mw/Mn	Mz/Mw	Range Value	Percentage
21,3417 - 23,9333	2	21,3417 - 29,4750	23.2500	1119	1086	1138	1203	1138	1.0987	1.1271	118014	14.61
23,9333 - 26,3250	2	21,3417 - 29,4750	25.0667	327	307	333	366	333	1.0862	1.0982	397223	49.17
26,3250 - 29,4750	2	21,3417 - 29,4750	27.1417	173	179	180	181	180	1.0042	1.0053	292647	36.22

I 13 Ulva 17



CH	Mp	Mn	Mw	Mz	Mv	Mw/Mn	Mz/Mw	Range Value	Percentage
2	---	254	378	709	378	1.4896	1.8784	785982	---

Range	CH	Baseline Range	tR	Mp	Mn	Mw	Mz	Mv	Mw/Mn	Mz/Mw	Range Value	Percentage
21,0417 - 23,7667	2	21,0417 - 29,5417	23.2750	1097	1092	1187	1337	1187	1.0868	1.1264	88302	12.00
23,7667 - 24,5750	2	21,0417 - 29,5417	24.2750	544	565	577	590	577	1.0217	1.0218	67756	9.21
24,5250 - 26,2917	2	21,0417 - 29,5417	25.2833	293	280	293	306	293	1.0440	1.0449	267414	36.33
26,2917 - 29,5417	2	21,0417 - 29,5417	27.2083	172	179	180	181	180	1.0049	1.0048	312511	42.46

Figure 105 GPC and FT-IR analysis of Ulva derived fungi extracts.



Jan Jacob

From Holdfasts to Harvest: Advancing Ulva Cultivation Techniques for Scalable Biomass Production

05/03/2024 to 30/08/2024

Ulva
The Future Wheat of the Sea

Start and end date: 05/03/2024 to 30/08/2024

Grantee name: Jan Jacob

DESCRIPTION OF THE WORK CARRIED OUT DURING THE STSM

During my research stay in Gran Canaria, a series of experiments were conducted to assess the material properties, algae attachment behaviour, and growth dynamics of Ulva under varying environmental conditions. This work aimed to explore both the structural resilience of potential substrates and the optimal conditions for maximizing Ulva growth.

In the first set of experiments, the focus was on evaluating the durability and buoyancy of different mobile floaters (holdfasts) of different materials (PLA, carbon Fiber PET and wood) when exposed to natural environmental conditions over several months. The results demonstrated that PLA and C-PET structures maintained their integrity without any noticeable deterioration or change. In contrast, untreated wooden structures lost buoyancy over time. The added weight of the attached biomass significantly compromised the floating capacity, raising concerns about the feasibility of using wood in floating cultivation systems. These findings suggest that while PLA and C-PET shows promise as a long-term substrate in aquatic environments, wooden materials may require design modifications or periodic maintenance to remain viable.

The subsequent experiments examined the sporulation and attachment success of Ulva spores in controlled tank conditions. In the first trial using a static 100-liter tank, attachment rates were nearly 100% as spores predominantly adhered to the tank walls, which provided stable surfaces for growth. However, in a follow-up experiment where floaters, mobile holdfasts structures, were introduced, only about 20% of the walls showed algae attachment. Interestingly, the sporulation pattern suggested that the movement and mobility of the floaters impeded effective attachment, with algae primarily accumulating on the upper cone-shaped floater near the water surface. This implies that static surfaces offer more favourable conditions for Ulva attachment compared to floating structures. Future experiments will aim to refine these findings by immobilizing floaters during the sporulation phase.

Further trials investigated the influence of environmental factors, particularly aeration and irradiance, on *Ulva* growth rates in photobioreactors. The highest daily biomass growth rates reached up to 50%. However, growth rates declined over time due to multiple factors, including increased biomass density, resistance to aeration, and light attenuation. Observations indicated that excessive accumulation of algae at the water surface and inadequate circulation led to suboptimal growth conditions, particularly at the top of the photobioreactors where light was most limited. Continuous and efficient aeration, ensuring uniform movement of algae throughout the water column, proved critical in sustaining high growth rates.

In the final experiment, irradiance levels were manipulated to assess their impact on growth and color changes in *Ulva*. The highest irradiance treatment resulted in highest growth and biomass yield, although a noticeable color shift towards less vibrant green was observed in higher irradiance treatments (maximum irradiance levels at 690 and 430 µmol photons m²/s). This discoloration could be linked to stress-induced sporulation and potential self-dissolution a phenomenon commonly observed in larger cultivation systems. The reduced growth after initial peak rates is likely attributed to increased self-shading as algae biomass expanded within the PBRs.

In conclusion, this research highlights the importance of selecting suitable substrate materials, optimizing environmental conditions, and understanding the interactions between physical movement and algal attachment. These findings contribute valuable insights for scaling up *Ulva* cultivation in controlled environments.

DESCRIPTION OF THE STSM MAIN ACHIEVEMENTS AND PLANNED FOLLOW-UP ACTIVITIES

The primary objective of my research stay in Gran Canaria was to investigate key factors influencing the cultivation of Ulva, with a focus on substrate durability, attachment success, and optimal growth conditions.

During the STSM, I conducted several experiments that provided valuable data and insights into these areas, contributing directly to the goals of promoting sustainable aquaculture practices.

PLA and C-PET structures proved resilient and maintained their integrity over time. Additionally, the experiments highlighted the limitations of using mobile floating holdfasts for Ulva attachment, with static surfaces proving more effective. Insights into optimal growth conditions, particularly in terms of irradiance and aeration, were also gained, further enhancing our understanding of how to maximize Ulva productivity. This information is crucial for refining substrate choices and factors that are possible to influence in future cultivation designs.

The STSM not only met its planned objectives but also delivered actionable results that will be directly applied in ongoing and future research efforts. These findings align with the Action objectives by providing practical recommendations for improving seaweed cultivation systems. The knowledge acquired during this mission has broadened my understanding of seaweed cultivation, and the work with the host institution has been very insightful.

Plans for future collaborations have been agreed upon, focusing on refining cultivation protocols and exploring the larger scale applications of Ulva. The results from this STSM might contribute to upcoming publications and align closely with the overall project goals. This stay has been instrumental in building long-term partnerships, ensuring that the insights gained will be translated into impactful advancements in sustainable aquaculture.



Francisca Marques

Food-grade non-conventional extraction of Ulva sp., single or blended: analysis of pigments and antioxidant capacity

13/04/2024 to 01/05/2024

Ulva
The Future Wheat of the Sea

Start and end date: 13/04/2024 to 01/05/2024

Grantee: Francisca Marques, Lipidomics Laboratory, Department of Chemistry, University of Aveiro
Francisco J. Barba, Department of Preventive Medicine and Public Health, Food Science, Toxicology and Forensic Medicine, University of Valencia
Rosário Domingues, Lipidomics Laboratory, Department of Chemistry, University of Aveiro

Marine algae are recognized as a reservoir of valuable compounds with a wide range of high-end industrial uses. These compounds are intended to be extracted without losing their quality and properties. Emerging technologies, e.g.: pulsed electric field (PEF)-assisted extraction, supercritical fluid extraction (SFE) and accelerated solvent extraction (ASE), have been proposed as sustainable and efficient alternatives to the conventional techniques for the recovery of compounds from natural matrices.
This STSM was conducted at the University of Valencia, focusing on obtaining extracts from *Ulva sp.*, Porphyra spp., and a blend of *Ulva sp.* and Porphyra spp. (BLEND) by different food-grade approaches, including PEF, SFE, and ASE. Additionally, PEF pre-treatment was combined in a sequential step with ASE (PEF+ASE) or SFE (PEF+SFE). Samples of *Ulva sp.* harvested in 2020 and 2023, Porphyra spp. harvested in 2019 and BLEND were provided by ALGAplus Ltd. (Ílhavo, Portugal). After the extraction approaches have been performed, PEF aqueous extracts were stored at -20 ºC for further analysis, and the extracts obtained by ASE and PEF with ethanol were characterized in terms of pigment content (chlorophyll a, chlorophyll b and total carotenoids) and antioxidant capacity using the Trolox Equivalent Antioxidant Capacity (TEAC) assay. The extracts obtained by ASE and SFE from *Ulva sp.* harvested in 2023 were the richest in the analyzed pigments. *Ulva sp.* extracts (2020 and 2023) obtained by PEF showed to have the best antioxidant capacity. Further characterization analyzes are required and will be carried out in the University of Aveiro.
The STSM contributed to new scientific knowledge about the efficacy of innovative extraction technologies, including PEF, SFE, and ASE, for obtaining valuable compounds from *Ulva sp.* Additionally, we will evaluate whether blending *Ulva sp.* with other seaweed species, such as Porphyra spp., enhances the bioactive potential of the extracts. We also assessed the impact of storage duration on the stability of compounds in *Ulva sp.* samples (*Ulva* 2020 vs. *Ulva* 2023). This research offers promising directions for optimizing the extraction of high-value compounds from *Ulva sp.*, potentially benefiting various high-end industrial applications.
Furthermore, carrying out this STSM was fundamental to the advancement of my career as a young researcher, providing me with valuable hands-on experience and enhancing my expertise in cutting-edge extraction technologies.

DESCRIPTION OF THE WORK CARRIED OUT DURING THE STSM

The purpose of this STSM, conducted in the Laboratory of Nutrition and Food Science at the Faculty of Pharmacy, University of Valencia (Spain), under the supervision of Professor Francisco J. Barba, was to obtain extracts from *Ulva sp.*, Phorphyra spp., and a blend of *Ulva sp.* and Porphyra spp. (BLEND) through non-conventional technologies and characterize the extracts in terms of pigments content and antioxidant capacity. Different food-grade approaches were used to obtain the extracts, including:

a) pulsed electric field (PEF)-assisted extraction,
b) accelerated solvent extraction (ASE) with ethanol,
c) supercritical fluid extraction (SFE) with ethanol,
d) ASE using samples pretreated by PEF (PEF+ASE),
e) SFE using samples pretreated by PEF (PEF+SFE).

Two replicates were performed per each extraction approach. The seaweed samples are then designated by species followed by the harvest year (e.g. *Ulva* 2020). The PEF treatment was performed in a PEF-Cellcrack III equipment on (1 g) samples of *Ulva* 2020, Porphyra 2019, and BLEND. After treatment, the remaining solids were freeze-dried to be reextracted by ASE or SFE. The resulting aqueous extract was stored at −20°C for future characterization. ASE was performed on samples (1 g) of *Ulva* 2020, *Ulva* 2023, Porphyra 2019, BLEND, PEF-pretreated Ulva, and PEF-pretreated BLEND using an accelerated solvent extractor ASE 200 Dionex. SFE was conducted using a JASCO system. Due to the time-consuming nature of SFE, it was only performed on samples of *Ulva* 2020 (1.5 g), *Ulva* 2023 (2.3 g), Porphyra 2019 (1 g), and PEF-pretreated *Ulva* (1.5 g).

Unfortunately, the Porphyra 2019 sample was lost. Subsequently, the extracts obtained by ASE and SFE were dried using a rotary evaporator.

Chlorophyll a, chlorophyll b, and carotenoid contents were then estimated spectrophotometrically. Aliquots of the extracts were diluted in ethanol and the absorbance was measured at 470, 648, and 664 nm. The obtained data indicated that the SFE (Chla=189.7, Chlb=162.9, Car=13.7 mg/g extract) and ASE (Chla=114.7, Chlb=73.4, Car=3.2 mg/g extract) *Ulva* 2023 extracts exhibited the highest amounts of pigments, whereas the remaining extracts interestingly showed an absence of carotenoids. The antioxidant capacity was evaluated by Trolox equivalent antioxidant capacity (TEAC) assay in the following extracts: ASE *Ulva* 2020, ASE Porphyra 2019, ASE Blend, PEF+ASE Ulva, PEF+ASE BLEND, PEF *Ulva* 2020, and PEF *Ulva* 2023. The highest antioxidant capacity was observed in the *Ulva* 2020 and 2023 extracts obtained by PEF (61.1–57.8 µmol TE/g extract).

The obtained extracts will be analysed in the home institution, University of Aveiro, for the detection of healthy and bioactive lipids. Thus, with this work, we will be able to determine the most effective approach to extract compounds of interest from *Ulva sp.* samples. Additionally, it will be possible to evaluate whether the mixture of *Ulva sp.* with other seaweed species (e.g.; Porphyra spp.) enhances the bioactive potential of the extracts, as well as evaluate the effect of shelf time on the stability of the compounds present in *Ulva sp.* samples (*Ulva* 2020 vs. *Ulva* 2023).

Regarding the progress made during the STSM, the proposed goals were achieved.

DESCRIPTION OF THE STSM MAIN ACHIEVEMENTS AND PLANNED FOLLOW-UP ACTIVITIES

The STSM was successfully executed, with the main planned tasks accomplished and valuable outcomes achieved. The STSM allowed me to acquire new training and knowledge in innovative extraction technologies, including pulsed electric fields (PEF), supercritical fluid extraction (SFE), and accelerated solvent extraction (ASE), distinct from those used at the Lipidomics Laboratory, University of Aveiro. Additionally, I developed expertise in characterizing extracts, enhancing my skill set.

The STSM involved the successful production of extracts from seaweeds, including *Ulva sp.*, using PEF, ASE and SFE, and characterization of the extracts focusing on pigment content and antioxidant capacity. Subsequent analysis of the composition of the extracts and evaluation of other bioactivities will be conducted at the Lipidomics Laboratory. If necessary, other extractions will be carried out by the host institution. Consolidating all these results will enable the preparation of a research article manuscript, showcasing the comprehensive findings and insights gained from the STSM's research activities.

Given the potential advantages of the innovative approaches (PEF, SFE and ASE), we intend to explore them at the University of Aveiro and incorporate them into our work, which will expand and raise our research capabilities.

The implementation of the STSM made a notable contribution to the primary objective of the COST Action CA20106, particularly aligning with the goals of Working Groups (WG) "*Ulva* as food and feed" (WG3) and "Bioactive chemicals and Ulva-associated microorganisms" (WG4). By providing essential work in these areas, the STSM directly supported the overarching aims of the CA20106.

Moreover, the STSM has been instrumental in advancing my career as a young researcher. The experience gained, along with the knowledge and skills acquired, will undoubtedly contribute to my professional development and future endeavors. Looking ahead, the robust network established with the experts from Professor Francisco Barba's team lays a strong foundation for future collaborative initiatives. This collaboration has the potential to foster continued advancements in research and facilitate the exchange of ideas and expertise across institutions, promising continued progress in the field of *Ulva* research and beyond.



Zorana Mutavski

Optimizing high-value compound extraction from Ulva sp. macroalgae: Enhancing efficiency through innovative pretreatment strategies on raw material

17/06/2024 to 05/07/2024

Ulva
The Future Wheat of the Sea

Start and end date: 17/06/2024 to 05/07/2024

Grantee name: Zorana Mutavski, PhD student

STSM work plan titled “Optimizing high-value compound extraction from *Ulva sp.* macroalgae: Enhancing efficiency through innovative pretreatment strategies on raw material” has been accepted as part of the COST action titled CA20106 - Tomorrow's 'Wheat of The Sea: Ulva, A Model for an Innovative Mariculture’. In July 2023, *Ulva lactuca* Linnaeus was collected from Zadar Kolovare (Adriatic Sea) and processed by researchers from the Faculty of Food Technology in Osijek. The dried samples were ground and prepared for extraction, beginning with supercritical CO2 extraction to remove non-polar components and enhance solvent penetration. The optimized ultrasound-assisted extraction (UAE) followed, aiming for a green, economically justified process. Various parameters such as sonication amplitude, time, and pulse were tested. The extracts were analyzed for yield, total phenols, carotenoids, and chlorophylls using gravimetric and spectrophotometric methods.
The optimal extract (UAE 3) was obtained under conditions of 100% sonication amplitude and pulse, following CO2 pre-treatment. This extract, rich in carotenoids and chlorophylls, is promising for pharmaceutical, cosmetic, and food industries due to its health benefits. The research led to the development of an efficient extraction model that aligns with sustainable marine resource use. Collaborative efforts between the Faculty of Food Technology in Osijek and the Institute for Medicinal Plants Research "Dr. Josif Pančić" under the STSM - CA20106 initiatives fostered knowledge exchange and laid a foundation for future research. The outcomes contribute to marine biotechnology innovations and the development of health-promoting products.

DESCRIPTION OF THE WORK CARRIED OUT DURING THE STSM

The sampling of Ulva lactuca Linnaeus in Zadar Kolovare (Adriatic Sea) was carried out in July 2023, followed by identification, and drying. Researchers from the Faculty of Food Technology in Osijek provided the raw material. At the beginning of the STSM, the dried samples were ground into powder in a laboratory mill and prepared for the extraction process (Figure 106).



Figure 106 Dried and grounded Ulva lactuca

The first step was pre-treatment, which included supercritical CO2 extraction (Figure 107), aimed at separating non-polar components from the raw material and destroying cells to facilitate the penetration of the solvent into the further upgrading process. The process parameters were pressure 300 bar, temperature 40℃ and extraction time 1h. The raw material after the separation of CO_2 from the process is kept for further extraction.

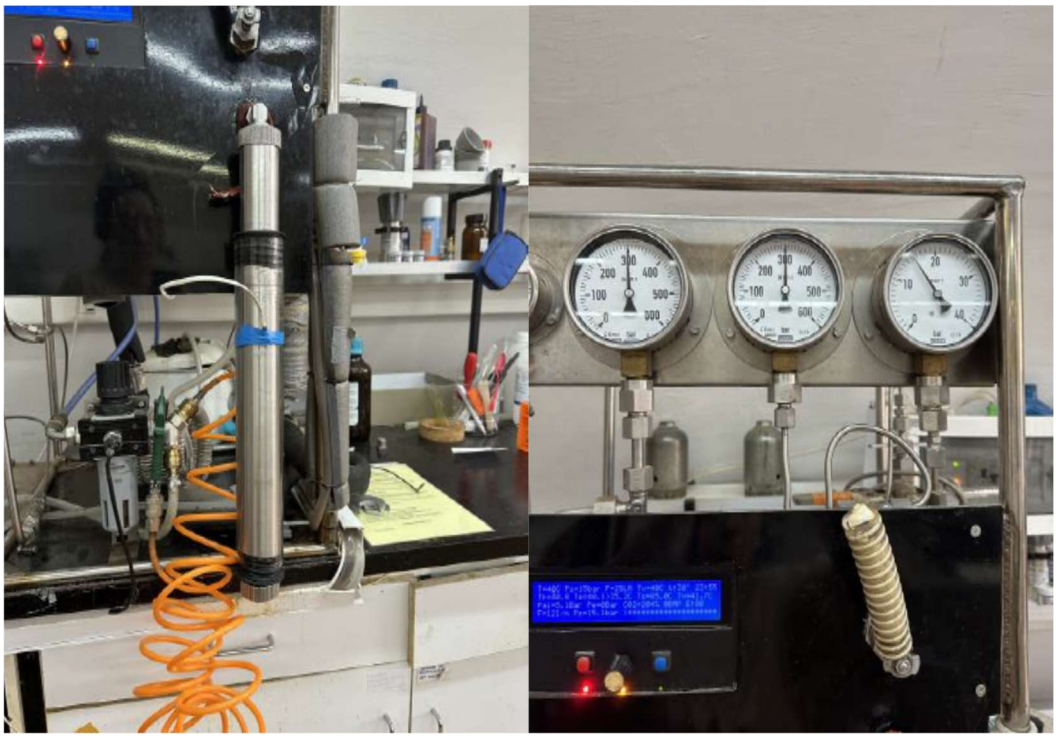


Figure 107 Equipment for supercritical CO_2 extraction

The second step of the STSM plan is to optimize the ultrasound-assisted extraction of treated and untreated *Ulva lactuca* (Table 1).

Table 9 Optimization of ultrasound-assisted extraction – process parameters

Samples	Amplitude (%)	Puls (%)	Time (s)
UAE 1	20	20	330
UAE 2	60	60	330
UAE 3	100	100	330
UAE 4	100	20	330
UAE 5	20	100	330
UAE 1"	20	20	330
UAE 2"	60	60	330
UAE 3"	100	100	330
UAE 4"	100	20	330
UAE 5"	20	100	330

Samples marked with " were obtained by ultrasound-assisted extractions of defatted *Ulva lactuca* (after pretreatment with supercritical CO_2 extraction)

The goal of the extraction process was to be economically justified and environmentally friendly, based on the principles of "green extraction". The effects of several extraction parameters: amplitude of sonication from 20 to 100%, time of 330s, and pulse from 20 to 100% on the extraction efficiency of ultrasound-assisted extraction were examined (Figure 108).

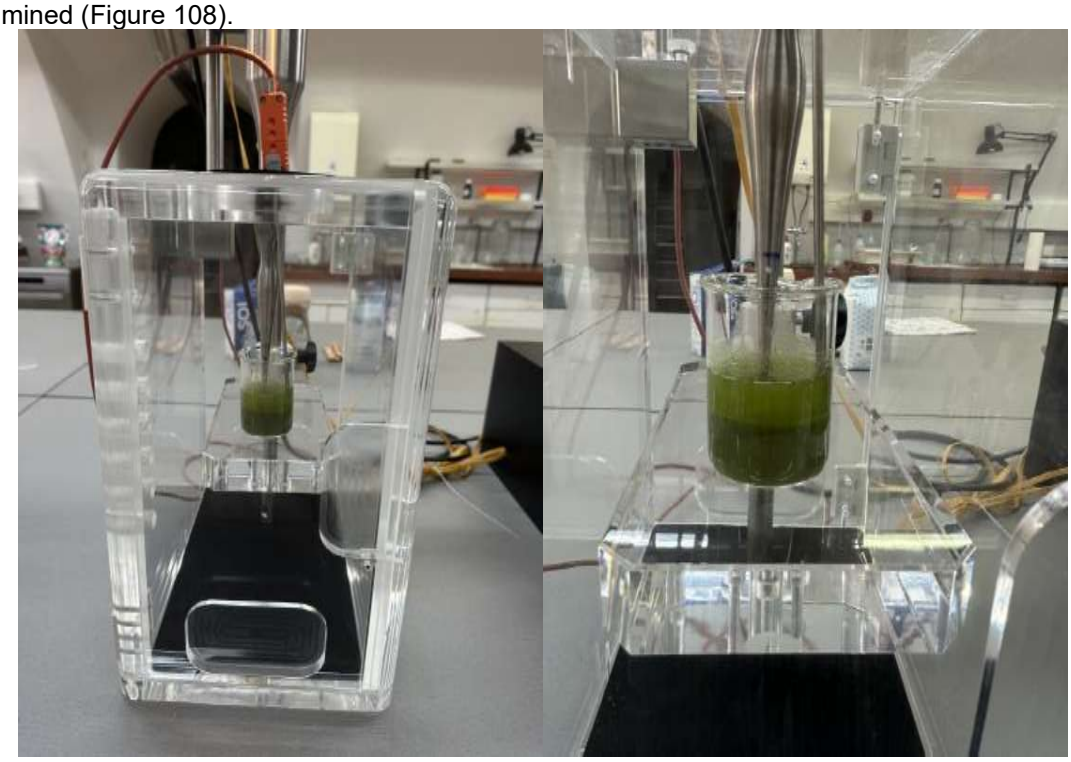


Figure 108 Optimisation of ultrasound-assisted extraction

After filtration (Figure 109), the obtained samples were analysed gravimetrically, and spectrophotometrically; the extraction yield, the content of total phenols, total carotenoids, and chlorophyll a and b of all samples were determined.

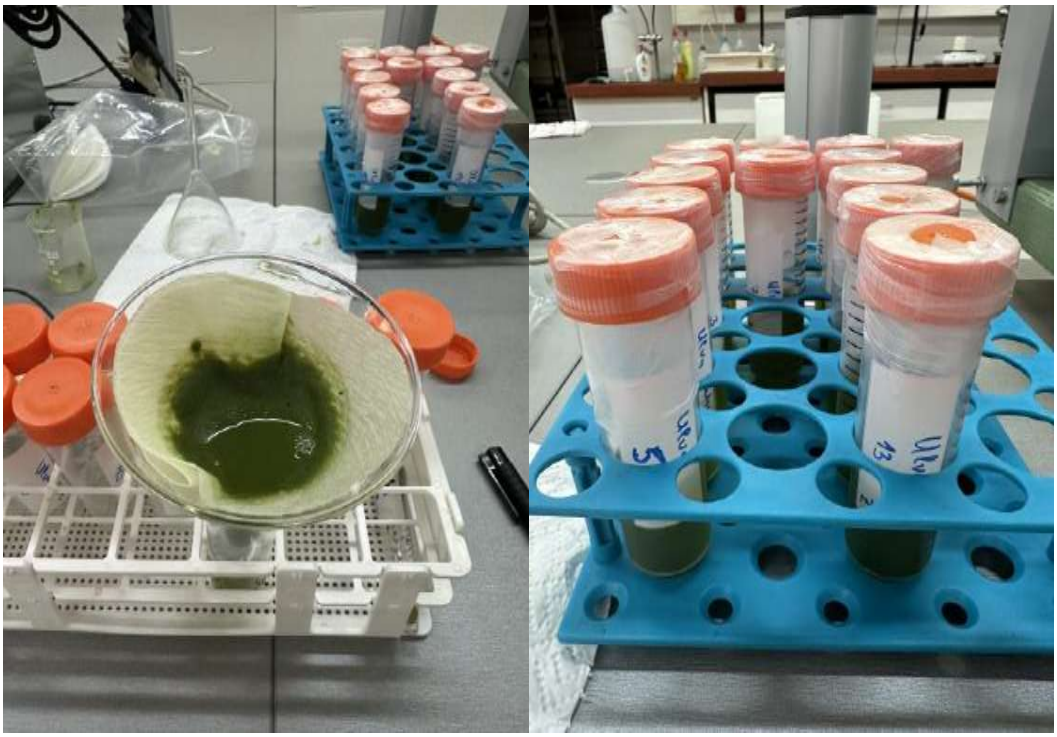


Figure 109 Filtration of Ulva lactuca extracts

The obtained UAE extracts of the macroalga Ulva lactuca were subjected to evaporation in a vacuum concentrator SpeedVac SPD1030 (ThermoFisher Scientific, Massachusetts, USA) and diluted in methanol to a concentration of 10 mg/mL. The samples were then filtered through a PTFE filter with pores of 0.20µm directly into cuvettes for spectrophotometric measurement on a Boeco S-220 UV/VIS spectrophotometer (Hamburg, Germany).
Absorbances were measured at wavelengths of 665.2nm (maximum absorbance for chlorophyll a), 652.4nm (maximum absorbance for chlorophyll b), and 470nm (maximum absorbance for carotenoids). The total concentrations of chlorophyll a, chlorophyll b, and carotenoids were calculated according to Lichtenthaler and Buschmann (2001). The amounts of pigments are expressed as mg/g of dry extract.

References

Lichtenthaler, H. K., & Buschmann, C. (2001). Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. Current protocols in food analytical chemistry, 1(1), F4-3.

DESCRIPTION OF THE STSM MAIN ACHIEVEMENTS AND PLANNED FOLLOW-UP ACTIVITIES

The optimization of the extraction process was carried out as a preliminary investigation based on the results of the extraction yield (EY), the total phenolic content (TPC), the content of carotenoids, and the content of chlorophyll a and b. Table 10 shows the contents of total phenols, carotenoids, and chlorophyll a and b. Based on the results, the sample UAE 3" was selected as optimal (TPC - 2.3174 mg GAE/g DE; carotenoids – 0.5150 mg/g DE; chlorophyll A – 7.2889 mg/g DE; chlorophyll B – 13.8729 mg/g DE). The conditions under which the extraction was carried out were: Sonication amplitude of 100% and pulse value of 100%, with pretreatment in the form of supercritical CO2 extraction. The constant parameters also included an extraction time of 330 s, 50% EtOH as a solvent, and a ratio of raw material to solvent of 1:10.

Table 10 Chemical properties of obtained ultrasound-assissted extracts of Ulva lactuca

Extracts	EY (%)	TPC (mg GAE/g E)	Carotenoids (mg/g E)	Chlorophyll A (mg/g E)	Chlorophyll B (mg/g E)
UAE 1	14.50	0.8730	0.1940	2.4140	4.6026
UAE 2	11.29	1.0154	0.2166	3.7195	7.0155
UAE 3	12.22	1.5718	0.3493	6.4902	10.3501
UAE 4	13.44	0.3466	0.0770	2.0370	4.3897
UAE 5	11.39	0.9522	0.2116	3.2347	6.3249
UAE 1"	10.95	2.1335	0.4741	3.7045	6.1687
UAE 2"	15.06	1.4284	0.3174	2.9899	4.9813
UAE 3"	**11.86**	**2.3174**	**0.5150**	**7.2889**	**13.8729**
UAE 4"	14.57	1.8955	0.4212	2.4598	4.1685
UAE 5"	15.39	2.0618	0.4582	2.7341	4.5380

Samples marked with " were obtained by ultrasound-assisted extractions of defatted *Ulva lactuca* (material after pretreatment with supercritical CO_2 extraction)

Table 10 shows that the pretreatment affected the increase in total phenolics and pigments content, while this trend was not observed in the extraction yield. Furthermore, if we look at the samples UAE 4 and UAE 5, we

can say that the 100 % pulse had a greater impact on the extractions than the 100 % amplitudes, considering that in the sample UAE 5, the measured pigment concentrations were higher.

The main results of this STSM:

- Development of a high-quality extract: An optimized liquid extract of Ulva lactuca rich in carotenoids, and chlorophylls was successfully produced. This extract is promising for the pharmaceutical, cosmetic, and food industries due to its potential health benefits.
- Improved extraction model: A pre-treatment model has been developed to improve the extraction processes for *Ulva lactuca* macroalgae. This model promotes more efficient and cost-effective industrial applications and is in line with the principles of sustainable use of marine resources.
- Knowledge exchange and collaboration: There was a productive exchange of knowledge between researchers from different institutions. Sharing expertise and techniques strengthened the research and fostered an environment for future collaborations, propelling complex research projects forward.

The collaboration between the Faculty of Food Technology in Osijek and the Institute for Medicinal Plants Research “Dr. Josif Pančić” under the STSM - CA20106 initiatives establishes a robust foundation for international cooperation in green technologies, biowaste valorization, and particularly macroalgae research. The data generated from this research will contribute to a collaborative scientific publication within the CA20106 framework. Future collaborations on this STSM topic and new areas are highly anticipated.

The STSM has significantly advanced research on *Ulva lactuca* extracts and strengthened institutional cooperation. Planned follow-up activities aim to ensure ongoing progress, broader dissemination, and practical application of the research outcomes. This initiative promises innovations in marine biotechnology and the development of new products with health-promoting properties.



Ignacio Moreu Badia

The Influence of Water Source and Irradiance on Ulva Ecophysiology

01/07/2024-31/07/2024

Ulva
The Future Wheat of the Sea

Start and end date: 01/07/2024-31/07/2024

Grantee name: Ignacio Moreu Badia

DESCRIPTION OF THE WORK CARRIED OUT DURING THE STSM

The aim of this study was to assess the physiological response of Ulva to different water sources and irradiances in land-based facilities. The water used to cultivate Ulva was fish effluent (FSW) from a local fish farm (Aqualvor), and artificial seawater (ASW) made with salt extracted from a "salina" from Cadiz. Both types of water were supplemented with inorganic fertilizers, achieving a final concentration of 2mM of NH4 and NO3, and 0,2 mM of PO4, simulating a recirculated fish farm water with high nutrient concentration. Additionally, two levels of irradiance were stablished for each water treatment, full (100%) and attenuated (20%) sunlight. Sunlight was reduced with a grey mesh.

Ulva was incubated for 5 days in closed mesocosms (4 L) placed inside tanks with constant water flow to maintain the temperature. Everyday water samples were taken to analyse pH and nutrient concentration, but only day 1 and 3 the water nutrient concentrations (NH4, NO3 and PO4) were assessed using spectrophotometer quantification protocols (Solórzano 1969, Garcia Robledo et al., 2014, Grasshoff et al.,1999).

Photosynthetic performance was monitored everyday using a Pulse-Amplitude-Modulation (PAM) Fluorometer. Rapid light curves were conducted to determine the maximum quantum yield (Fv/Fm), Electron Transport Rate (ETR), photosynthetic efficiency (αETR), Saturation and Optimal irradiance (Ek and Eopt).

Photosynthetic rate (mg O2/L/min/g FW) was also assessed day 5 of incubation. Using 4 oxygen proves and PiroScience software, each replicate was incubated in dark and light condition for 20 minutes in a sealed incubation chamber. Gross photosynthesis and Respiration were determined and used to calculate the net photosynthesis rate.

After incubation, samples of water and biomass from each replicate were collected for subsequent analyses. In the case of samples collected for microbiome analyses, 10 mL of water and approximately 50 mg of Ulva biomass were collected in Eppendorf tubes. Similarly, approximately 8 g of Ulva biomass was stored at -80°C for proteins, pigments, antioxidants, lipids and carbohydrates determination using advanced extraction methods and quantification with HPLC or spectrophotometer. All water samples will be reanalysed manually or with a Technicon Auto-Analyzer device. Microbiome analysis of water and Ulva will be conducted by the host institution.

Deviations from original plan:

The initial objective was to compare the growth and physiological response of Ulva cultivated using organic fertilizer of an enriched fish effluent, versus inorganic fertilizers supplemented in artificial or natural seawater. However, the original plan was not feasible because none of the 3 different effluents tested contained enough nutrients to maintain macroalgae cultures (≤0.02 mM NH4, 0 mM NOx, 0.001 PO4).

Despite that, the effect of the water source in Ulva physiological response was yet tested in the new experimental design. Additionally, 2 different levels of irradiance were also tested to see the effect of water under different light conditions. Oxygen evolution in dark and light incubation were also developed, nonetheless were not originally planned.

References:

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DESCRIPTION OF THE STSM MAIN ACHIEVEMENTS AND PLANNED FOLLOW-UP ACTIVITIES

Regarding action CA20106, these trials will expand our understanding of Ulva biology and explore its applications in aquaculture, focusing on biomass production, nutracosmeceutical uses and ecosystem services (WG1, WG2, WG3, WG4 and WG5).

The expected outcome of this study is a better understanding of Ulva physiology cultivated in land-based facilities. Preliminary results of this study (Figure 110) indicate a better performance of Ulva when cultivated using fish effluents. Further microbiological and biochemical analyses will help to identify the underlying mechanisms explaining these differences on Ulva ecophysiology functioning in multitrophic systems (WG1), as well as its nutracosmeceutical potential (WG3 and WG4). Furthermore, the knowledge provided by this study will help improving macroalgae cultures in the home institution (WG2), where natural seawater is unavailable.

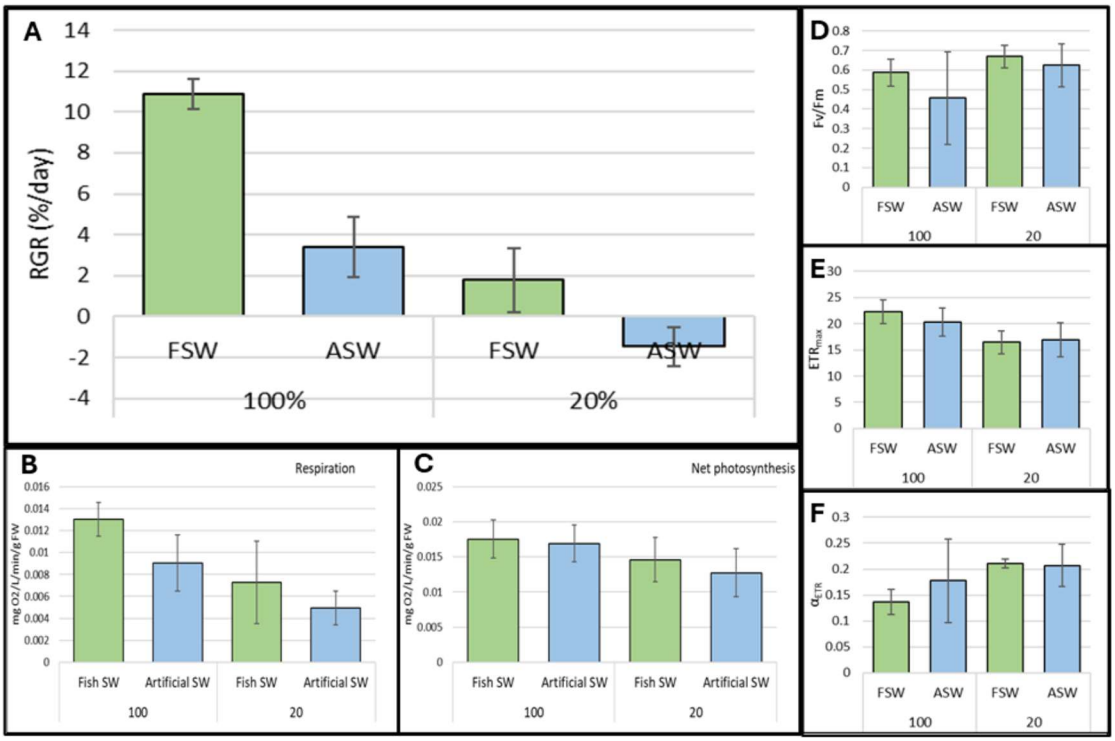


Figure 110 Preliminary results of growth (A), respiration rates (B), net photosynthesis rate (C) and photosynthetic performance (Maximum quantum yield, Fv/Fm (D), maximum electronic transport rate, ETRm (E) and photosynthetic efficiency (F). Incubation was under 100% or 20% sunlight, in Fish effluent (FSW) or artificial seawater (ASW).

This STSM facilitated the collaboration between University of Malaga and Centre of Marine Sciences (CCMAR; University of Algarve), two CA20106 partner institutions, encouraging ongoing cooperation within the cost-action network.

Additionally, the STSM enabled the grantee to acquire essential methodologies for monitoring macroalgae physiological response in land-based aquaculture systems, such as water nutrient quantification or photosynthetic performance (PAM and O2 evolution). The findings of the experiment will support the grantee Phd research on macroalgae cultures using multitrophic aquaculture systems.

Some initiatives associated with the outcomes of this STSM include designing a pilot multitrophic RAS system, integrating Ulva and fish (Chelon labrosus) in the University of Málaga land-based aquaculture facilities (IBYDA, Biotechnology and Blue Development Institute).

Future collaborations

The grantee has stablished possible future collaboration with the host institution and local fish farms to develop large scale multitrophic aquaculture systems, integrating seabream, sea cucumber and macroalgae in semi-natural fishponds in south of Iberian Peninsula.

Expected future publications:

Moreu et al. Eco-physiological response of Ulva cultivated with fish effluent or artificial seawater: Effect on Growth, Photosynthesis, Bioremediation, Microbiome and Nutritional properties.

Contribution of STSM to the SeaWheat Objectives:

- Support the creation and enhancement of the SeaWheat network and capacity building efforts, involving the CCMAR, University of Málaga, as well as companies (Aqualvor fish farm).
- Develop and disseminate new knowledge through SeaWheat Core Activities in WG1, WG2, WG3, WG4 and WG5, focusing on research related to Ulva ecophysiology and their potential in bioremediation and nutracosmeceutical purposes.
- The Early Career Investigator (ECI) will disseminate the results of the COST action through publications, COST channels, and other relevant scientific and industrial forums.
- Substantial impact to ECI career development by promoting collaborations involving local fish farms (Aqualvor) the CCMAR and University of Málaga, in addition to building knowledge which support the grantee Phd and enhance seaweed aquaculture sector.
- STSM outcome will provide a substantial support for Ulva land-based aquaculture in Málaga (IBYDA) and will reveal possible commercial applications with nutracosmeceuticals purposes.



Ana Sofia Pereira Moreira

Sequential extraction of lipids and oligosaccharides from Ulva spp. using novel and green methodologies - a biorefinery approach

06/07/2024-14/07/2024

Ulva
The Future Wheat of the Sea

Start and end date: 06/07/2024-14/07/2024

Grantee name: Ana Sofia Pereira Moreira

DESCRIPTION OF THE WORK CARRIED OUT DURING THE STSM

The purpose of this STSM, conducted in the Laboratory of Nutrition and Food Science at the Faculty of Pharmacy, University of Valencia (Spain), under the supervision of Professor Francisco J. Barba, was to develop a multistep process biorefinery pathway to obtain bioactive extracts enriched in oligosaccharides and lipids from green seaweed *Ulva spp*. using novel and green methodologies. According to the experience of Professor Francisco Barba and his team and preliminary results using *Ulva spp.,* the methods selected were supercritical fluid extraction (SFE) and accelerated solvent extraction (ASE).

A multistep process biorefinery pathway using food-grade solvents (ethanol, water and CO_2) was defined to recover non-polar components as lipids and polar components as polysaccharides (ulvans) and their oligosaccharide derivatives, combining:

a) supercritical fluid extraction (SFE) with CO2 and ethanol (EtOH)
b) accelerated solvent extraction (ASE) with ethanol (90°C) using samples pretreated by SEF
c) ASE with water (90°C) using samples pretreated by SFE and ASE alone

For comparison of the results, the same amount (4 g) of biomass was extracted only using SFE with ethanol. Also, the same amount of biomass (4 g) was used for ASE with ethanol (90°C), followed by twice sequential extractions using ASE with water (90°C). All the extractions performed are summarized in Figure 111 Figure 111.

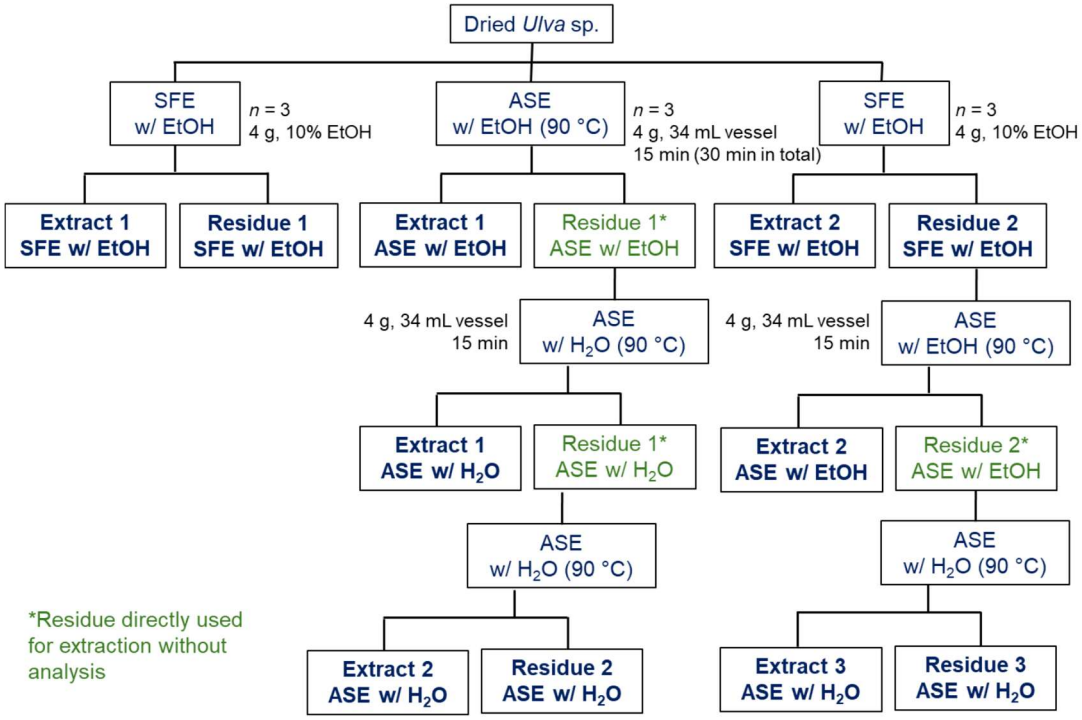


Figure 111 Representation of the extraction approaches based on the use of innovative and green technologies, supercritical fluid extraction (SFE) and accelerated solvent extraction (ASE), to recover lipids and polysaccharides (ulvans) and their oligosaccharide derivatives from Ulva spp. The respective extracts and solid residues were named as indicated in this scheme.

Three replicates were performed per each extraction approach using 4 g of dried Ulva spp. biomass. The sample (in powder) was provided by ALGAplus, Ílhavo, Portugal (batch U1.00323MF0101), a Portuguese company that cultivates organic certified seaweed under an integrated multi-trophic aquaculture (IMTA) system. After treatment, the ethanolic extracts obtained by SFE and ASE were dried using a rotary evaporator. The aqueous extracts obtained by ASE and the remaining solids were stored at −20°C until freezedrying. The solid residue obtained by SFE with ethanol (residue 1_SEF w/ ethanol) was left to air dry in the fume hood overnight. ASE experiments were performed using an accelerated solvent extractor ASE 150 Dionex with a vessel of 34 mL. SFE was conducted using a JASCO system using a vessel of 5 mL. The obtained extracts and final solid

residues will be analysed in the home institution, University of Aveiro, for the determination of the composition of lipids, polysaccharides (ulvans) and oligosaccharides. Thus, with this work, we will be able to develop a multistep biorefinery approach to obtain different compounds of interest from Ulva spp. samples with recognized bioactive properties and potential multiple applications. Regarding the progress made during the STSM, the proposed goals were achieved.

DESCRIPTION OF THE STSM MAIN ACHIEVEMENTS AND PLANNED FOLLOW-UP ACTIVITIES

The STSM was successfully completed, with the main planned tasks accomplished and valuable outcomes achieved. The STSM allowed me to acquire new training and knowledge in innovative and green extraction technologies, including supercritical fluid extraction (SFE) and accelerated solvent extraction (ASE). These techniques are not available at the Lipidomics Laboratory, University of Aveiro (my home institution). The STSM involved the development of a multistep process biorefinery pathway with use of food-grade solvents (ethanol and water) with the successful production of extracts obtained from Ulva spp. Subsequent analysis of the composition of the extracts, as well as the initial biomass and final solid residues, will be conducted at the Lipidomics Laboratory, University of Aveiro. If necessary, other extractions will be carried out by the host institution. By consolidating all these results, we will be able to prepare a research article manuscript that showcases the comprehensive findings and insights gained from the STSM's research activities, particularly in the framework of COST Action CA20106 (SeaWheat). Considering the potential advantages of the innovative and green approaches (SFE and ASE), we plan to explore these methods at the University of Aveiro and integrate them into our lab work, thereby expanding and enhancing our research capabilities. The implementation of this STSM made a notable contribution to the main objective of the COST Action CA20106, focused on to make a step-change towards a green economy based on Ulva mass production and utilization within the European community and beyond. In specific, it will contribute to the deliverables (D) of CA20106 working groups, WG3 (Ulva as food and feed) and WG4 (Bioactive products). By providing essential work in these topics, this STSM directly supported the overarching aims of the CA20106. Moreover, the STSM has been pivotal in advancing my career as a young researcher. The experience gained, along with the knowledge and skills acquired, will significantly contribute to my professional development. Also, the network established with Professor Francisco Barba and his team provides me new opportunities for future collaborative initiatives/projects in an international context. This collaboration holds the potential to foster continued advancements in research, facilitate the exchange of ideas and expertise across institutions, and promote continued progress in the field of Ulva research and beyond.



Fusun Agkul

Using of Ulva and other annual seaweeds as bioindicators (biomarkers) of Black Sea waters along the Turkey and Greek coasts

08/07/2024 to 19/07/2024

Ulva
The Future Wheat of the Sea

Start and end date: 08/07/2024 to 19/07/2024

Grantee name: Fusun Agkul

DESCRIPTION OF THE WORK CARRIED OUT DURING THE STSM

STSM has 4 Work Packages and until this time WP1 and WP2 were carried out.

WP1. Collecting indicative Ulva and other annual seaweed samples from Dardanelles Straits (Turkey) and Eastern Macedonian coasts (Greece).

After receiving COST support, Ulva samples were collected simultaneously from the dates and stations determined together with our Greek partner, in accordance with the purpose of the study. A sufficient amount of *Ulva* samples *(U. lactuca, U. rigida, U. linza, U. compressa, U. intestinalis*) was collected (6-13 April 2024) for the analysis, cleaned, dried and delivered to the Greek partner. Likewise, relevant samples were received from the Greek partner for analysis to be carried out in Turkey.



Figure 112 Field study in Turkey

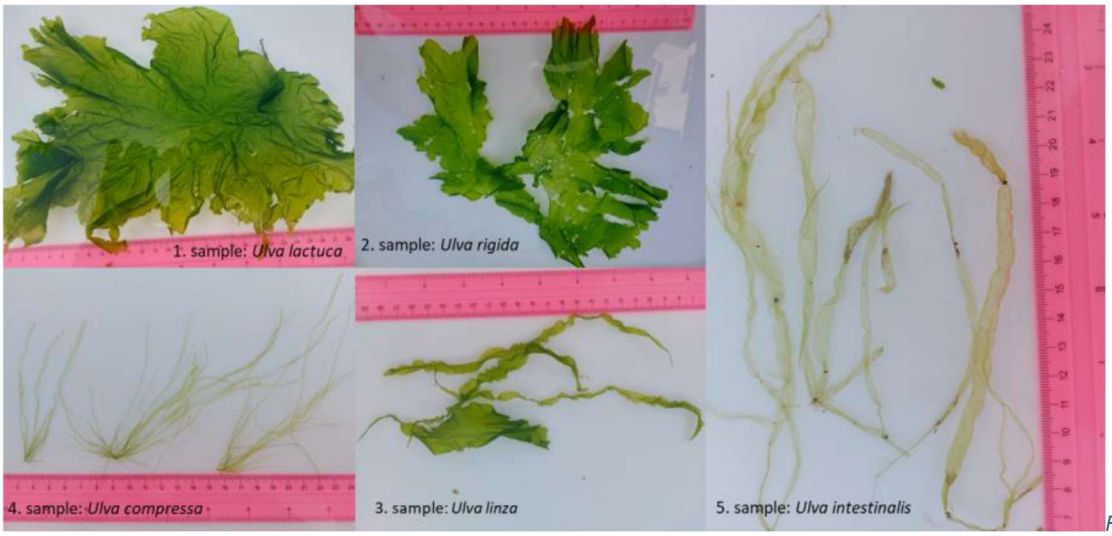


Figure 113 Collected Ulva samples from Turkey side

WP2. Measuring key physical [temperature, salinity, pH, O2(%)] and eutrophication (Dissolved nutrients) parameters in sampling areas, in Turkey and Greece.

Relevant measurements were made during the field study and the results are given in Table 11.

Table 11 Physical parameters in sampling sites

Country	**Turkey**	**Turkey**	**Turkey**	**Greece**	**Greece**	**Greece**
Stations	Site 1 (Hamzakoy)	Site 2 (Eceabat)	Site 3 (Kepez)	Site 1 (Folia)	Site 2 (Vrasidas)	Site 3 (Americanos)
Map Coordinates	40.411771, 26.679695	40.18624, 26.35932	40°06'14.3"N 26°23'46.7"E	40.752811° , 24.166531°	40.826808°, 24.335289°	40.905119°, 24.365356°
Date	April 6, 2024	April 10, 2024	April 11, 2024	April 25, 2024	April 30, 2024	May 14, 2024
Temperature (°C)	15	14	14	17,6	17,9	18,7
pH	8,22	8,22	7,98	8,88	8,981	9,028
Conductivity (mS/cm)	38,92	39,55	40,45			
Salinity (‰)	24	23	23	35,9	35	35,2
Dissolved O_2 (mg/L)	8,23	8,11	9,05			
Dissolved O_2 (%)	99,6	98,2	109,5	99,4	96,1	102,2
K^+	260 ppm	260 ppm	240 ppm			
Na	8100 ppm	7500 ppm	7800 ppm			

We discussed the station photos we had taken during the field work when exchanging Ulva samples with our Greek partner Dr. Sotiris Orfanidis, and the detailed photos of the Ulva samples. We also evaluated the physical parameters we had and made conclusions.

I visited to Fisheries Research Institute (INALE) and got information about the equipment and research laboratories. We talked about new studies that will further enrich and develop our cooperation and what we can do in the future.

We shared a presentation on the biotechnological applications of Ulva and other macroalgae and what we have done in this area and other studies in this field with Dr. Sotiris and other interested parties at the institute and talked about new studies that can be done together.



Figure 114 Photos of the research visit

DESCRIPTION OF THE STSM MAIN ACHIEVEMENTS AND PLANNED FOLLOW-UP ACTIVITIES

After the exchange of samples between the two partners, the last two of the 4 work packages will be carried out in the coming months.

WP3. Biochemical (Protein, aa, total lipid, FAMEs) compositions of samples (analysis in Turkey).

Total Protein, Total lipid, aminoacids and fatty acids composition of Greek and Turkey samples will be detected in Turkey.

WP4. Metal content of annual macroalgae, i.e. Ulva, via Inductively Coupled Plasma Mass Spectrometry (ICP-MS/MS) as a function of the environmental conditions (analysis in Greece).

Metal content of Greek and Turkey samples will be detected in Greece. All data will be evaluated together after the results of the last 2 work packages are received.The overall aim of SEAWHEAT is to bring together experts in the field of marine biotechnology, to provide a platform for sharing experience, knowledge and technologies, and to design a roadmap for a more efficient and rapid development of macroalgae biotechnology research in Europe and beyond.

With this cooperation, we have carried out a study that will serve the main goals of this COST action. With the simultaneous collection and analysis of Ulva species from the Dardanelles Strait (Turkey) and the Eastern Macedonian coast (Greece), we will obtain an important data set in this field and data that will be used in our future studies.

With the results of this STSM, 1-2 manuscript will be submitted. In addition, this study will provide a record of the Aegean Sea, which is becoming more important by the day, and the data from the above work packages will be published in the literature and will inform future studies in this area.



Leonardo Bruni

Microbiome analysis of Ulva ohnoi cultured in IMTA-RAS systems

28/07/2024 to 30/08/2024

Ulva
The Future Wheat of the Sea

Start and end date: 28/07/2024 to 30/08/2024

Grantee name: Leonardo Bruni

DESCRIPTION OF THE WORK CARRIED OUT DURING THE STSM

The first main focus of the STSM was to establish a collaboration and put in common methodologies (bacterial DNA extraction, sequencing, bioinformatics analysis) to analyse and monitor bacterial communities in Ulva from the IMTA-RAS system in relation with other compartments of the system (water, biofilms, biological or mechanical filters, etc), and the culture conditions (e.g. light). The DNA extractions, crucial step in bacterial metabarcoding studies and highly prone to introducing biases, were performed on the Ulva samples collected in the IMTA-RAS system. By performing the activities by using the same infrastructure for samples coming from different locations, we made sure that the comparison between the microbiome of the cultured Ulva and the wild samples is possible. The activities have been:

- DNA extraction of the microbiome of 560 samples of:
  - (i) *U. ohnoi* in cultivation system in closed tanks,
  - (ii) *U. ohnoi* in IMTA-RAS in different conditions
- Amplification essays of subsets of the extracted DNA in order to verify the quality of the extracted material. Differently from the original working plan, the downward processing of the extracted samples will be done through online meetings once all the samples are sequenced. We agreed on this change to better manage the time of the STSM.

DESCRIPTION OF THE STSM MAIN ACHIEVEMENTS AND PLANNED FOLLOW-UP ACTIVITIES

In terms of benefits for the SeaWheat action, the results of the STSMS will align with the scientific objectives of WG1 and WG2, specifically enlarging the knowledge on epibiotic microbial interaction, which has been shown to be crucial for Ulva cultivation. A fine-tuned Ulva production originating from the new knowledge will facilitate product development and mitigate its environmental impact (WG 3 and 4).

The home institution has benefited from the STSM by ensuring that the processing of samples for next generation sequencing is carried out in a state-of-the-art facility with qualified staff and according to a fast and efficient protocol. Both institutions have strengthened the existing collaborations to ensure a long-term relationship, and the stay specifically allowed me to reinforce the collaboration with Dr Engelen's group, as I visited the CCMAR-Ramalhete Experimental Station, which is part of the TNA network and allows for future joint experiments between Dr Engelen's group, expert in algae, and me, expert in fish.

Furthermore, I produced DNA the samples to be sequenced and once they will be sequenced and processed, the data will lead to one or more open access publication in international peer-reviewed journals (submitted by one year from now). Follow-up activities after the end of the STSM will be ensured to bioinformatically process the data and to write the common papers.

Finally, in addition to being an essential step of the project carried out in the home institution, the stay at CCMAR allowed me to apply an efficient DNA extraction method, to strengthen my academic network, and it gave him the opportunity to publish a paper on a topic that is dear to the action CA20106, i.e., the microbiome of Ulva. Concluding, the stay brings him closer to the academic maturity and richness necessary for an internationally acknowledged scholar.



Sara M. García Poza

Testing nitrogen assimilation capacity of different strains of Ulva sp. to evaluate their potential as biofilters for bioremediation of different effluents

03/08/2024 to 24/08/2024

Ulva

The Future Wheat of the Sea

Start and end date: 03/08/2024 to 24/08/2024

Grantee name: Sara M. García Poza

DESCRIPTION OF THE WORK CARRIED OUT DURING THE STSM

Home Institution: Canary Islands Institute of Technology, Spain.
Host Institution: Aarhus University, Denmark.

Main goals and aims:

During this STSM, the focus was on culturing the *Ulva compressa* strain, provided by SeaWheat SME partner Pure Algae Denmark, over a 16-day period using land-based Recirculated Aquaculture Systems (RAS). The growth of the macroalgae was monitored daily, and regular biomass and water samples were collected. These samples were analyzed to assess the nutrient removal capacity of *Ulva compressa*, with the aim of evaluating its potential effectiveness in water treatment and aquaculture systems.

Prior to the STSM the seaweed were cultivated with the objective to achieve the biomass concentration of 2 g/L. The cultivation trial was conducted for a period of 17 days. Most of the time was dedicated to learning cultivation methods, and study the efficiency of Ulva spp. strain capacity of absorbing nitrogen.

The cultivation system consisted of eight aquariums, each with a capacity of 4 liters, arranged on two shelves within a Recirculated Aquaculture System (RAS). In the four upper aquariums, no biomass was removed, leading to an increase in biomass concentration each day. In contrast, biomass was removed daily from the four lower aquariums to maintain a consistent concentration of 0.5 g/L. The biomass from each aquarium was weighed daily, and water samples were collected. Simultaneously, biomass samples were taken from the four lower aquariums for subsequent analysis of nitrogen content stored in their tissues. Some of the aquariums were equipped with light sensors that recorded measurements every hour. Each day, the aquariums were emptied for two hours to allow the sensors to take light measurements without the presence of biomass, enabling the correlation of this data with light absorption. Nutrients were added to the main RAS tank (supplying all aquariums) once a week. The biomass samples were weighed, dried, and ground for later analysis. A total of 67 water samples were collected and frozen at -20ºC for future analysis.

In addition to these tasks, I assisted the rest of the team with their ongoing work. I supported PhD student Kristoffer Larsen-Ledet with the temperature-related experiments he was conducting on the same strains cultivated in other aquariums. I also participated in field trips organized by the department, including one to sample and harvest a green tide of Ulva sp. in Skive Fjord during two days. In addition, I made an industrial visit to Pure Algae, an SME dedicated to providing sustainable solutions for land-based seaweed cultivation.

Deviations from the original plan:

Although the macroalgae were introduced into culture before the start of the STSM, the biomass concentration in the aquariums did not reach the initially expected level of 2 g/L. Instead, the experiment began with a lower biomass concentration of 0.5 g/L.

It was not possible to take daily PAM measurements because the equipment was not available at the research center.

The cultivation trial was done with just one strain from the Pure Algae Denmark partner.

DESCRIPTION OF THE STSM MAIN ACHIEVEMENTS AND PLANNED FOLLOW-UP ACTIVITIES

Achievements:

This STSM provided the grantee with valuable experience in culturing Ulva sp. strains within an indoor Recirculated Aquaculture System (RAS). The methodologies learned will be applied in the grantee's future research and will serve as a foundation for ongoing work with Ulva strains in the Canary Islands. The knowledge gained in sampling water and Ulva tissue for determining nitrogen uptake efficiency will be implemented in upcoming projects for scaling-up and in outdoors conditions at the Canary Islands Institute of Technology.
Additionally, the results from this STSM can be utilized by PhD student Kristoffer Larsen-Ledet in his research studies.
The cultivation trials will contribute to a deeper understanding of Ulva biology (WG1) and its application potential in aquaculture, both for biomass production and ecosystem services (WG2 and WG5) associated with CA20106.
The findings from this study aim to advance the objectives and deliverables of the COST Action CA20106, specifically supporting deliverable of CA20106 D5.1. This includes generating knowledge and fostering cooperation to enhance the ecosystem services provided by Ulva spp., particularly in nutrient and carbon removal and recycling.
The study contributes to the understanding of the Ulva genus's ability to grow in and remove nitrogen from nutrient-rich process streams, functioning as a biofilter to remediate wastewater from various industries.
Lastly, this STSM facilitated the exchange of knowledge between two CA20106 partner institutions—the Canary Islands Institute of Technology and Aarhus University—as well as an associated SME (Pure Algae). This collaboration encourages future cooperation within the COST Action network.

Already planned future collaborations:

Daily monitoring data, along with water and nutrient samples, were collected and processed by the grantee during the STSM. However, the nutrient samples still need to be analyzed by Aarhus University. Once these results are available, the grantee will analyze the data with the aim of enhancing our understanding of the life cycle of macroalgae in the Ulva genus. This data may also contribute to future publications involving both institutions.
Additionally, meetings have been scheduled in September to evaluate the data and follow up on the activities carried out during the STSM and a team's platform was create for sharing files, data and photos.

Contribution of STSM to the Seawheat Objectives:

1. Contribute to generating and strengthening the SeaWheat network and capacity building (Canary Islands Institute of Technology and Aarhus University, Pure Algae (SME))
2. Generate and share new knowledge in relation to SeaWheat Core Activities in WG2 and WG5, producing new knowledge on Ulva strains and applicability in large-scale specialized Integrated Multi-Trophic Recirculating Aquaculture System (IMRAS) cultivation.
3. Significant contribution to the grantee development in the cross field between science and industry.
4. Information exchange: The results of the STSM will be shared between both institutions, with an effort to generate open-access publications.



Kristoffer Larsen-Ledet

Temperature Effects on the Chemical Composition of Ulva: Perspectives on Biomass Quality in Land-Based Cultivation Systems

11/08/2024 to 03/09/2024

Ulva
The Future Wheat of the Sea

Start and end date: 11/08/2024 to 03/09/2024

Grantee name: Kristoffer Larsen-Ledet

DESCRIPTION OF THE WORK CARRIED OUT DURING THE STSM

Main Goals and Aims:

This Short-Term Scientific Mission (STSM) aimed to train the grantee in biochemical methods with a focus on how cultivation temperature might influence the biochemical composition of Ulva biomass using spectrophotometry and ethanolic extractions.

Summary of Activities:

Before the STSM, a cultivation experiment was conducted at Aarhus University. A single Ulva strain was cultivated at eight different temperatures relevant to potential aquaculture systems. Fifty-one biomass samples were freeze-dried and transported to Galway, Ireland, for extractions and analysis.
In Galway, initial ethanolic extractions were performed on all samples in triplicate. This was followed by various spectrophotometric assays to quantify the protein content, free amino acids, starch content, and ulvan content of the biomass. The methods used during the STSM followed Simon et al. (2024). The acquired results underwent quality control, and assays were repeated if necessary.
Initial data analysis showed a clear effect of cultivation temperature. Protein and amino acid content were higher at temperature extremes (both low and high) and lower at medium temperatures. Conversely, starch content was lower at temperature extremes while ulvan content decreased with temperature. All work during the STSM was supervised by Dr. Ronan Sulpice's team.

Deviations from Original Plan:

More work and assays were conducted than anticipated in the original STSM application. This included a full screening of ulvan, starch, amino acids, protein, and pigment contents of control samples (36 samples) for the grantee to practice the protocols. Additionally, the original application only anticipated assays of free amino acids and ulvan, but this was expanded to include protein and starch content.

References:

Simon, C., Fort, A., Jouanneau, D. et al. Fast screening method to identify salinity tolerant strains of foliose Ulva species. Low salinity leads to increased organic matter of the biomass. J Appl Phycol 36, 2161–2172 (2024)

DESCRIPTION OF THE STSM MAIN ACHIEVEMENTS AND PLANNED FOLLOW-UP ACTIVITIES

Achievements:

This Short-Term Scientific Mission (STSM) enabled the grantee to learn several biochemical methods and protocols applicable to various algae species and strains. The results obtained during this STSM will be used for joint scientific publications with members of Aarhus University and NUI Galway.
Additionally, these results provide valuable insights into the quality of Ulva produced at different cultivation temperatures. Understanding temperate Ulva biology (WG1) is crucial for further organism research and for potential Ulva cultivation aspects. The results acquired during this STSM can be used to optimize Ulva cultivation which can be based on the targeted biochemical composition and might promote higher yields of either protein or ulvan depending on the utilization purposes (WG2 and WG5).
Lastly, the STSM facilitated knowledge exchange between two CA20106 partner institutions – Aarhus University and NUI Galway – encouraging future cooperation within the cost-action network.

Already Planned Future Collaboration:

This STSM strengthened an ongoing collaboration on the biochemical analysis of Ulva species for optimized cultivation under specific conditions. Given the importance of biochemical composition for using Ulva as a food source or pharmaceutical product, similar scientific research stays are expected in the future.

Expected Publications:

1. Larsen-Ledet K et al. Optimized cultivation of two specific Ulva strains under relevant RAS conditions, ensuring optimal yield, biomass quality, and emission capture.
2. Larsen-Ledet K et al. Up-scaling ecosystem services of cultivating selected Ulva strains in large-scale RAS.

Contribution of STSM to the SeaWheat Objectives:

1. Contribute to generating and strengthening the SeaWheat network and capacity building (Aarhus University, NUI Galway, and PureAlgae Denmark (SME)).
2. Generate and share new knowledge related to SeaWheat Core Activities in WG1, WG2, and WG5, producing new information on Ulva biology and its applicability in large-scale specialized IMRAS cultivation.
3. Early Career Investigator (ECI) will produce and disseminate COST action results via publications, COST channels, and other relevant science-industrial fora (including the Nordic Seaweed Conference 2024, and the International Seaweed Symposium 2025).
4. Significant contribution to ECI career development in the cross-field between science and industry (Aarhus and Galway universities, PureAlgae (SME)), introducing new opportunities in academia and the seaweed industry sector.
5. Sharing of information – outcomes of the STSM will be shared via open access publications.



Pınar AKDOĞAN ŞİRİN

Molecular identification of Ulva species distributed in the Black Sea Region (Turkey)

13/08/2024 to 07/09/2024

Ulva

The Future Wheat of the Sea

Start and end date: 13/08/2024 to 07/09/2024

Grantee name: Pınar AKDOĞAN ŞİRİN

DESCRIPTION OF THE WORK CARRIED OUT DURING THE STSM

The main objective of this STSM is to conduct the molecular analysis and identification of Ulva species collected from the Black Sea region (Turkey), in line with the previous working plan of the COST Action 'European Ulva Sampling Initiative (EULVA).' For this purpose, Ulva samples were collected from 17 locations across 5 different cities along the Turkish Black Sea coast (Table 1). The DNA extractions, PCR amplifications of tufA gene region, sequencing, and phylogenetic analyses of the collected 51 macroalgae samples were carried out under the supervision of Prof Dr Olivier De Clerck at Ghent University.

Table X. Sampling location details

No.	Location Name / City	GPS Coordinates
1	(Saklı Bahçe) Çaytepe, Ordu	41.12625277102386, 37.69242395829241
2	Hoynat Island (Hoynat Adası), Ordu	41.117659498639085, 37.72874153785391
3	Çeşmeönü, Ordu	41.096539055725934, 37.78045724216704
4	Station 1, Samsun	41.32796208435847, 36.306985018508605
5	Station 2, Samsun	41.406677658232155, 36.18998162121474
6	Station 3, Samsun	41.45322041154401, 36.14028551250243
7	Station 4, Samsun	41.63434388178403, 35.52342102175076
8	Abana, Kastamonu	41.98085861417419, 34.048060413922198
10	Merkez (City Center), Sinop	42.01897887919942, 35.164002156956336
11	Gerze, Sinop	41.79823775047528, 35.19413381659321
12	Gülyalı, Ordu	40.96823837191166, 38.059240705645394
13	Giresun City Center, Küçüklü Beach	40.93639924240079, 38.29706046083124
14	Aşıklar Bay (Aşıklar Koyu), Giresun	40.97069386901714, 38.63249068495459
15	Görele, Giresun	41.04029525918476, 38.93850731027655
16	Eynesil, Giresun	41.047722137675614, 39.10055765006961
17	Efirli, Ordu	41.02854638412896, 37.80375726870544
18	Fatsa, Ordu	41.062681398392876, 37.47539583028415

DESCRIPTION OF THE STSM MAIN ACHIEVEMENTS AND PLANNED FOLLOW-UP ACTIVITIES

As a result of the analyses, six different Ulva species (*Ulva compressa, Ulva australis, Ulva flexuosa, Ulva gigantea, Ulva lacinulata, Ulva laetevirens*) were identified in the locations where macroalgae samples were collected. *U. compressa* was found to be the most prevalent species in the sampling locations, followed by *U. lacinulata* as the second most common macroalgae species (Table 2). Gel photographs of the sampled macroalgae are shown in Figure 1.

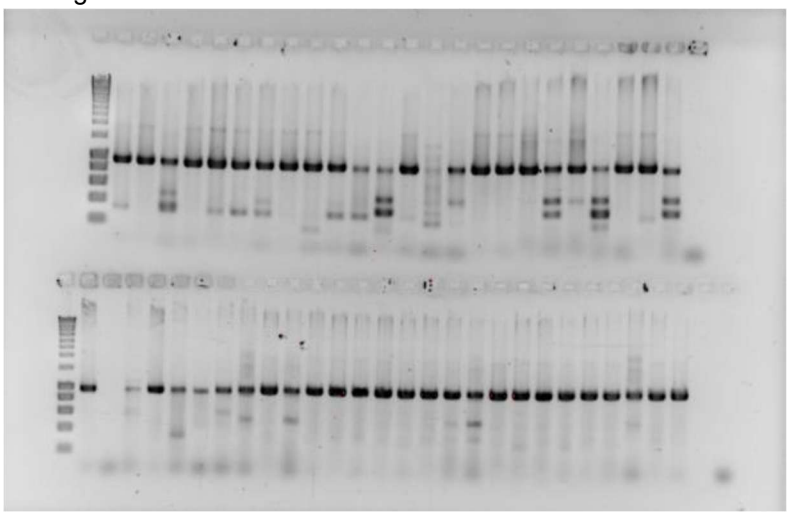


Figure 1 Gel photographs of the macroalgae

The data obtained from this STSM contribute significantly to both the literature and COST Action 20106.
In future studies, it is planned to conduct sampling in areas of the Black Sea region where macroalgae sampling has not yet been carried out.

Table 2. *Ulva species distributed in the Black Sea Region*

No.	Location Name / City	Ulva Species Present
1	(Saklı Bahçe) Çaytepe, Ordu	*Ulva compressa, Ulva laciniulata*
2	Hoynat Island (Hoynat Adası), Ordu	*Ulva compressa, Ulva laetevirens*
3	Çeşmeönü, Ordu	*Ulva compressa, Ulva laciniulata*
4	Location 1, Samsun	*Ulva compressa*
5	Location 2, Samsun	*Ulva compressa, Ulva gigantea*
6	Location 3, Samsun	*Ulva compressa*
7	Location 4, Samsun	*Ulva compressa*
8	Abana, Kastamonu	*Ulva compressa*
10	Merkez (City Center), Sinop	*Ulva laciniulata*
11	Gerze, Sinop	*Ulva flexuosa*
12	Gülyalı, Ordu	*Ulva compressa*
13	Merkez Küçüklü Beach, Giresun	*Ulva compressa, Ulva laciniulata*

No.	Location Name / City	Ulva Species Present
14	Aşıklar Bay, Giresun	*Ulva compressa, Ulva laciniulata*
15	Görele, Giresun	*Ulva compressa, Ulva australis*
16	Eynesil, Giresun	*Ulva compressa, Ulva australis*
17	Efirli, Ordu	*Ulva compressa, Ulva australis*
18	Fatsa, Ordu	*Ulva compressa, Ulva laciniulata*



Hermann Holbl

Morphogen triggered changes in the transcriptome of Ulva mutabilis

30/03/2025 to 19/04/2025

Ulva
The Future Wheat of the Sea

Start and end date: 30/03/2025 to 19/04/2025

Grantee name: Hermann Holbl

The Short-term Scientific Mission (STSM) was focused on the model system of *Ulva mutabilis* and its associated beneficial bacteria, *Maribacter* sp. strain MS6 and *Roseovarius* sp. strain MS2 (Blomme et al. 2023; Wichard 2023). *Ulva*'s cell differentiation is regulated by (−)-thallusin, an algal growth- and morphogenesis-promoting factor (AGMPF) produced by *Maribacter* (Alsufyani et al. 2020, Dhiman et al. 2022, Wienecke et al. 2024). In the absence of bacteria, *Ulva* lacks rhizoids, and deformed cell walls with protrusions are visible, emphasizing the crucial role of bacterial signalling in algal morphogenesis (Spoerner et al., 2012). I have already demonstrated that *Ulva* calli inoculated with (−)-thallusin or *Maribacter* sp. MS6 exhibited rhizoid formation and significantly increased tissue growth with no cell wall protrusion compared to nonactive treatments (**Fig. 110**). Experiments with treated axenic *U. mutabilis* calli were conducted before the STSM to investigate metabolic and transcriptomic changes caused by the presence or absence of thallusin. The goal of this STSM was to acquire bioinformatic workflows for transcriptome analyses and to understand best practices for *Ulva* data interpretation to analyse RNA sequencing data from the experiments with sampling times after 3 days and 28 days. In the framework of my PhD project, this data will be merged with endo-metabolome data for a comprehensive analysis of molecular processes associated with thallusin signalling in *Ulva*.

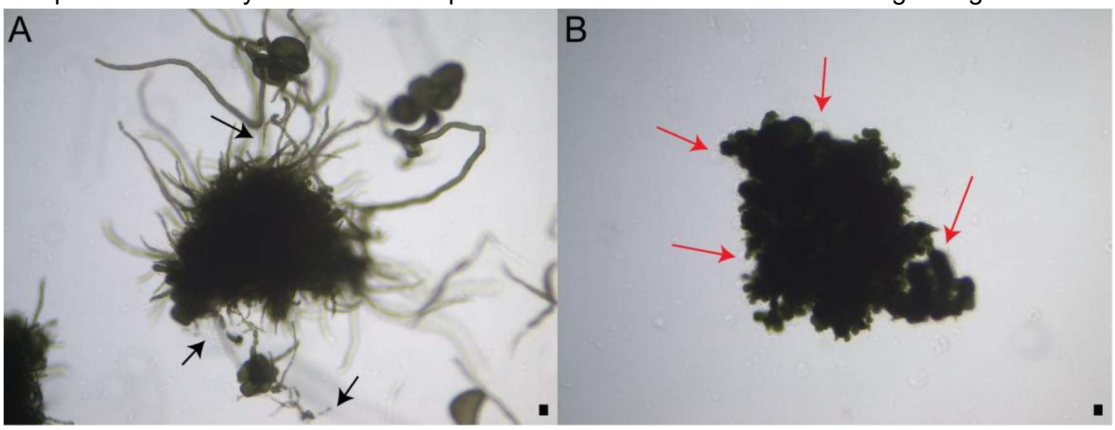


Figure 115 (A) Treatment - specimen of culture treated with Roseovarius sp. and exposed to (−)-thallusin for 28 days. This picture also represents cultures inoculated with Maribacter sp. instead of thallusin. Black arrows show rhizoid formation. (B) Control experiment - specimen of culture treated with Roseovarius sp. and the inactive (+)- thallusin and exposed for 28 days. This picture is representative of other treatments that were inactive regarding morphology changes. Red arrows show cell wall protrusions. Scales represent a length of 50 µm.

DESCRIPTION OF THE WORK CARRIED OUT DURING THE STSM

Description of the activities carried out during the STSM. Any deviations from the initial working plan shall also be described in this section.

During the STSM at Ghent University in Prof. Olivier de Clerck´s research group, I learned extensive processing steps to analyse transcriptome data using the most up-to-date reference genome of *U. mutabilis*. Thus, I established a data processing workflow utilising the high-performance computing (HPC) cluster of Ghent University to convert the paired-end sequencing reads into count data necessary for the analysis of differentially expressed genes (DEG).

This workflow is composed of the following steps:

- QC of reads
- Trimming of low-quality reads
- Mapping to the reference genome of *Ulva mutabilis* and receiving aligned reads
- Counting of aligned reads to the respective gene fit

This workflow can be easily adjusted for future analyses on the HPC cluster in Jena, as both utilize the same management system, Simple Linux Utility for Resource Management (SLURM).

Further, a workflow in the programming language R for the analysis of DEG was created during the STSM. Here we used "EdgeR" to normalize for sequencing depth and compare between different treatments sampled after 3 days of inoculation with thallusin, it's derivatives *or Maribacter* sp. (MS6). AMDS plot of the dataset sampled

at day 3 is shown in the description of the STSM main achievements (Fig. 112). To assess differences in the transcriptome due to the presence of the morphologically active (−)-thallusin, we searched for genes significantly up- or downregulated in treatments inoculated with (−)- thallusin as well as in *Maribacter* sp. but not in controls with (+)-thallusin and *Roseovarius* sp. only. In this comparison, we received 84 genes that were differentially expressed with a Benjamini-Hochberg corrected p-value below 0.05 and log base 2 transformed fold change of at least 1. Afterwards, gene enrichment analyses based on GO-Terms with the package "topGO" were conducted. With the list of regulated genes, we will now deduce further information about the molecular processes involved in (−)- thallusin signalling, e.g., by blast search or genetic modification of genes of interest. An overview of the entire workflow is illustrated in Figure 111.

During the STSM, our primary focus was on developing scripts to analyze my data. Specifically, we concentrated on identifying core genes associated with the response to (−)-thallusin. In addition to the dysregulated genes, the following supplementary insights can be obtained:

- Mediated changes in the transcriptome by *Maribacter* sp. through cell-cell contact
- Impact of *Roseovarius* sp. on mRNA regulation
- Effect of the partially active derivative of thallusin on gene regulation
- Changes in regulation over time between 3 days and 28 days of exposure.

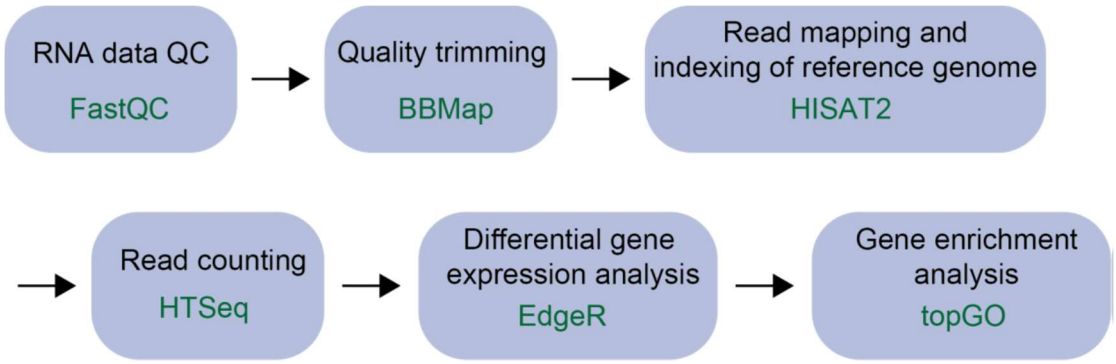


Figure 116 Overview of the processing and analysis steps performed during the STSM in Ghent. The software tools used for each step are highlighted in green.

Since the mRNA data is stored in the HPC cluster in Ghent, I can share it anytime for improvements of the current reference genome and de novo transcriptome assembly. The high sequencing depth (mostly above 30 million raw reads) and the very high mapping rate of ~95% to the recent annotated reference genome of *Ulva mutabilis* slender suggest that my transcriptome dataset is well-suited for this purpose.

DESCRIPTION OF THE STSM MAIN ACHIEVEMENTS AND PLANNED FOLLOW-UP ACTIVITIES

In total, the STSM at Ghent University was very successful. To demonstrate the productive STSM, a MDS plot based on normalised read counts representing sample variability is illustrated (Fig. 112). The referred samples were taken 3 days after inoculation. The plot shows much higher variability between treatments compared to variability between biological replicates, supporting the experimental design. Moreover, the morphologically active treatments ((−)-Thallusin and *Maribacter* sp.) cluster together on the positive side of dimension 1, whereas the negative treatments (negative control, *Roseovarius* sp. only, (+)-thallusin) are located at the negative side of dimension 1. Only the treatment with the partially morphologically positive derivative of thallusin, being more similar to the negative (+)-thallusin, does not align entirely with our expectation. This can be explained by the fact that the derivative was tested on axenic gametes beforehand and not on axenic calli, describing the activity of this specific derivative.

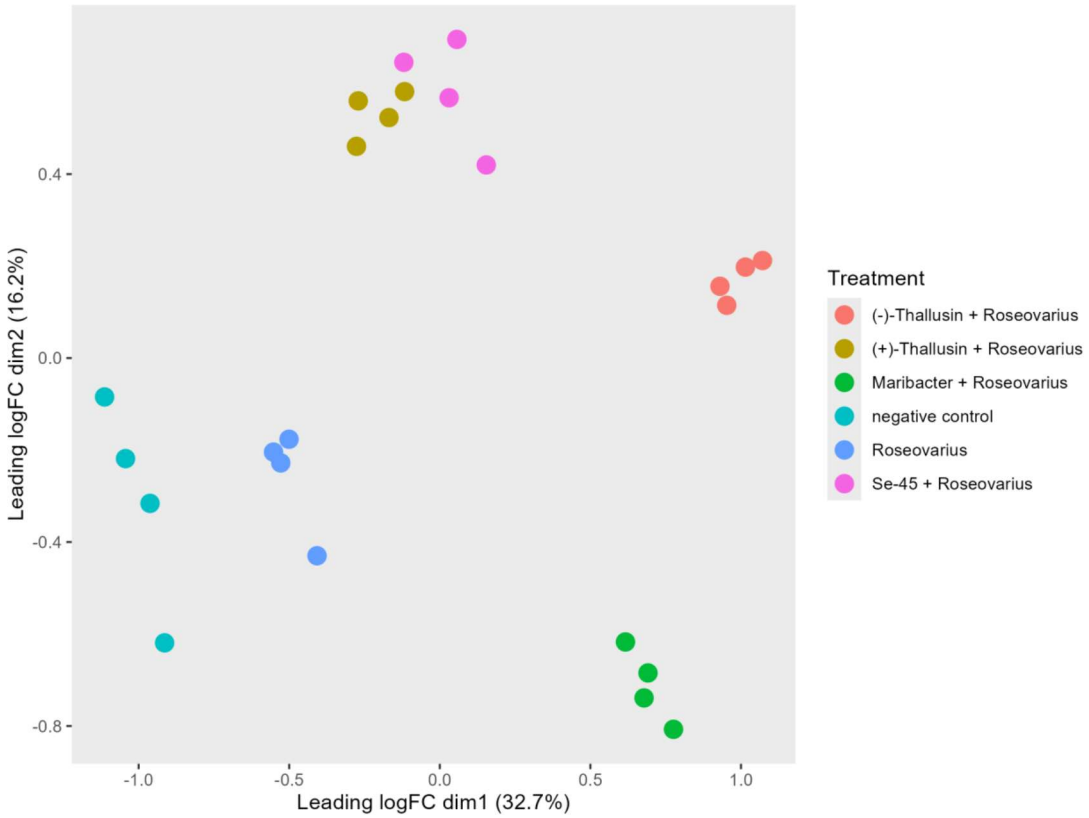


Figure 117 MDS plot of transcriptome data from calli with 3-day exposure time of thallusin, the partially active derivative of thallusin (Se-45, Dhiman et al. 2022) or Maribacter sp.

In detail, the following goals were achieved, and prospective activities are planned:

(1) With the support of Prof. Olivier de Clerck's research group, I created a transcriptome processing and analysis workflow that takes into account the properties of my sequencing data and experimental design. In the following weeks, this workflow will be amplified to extract more knowledge from the experiment (see description of work carried out) and appropriately visualise the data. Knowledge retrieved from my experiments is connected to bacterial–Seaweed interactions on the molecular level **(COST Action Task 1.2 of WG1**).

(2) In a conservative approach, 84 genes were identified to be significantly regulated in treatments containing (−)-thallusin compared to the controls after a 3-day exposure time. These genes, regulated by (−)-thallusin, enable the targeting of specific metabolites crucial for the morphogenesis of *Ulva* (**COST Action Task 4.1 of WG2**). Additionally, we aim to evaluate the role of these genes on morphology by using gene knockout mutants in collaboration with the Phycology Department of Ghent University.

(3) Since my RNA-seq data is stored on the local HPC in Ghent, specific data can be easily shared for improvements on the *Ulva mutabilis* slender genome. This results in a more comprehensive version of the current reference genome, by improving the reference genome, the whole scientific community benefits from working on *Ulva*.

We aim to publish our results in scientific journals. I highly appreciate the support of the Phycology research group at Ghent University during my STSM.

The main results of this STSM:

The introduction to transcriptome analysis that I received during my STSM in Ghent has equipped me to expand the analysis workflow we developed and extract further insights from my experiments at the University of Jena. We ensured the generation of high-quality data and successfully conducted differential gene expression analyses on a subset of the dataset to assess the effect of (−)-thallusin on gene regulation. Our ongoing goal is to integrate transcriptomic and metabolomic data to gain a more comprehensive understanding of thallusin signalling in *Ulva*. This STSM also strengthened the collaboration with Prof. Olivier De Clerck's research group,

with future projects involving gene knockout mutants already in planning. The outcome of the STSM directly contributes to the **Delivery 1.1.b** of the COST Action entitled "*Ulva* microbiome, interactions with bacteria on and in the seaweed fronds, affecting *Ulva's* growth and development".

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Karina Balina

Enhancing LCA for Ulva Aquaculture: Integrating Environmental Interactions and Ecosystem Services

17/05/2025 to 30/05/2025

Ulva
The Future Wheat of the Sea

Start and end date: 17/05/2025 to 30/05/2025

Grantee name: Karina Balina

DESCRIPTION OF THE WORK CARRIED OUT DURING THE STSM

The STSM took place at GreenCoLab in Faro, Portugal, under the supervision of Dr. Lais Galileu Speranza. The mission aimed to explore how nutrient exchange and biogenic carbon flows could be integrated into Life Cycle Assessment (LCA) of Ulva cultivation systems. During the first week, I reviewed existing seaweed-related LCA studies with a particular focus on how marine ecosystem services, such as nitrogen removal and carbon uptake, could be addressed through impact categories like marine eutrophication and climate change. Special attention was given to the work of researchers, including research done by Jean-Baptiste Thomas, Marianne Thomsen, and Michelle Seghetta. This helped to identify possible system boundaries, functional units, and methodological gaps relevant for future seaweed-based assessments.

The first week also included a team meeting at GreenCoLab, where I presented my research and introduced the SeaWheat COST Action, as well as the opportunities available within the COST network. Additionally, I participated in an online meeting with GreenCoLab's sustainability partners, which provided an opportunity to share the STSM objectives and engage in discussions about other ongoing projects and initiatives.

During the second week, I participated in several hands-on workshops together with GreenCoLab Sustainability group: Dr. Lais Speranza, Sofia Navalho, and Alexandre Lima, focusing on practical experience using SimaPro and OpenLCA. We explored how each tool supports modelling of environmental systems, with particular emphasis on their flexibility and user experience. SimaPro was perceived as more intuitive and user-friendly, while OpenLCA allowed more customisation in handling background datasets and adjusting characterisation and normalisation factors, which are important features when dealing with less conventional systems such as macroalgae aquaculture.

Although the original application included the intention to work with empirical data from ongoing *Ulva* cultivation trials in Faro, this data was not yet available during the STSM due to unexpected delays. Therefore, no LCA modelling using original data was performed. Instead, the work focused on developing a conceptual framework, including the definition of key inventory needs, boundary assumptions, and methodological approaches for representing ecosystem services such as nutrient removal and carbon dynamics. The time spent on literature review, discussion, and tool comparison provided a strong base for follow-up modeling once the relevant data is accessible. Overall, the STSM created space for focused methodological development, interdisciplinary dialogue, and deeper understanding of the challenges and opportunities in applying LCA to seaweed systems, particularly in the context of the SeaWheat COST Action.

DESCRIPTION OF THE STSM MAIN ACHIEVEMENTS AND PLANNED FOLLOW-UP ACTIVITIES

The STSM provided an opportunity to advance methodological thinking around the integration of nutrient exchange and carbon balance into LCA for seaweed cultivation systems. One of the key achievements was the development of a conceptual framework that outlines how Ulva's contribution to nutrient uptake and biogenic carbon sequestration could be more systematically included in environmental assessments. Although it was not possible to build a complete model due to the unavailability of local empirical data, the groundwork was laid for future work once data becomes accessible.

A key contribution was the hands-on comparison of two leading LCA software tools – SimaPro and OpenLCA. This helped to identify the strengths and limitations of each for marine system modelling. OpenLCA offered greater flexibility for customising background data and impact methods, while SimaPro provided a smoother user experience for structuring conventional models. This insight will be valuable in selecting the most suitable tool for future modelling tasks involving novel impact pathways.

The mission also established valuable connections to broader European initiatives, notably the EABA LCA Task Force. The outcomes of this STSM will directly contribute to the ongoing development of the Task Force's white paper, which aims to summarise and harmonise current LCA practices across different algae production systems. Findings on ecosystem service modelling and software choice will help inform that effort. Continued collaboration with EABA members is planned, including joint discussions on methodological

challenges, allocation strategies, and marine-specific impact categories. Furthermore, the STSM has reinforced the need for a dedicated COST Action focused on algae LCA. While interest in algae-based products is growing rapidly, there is currently no coordinated European network that addresses the methodological complexities of assessing environmental impacts across diverse algae types, production conditions, and product applications. An algae-specific COST Action would fill this gap by creating a stable platform for interdisciplinary collaboration, method development, data sharing, and stakeholder engagement. The conceptual insights and network contacts developed during the STSM will help frame this proposal and facilitate the gathering of potential partners across academia, industry, and policy.

In the short term, I plan to continue collaborating with GreenCoLab's sustainability group to explore further the methodological challenges associated with LCA applications on seaweed. I also plan to present the results of this STSM at the final conference of SeaWheat to contribute to LCA-related activities within the Action. Overall, this STSM catalyzed strategic cooperation, yielding technical, institutional, and policy-relevant impacts that extend well beyond the two-week visit.

Ulva

The Future Wheat of the Sea



SeaWheat

COST Action CA20106

TOMORROW'S 'WHEAT OF THE SEA': ULVA, A MODEL FOR AN INNOVATIVE MARICULTURE

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