

SeaWheat

COST Action CA20106

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





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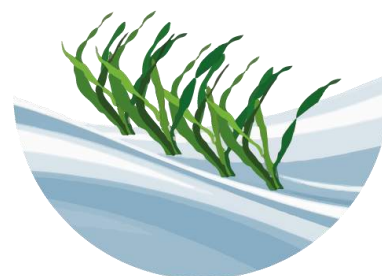
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TOMORROW'S 'WHEAT OF THE SEA': ULVA, A MODEL FOR AN INNOVATIVE MARICULTURE

Conference Program



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SeaWheat *Ulva*, Tomorrow's "Wheat of the Sea": A Model for an Innovative Mariculture



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TOMORROW'S "WHEAT OF THE SEA": *ULVA*, A MODEL FOR AN INNOVATIVE MARICULTURE

PRE-CONFERENCE EVENTS:

SEPTEMBER 12, 2022

Location: Aula Magna,

Faculty of Philosophy and Arts, University of Cádiz, Spain

12:00-14:00 Working Group and SMEs meeting*

Moderated by Prof. Muki Shpigel and Dr Rui Pereira

14:00-17:00 Working Groups and Core Group meeting*

Moderated by Prof. Muki Shpigel and Dr Thomas Wichard

17:30 COST Action Icebreaker & registration** with welcome comments from:

- Prof. Ignacio Hernández and Dr Erik-jan Malta
- Councillor Mrs. Rocío Saiz Guerrero, Executive Member, Council of City of Cádiz for Environment
- Prof. Dr María Jesús Mosquera Díaz, Vice Rector Science for Technology Policy of the University of Cádiz
- Mr Juan Manuel García de Lomas Mier, General Manager of the Andalusian Aquaculture Technology Center (CTAQUA)
- Prof. Muki Shpigel, Action Chair SEAWHEAT

Wine and refreshments will be provided

**The attendance list must be signed in person with Dr Leigh Livne*

***event is informal and participation is voluntary*

**Please use #seawheat #costaction #ulva
#algae in your social media posts**

Scientific Committee:

Chair: Dr Erik-jan Malta

Vice Chair: Dr Laurie C. Hofmann

Dr Ricardo Bermejo

Prof. Muki Shpigel

Dr Leigh Livne

Organising committee:

Dr Erik-jan Malta (CTAQUA, Spain, chair)

Prof. Dr Ignacio Hernández (Univ. Cádiz, Spain, chair)

Prof. Dr José-Lucas Pérez-Lloréns (Univ. Cádiz, Spain)

Ms. María Galindo (CTAQUA, Spain)

Ms María del Mar Agraso (Technical Director CTAQUA, Spain)

Dr María del Mar Barrios (P&O Director, CTAQUA, Spain)

Mr Manuel Macías (CTAQUA, Spain)

Dr Fini Sánchez García (Univ. Cádiz, Spain)

Dr Rui Pereira (A4F, Portugal)



08:00-09:00 REGISTRATION (Please sign the daily attendance list each day)

09:00-09:15 Conference Opening by Dr Erik-Jan Malta, Dr Laurie Hofmann, and Prof. Ignacio Hernández

09:15-10:00 Plenary speaker: Prof. Jang K Kim

"Bad" *Ulva* vs. "Good" *Ulva*: Green Tides Versus Nutrient Bioextraction

Chair: Dr Malta

10:00-10:30 Keynote speaker: Dr Ronan Sulpice

Ulva breeding: huge potential for increased yields, but care should be taken!

Chair: Dr Malta

10:30-10:50 *Coffee Break*

Ulva Biology Session - Chair Dr Ronan Sulpice

- 10:50-11:10 Prof. Olivier De Clerck (*online*): Towards a comprehensive overview of *Ulva* species diversity in Europe
- 11:10-11:30 Dr Sotiris Orfanidis: Photosynthetic and growth strategies of *Ulva lacunculata* to survive the seasonal fluctuating conditions in a eutrophic Mediterranean Sea Bay
- 11:30-11:50 Dr Lior Guttman: A bacterial-based process for decomposition of *Ulva* cell-wall polysaccharides in biorefinery
- 11:50-12:10 Dr Gabrielle Zammit: Sustained growth in culture of barcoded *Ulva* spp. germings for bioprocessing

12:10-12:40 Keynote Speaker: Dr Rui Pereira

Ulva production at commercial scale - engineering and beyond

Chair Prof. Ignacio Hernández

12:40-12:50 *Break*

Ulva in Aquaculture, Food and Feed Session- Chair Prof. Ignacio Hernández

- 12:50-13:10 Dr Sophie Steinhagen: Large-scale off-shore cultivation of *Ulva*: seasonality affects the biomass yield, performance, and biochemical profile of the biomass
- 13:10-13:30 Dr Juan Luis Pinchetti: Intensive tank cultivation of *Ulva* in the Canary Islands - the nitrogen influence

13:30-14:40 *Lunch Break* - Dr Leigh Livne is available to assist with COST reimbursement claims

Ulva in Aquaculture, Food and Feed Session 2 - Chair Dr Juan Luis Pinchetti

- 14:40-15:00 Prof. Dr Karel Keesman: Transition of aquaponics towards saltwater: Model-based Design of a Multi-Loop Maraponics System
- 15:00-15:20 Dr Georg Martin: Using *Ulva* to extract nutrients from finfish aquaculture effluents
- 15:20-15:40 Prof. Piero Addis: *Ulva* and its components as potential stimulants in aquaculture feeds: chemosensory response of a valuable sea urchin species

15:40 - 16:00 *Coffee Break and Exhibitions*

16:00 - 17:00 SME Presentations - Chair Prof. Muki Shpigel

- Dr Sotiris Orfanidis presents the database of SEAWHEAT publications
- Pure Algae - Esben Rimi Christiansen
- Algabase - Jose A. Callejo
- The Seaweed Company - Dr Stefan Kraan
- Seakura - Dr Yossi Tal
- Eranova - Xavier Marquat

17:00 - 18:00 Poster Exhibitions

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#costaction #ulva #algae in your
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08:00-09:00 REGISTRATION (Please sign the daily attendance list each day)

09:00-09:15 COST activities by Dr Leigh Livne

09:15-10:00 Invited Speaker: Dr Stefan Kraan: The opportunity and gaps in *Ulva* aquaculture
Chair: Prof. Bela H. Buck

10:00-10:20 *Coffee Break*

10:20-10:50 Keynote Speaker: Dr Sylvia Strauss
Opportunities for *Ulva* as a sustainable crop in human and animal consumption
Chair: Prof. Bela H. Buck

Ulva in Aquaculture, Food and Feed Session 3 - Chair Dr Sylvia Strauss

- 10:50-11:10 Dr Erik-jan Malta: State of the art of applications of *Ulva* and its extracts in fish and invertebrate aquaculture
- 11:10-11:30 Dr Orhan Tufan Eroldoğan: Effect of dietary non-processed algae meals on nutrient digestibility and inflammatory response of European seabass (*Dicentrarchus labrax*)
- 11:30-11:50 Dr Vincent van Ginneken: The seaweed *Ulva lactuca* as a nutritional 'tool' to prevent Chronic Degenerative welfare Diseases (CDDs) in the human population.
- 11:50-12:10 Dr Laurie C. Hofmann: Packaging solutions from seaweed
- 12:10-12:30 Dr Gül den Gökşen: Fabrication and Characterization of biodegradable film from *Ulva* spp.

12:30-14:00 *Lunch Break & 'Cooking with Ulva' demonstration by Chef Boaz Tsairi*

14:00 - 14:30 Keynote Speaker: Dr Thomas Wichard
Ulva: a model system in chemical ecology - potential applications in aquaculture
Chair: Dr Lior Guttman

Bioactive Products and Associated Microorganisms Session - Chair Dr Gabrielle Zammit

- 14:30-14:50 Dr Aschwin Engelen: *Ulva* microbial contrasts between lagoon and open coast habitats in southern Portugal
- 14:50-15:10 Dr Fatemeh Ghaderiardakani: Three become one in a tripartite community: Reassessing the collaborative adaptation of *Ulva* and its microbiome to cold temperatures in Antarctica
- 15:10-15:30 Dr José Pintado: Shedding light on the *Ulva* holobiont: the role of light in microbial interactions with implication in IMTA-RAS
- 15:30- 15:50 Prof. Antonio Meléndez-Martínez: Carotenoids as versatile compounds for the sustainable production of health-promoting products

16:00 *Coffee Break and Exhibitions*

16:20- 17:30 SME Presentations - Chair Prof. Muki Shpigel

- Olmix - Pi Nyvall Collén
- RecirkFisk - Ola Oberg
- Algae for Future - Dr Rui Pereira
- Blue-Green Technologies - Dr Vincent Van Ginneken
- Alga+ - Dr Helena Abreu & Dr Margarida Martins
- DAS - Dr Avi Griffel

17:30 - 18:30 Poster Exhibitions

20:30: SEAWHEAT dinner (not mandatory) at Hotel del Parador de Cádiz

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08:00-09:00 REGISTRATION (Please sign the daily attendance list each day)

09:00 - 09:30 Keynote Speaker: Prof. John Bolton (*online*)

Two decades of commercial integrated aquaculture of *Ulva* with abalone in South Africa

Chair: Dr Olympia Nisiforou

09:30-10:00 Keynote Speaker: Dr Annette Bruhn

Ecosystem Services of *Ulva*

Chair: Dr Olympia Nisiforou

10:00-10:20 *Coffee Break*

Ecosystem Services Session - Chair Dr Annette Bruhn

- 10:20-10:40 Dr Ricardo Bermejo: Report from the Eklipse Expert Working Group on Ecosystem Services provided by Seaweed Cultivation
- 10:40-11:00 Dr Andreia Freitas: Macroalgae as bioindicator of pharmaceutical's environmental contamination
- 11:00-11:20 Dr Alvaro Israel: Evaluating *Ulva* sp. cultivation for climate change mitigation: growth, carbon uptake and long-term storage of inorganic carbon
- 11:20 - 11:40 Prof. Ignacio Hernández: *Ulva*, seagrasses and ecosystem services: a tale of love and coldness

11:40-11:50 *Break*

11:50 - 12:20 Keynote Speaker: Dr Céline Rebours

Marine biotechnology: RRI, Convention of Biological Diversity, Nagoya Protocol, Access and Benefit-sharing Clearing-house.

Chair: Dr Lior Guttman

Legal, Social, and Regulation Aspects Session - Chair: Dr Céline Rebours

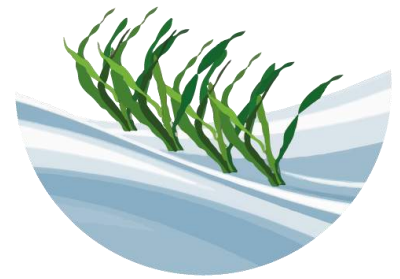
- 12:20 - 12:40 Dr Anna Fricke (*online*): *Ulva* as model organisms in a new approach to urban farming
- 12:40 - 13:00 Dr Karina Balina: Life Cycle Assessment of seaweed cultivation: Opportunities and challenges
- 13:00 - 13:20 Dr Avi Griffel: Risk Assessment of COST Project: Principles and Methods

Closing comments by Prof. Ignacio Hernández, Dr Erik-jan Malta, Prof. Muki Shpigel

BEFORE LEAVING, PLEASE CONSULT DR LEIGH LIVNE TO CHECK YOUR ATTENDANCE AND REIMBURSEMENT CLAIM

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Invited Speakers



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

Professor John Bolton



Emeritus Prof John J. Bolton

**Department of Biological Sciences,
University of Cape Town, South Africa**

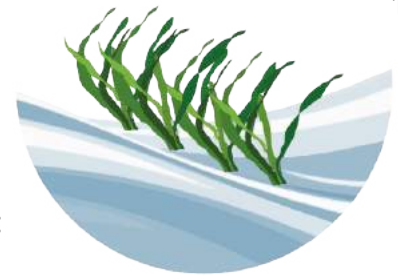
John J Bolton has been a leading marine plant scientist in South Africa for more than three decades. Work of his research group has greatly increased our knowledge of the biodiversity and taxonomy, biogeography, ecology, and resource use of South African seaweeds, and provided scientific backing to the 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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Two decades of commercial integrated aquaculture of *Ulva* with abalone in South Africa: feed and much more

John J Bolton^{1*}, Marissa Brink-Hull¹, Morgan J Brand¹, Mark D Cyrus^{1,2}, Brett M Macey^{1,2}

¹Department of Biological Sciences, University of Cape Town, Rondebosch 7701, South Africa

²Department of Forestry, Fisheries and the Environment, Cape Town, 8001 South Africa

*john.bolton@uct.ac.za



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 lacunculata* 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.





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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.

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



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Dr. Jang Kyun Kim



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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, Ministry 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.



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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 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 China, where the biomass was as high as about 3.5×10^6 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





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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.



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

Dr. Stephan Kraan



The Seaweed Company Ltd

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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 in 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.



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Ulva aquaculture: opportunities, exploitation, products and gaps

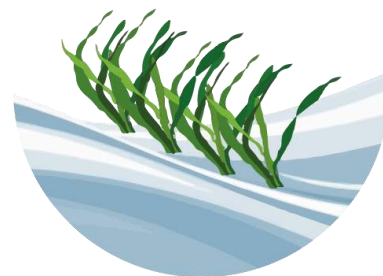
Authors: Dr Stefan Kraan*

Affiliations: The Seaweed Company Blue Turtle , Galway ireland

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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 earnest 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 sulphated 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.





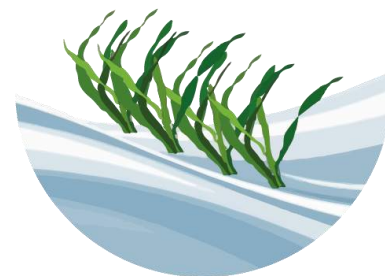
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Keynote Speakers



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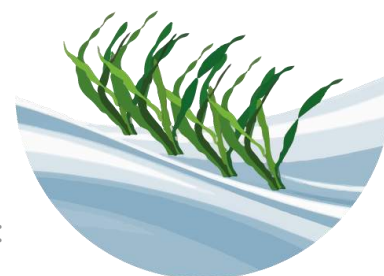
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Annette Bruhn

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.



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Ecosystem services of *Ulva*

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⁴Applied Marine Research, University of Applied Sciences Bremerhaven, 27568 Bremerhaven, Germany

⁵Wageningen University, Mathematical and Statistical Methods - Biometris, Wageningen, Netherlands

⁶University of Malaga, Department of Ecology and Geology, Blvr. Louis Pasteur, 31. 29010 Malaga, Spain

⁷Copenhagen University, Department of Food Science, Rolighedsvej 26, 1958 Frederiksberg Denmark

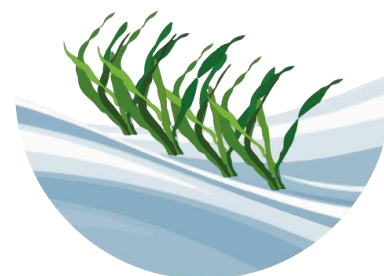
*anbr@ecos.au.dk

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.





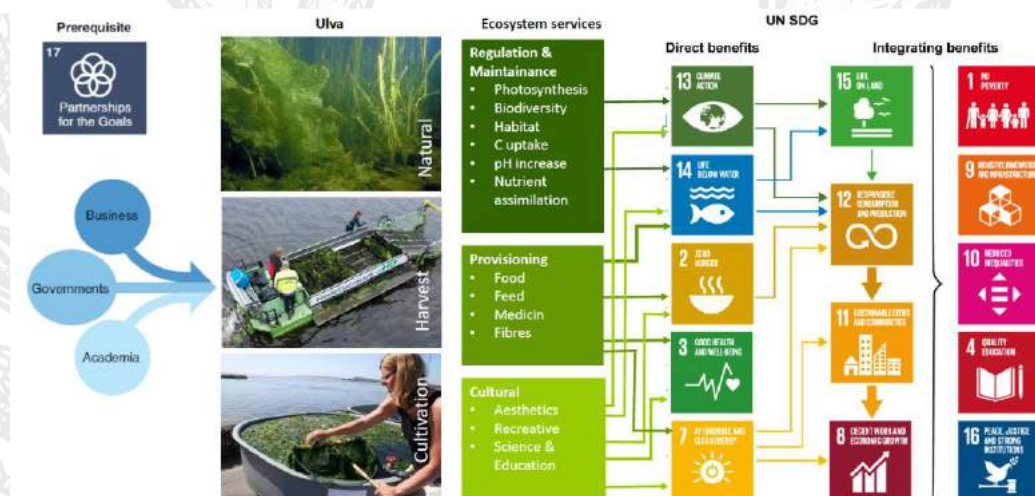
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In EU alone, 50 tonnes of cultivated *Ulva* and 217 tonnes of harvested *Ulva* is annually (2019) used for food, feed, fertiliser, biomolecules with focus on both complementary protein sources and unique fibres, such as ulvane. The corresponding biorefinery 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 instiutitions will accellerate innovation and development, while ensuring sustainability.

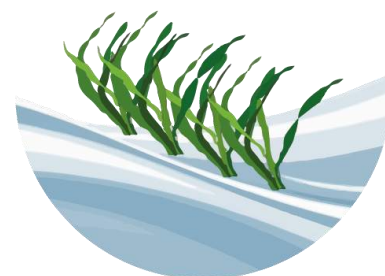
Graphical abstract



Ulva Ecosystem Services and their relations to the UN Sustainable Development Goals (SDGs).



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

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 (BRASDEC). In 2012 I decided to leave the Academic world and co-founded ALGApplus, 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.



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Ulva production at commercial scale, engineering and beyond...

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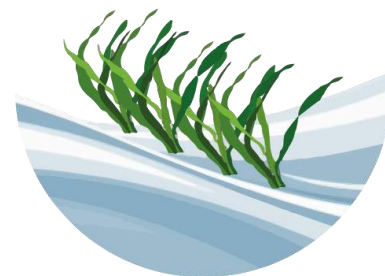
Producing *Ulva* for commercial applications, irrespectively of the scale of production, as many more challenges than engineering. In fact, planning your *Ulva* farm in an “engineer manner” is always a good practice, but can become more critical after a certain scale of production and depending on the 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 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 challenges, but it is just as important to know they exist.





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

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:

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Marine biotechnology: RRI, Convention of Biological Diversity, Nagoya protocol, Access and Benefit sharing Clearing house.

Céline Rebours^{1*}, Xenia T. Schneider², Belma Kalamujić Stroil³, Christiana Tourapi⁴, Susana P. Gaudêncio^{5,6}, Lucie Novoveska⁷ and Marlen I. Vasquez³

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TOMORROW'S 'WHEAT OF THE SEA': *ULVA*, A MODEL FOR AN INNOVATIVE MARICULTURE

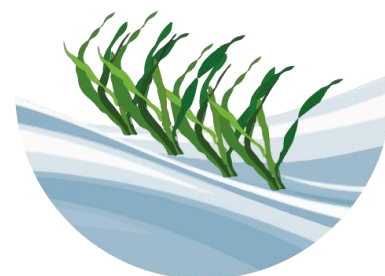
Blue Biotechnology (BB) is a multidisciplinary, knowledge- and capital-intensive technological area that significantly contributes to innovations in the pharmaceutical, medical, cosmeceutical, nutraceutical, aquacultural, agricultural, and energy sectors. The BB sector in Europe includes non-traditionally 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. The results of the qualitative survey conducted at the 2ndMC 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.

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 \(mdpi.com\)](#)



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

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.



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Opportunities for *Ulva* as sustainable crop in human and animal consumption

Dr. Sylvia Strauss

The Seaweed Company
sylvia.strauss@theseaweedcompany.com

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.





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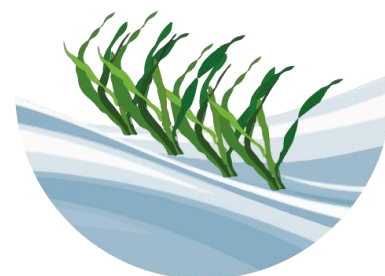
TOMORROW'S 'WHEAT OF THE SEA': ULVA, A MODEL FOR AN INNOVATIVE MARICULTURE

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.



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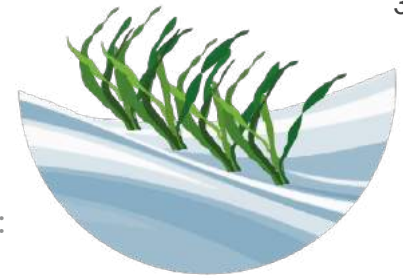
TOMORROW'S 'WHEAT OF THE SEA': ULVA, A MODEL FOR AN INNOVATIVE MARICULTURE

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.



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Ulva breeding: a huge potential for increased yields, but care should be taken!

Antoine Fort^{1,2}, Clara Simon¹, Alisha Nelly¹, Masami Inaba¹, Kevin Cascella³, Philippe Potin³, Marcus McHale¹ & Ronan Sulpice^{1*}

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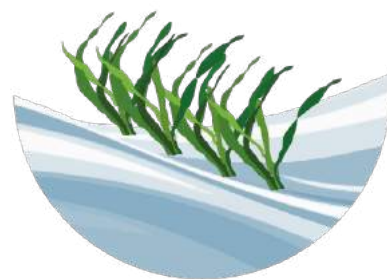
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Sea lettuce (*Ulva* spp.) is recognised for its potential in food, pharmaceutical, nutraceutical, biorefinery and bioremediation industries and is increasingly being cultivated in aquaculture. The demands of industrial applications vary widely in terms 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.





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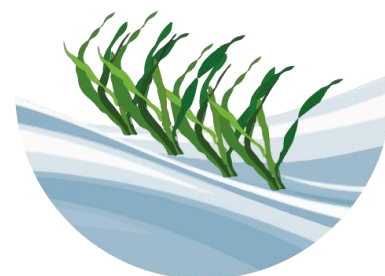
TOMORROW'S 'WHEAT OF THE SEA': ULVA, A MODEL FOR AN INNOVATIVE MARICULTURE

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.



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

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 Skłodowska-Curie Innovative Training Network [ALFF]. He works as an Associate Editor for the journal *Botanica Marina*.



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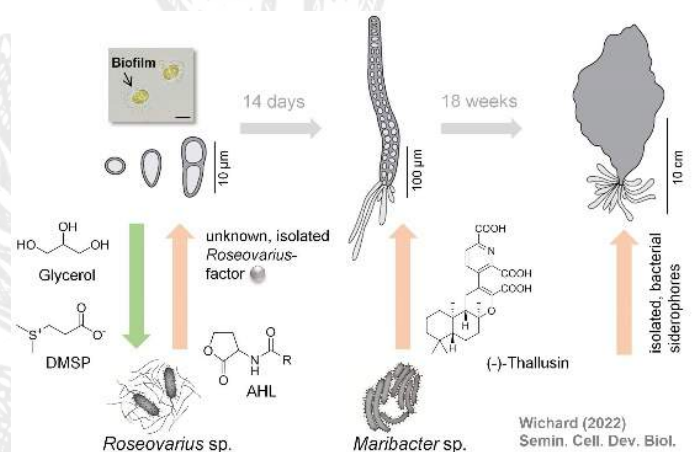


The green macroalga *Ulva* - a model system in chemical ecology and its potential applications in aquaculture

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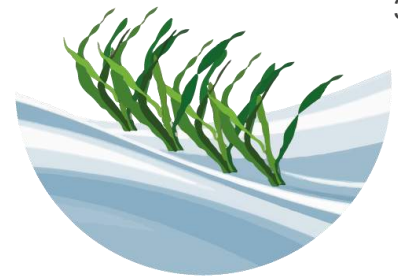
The marine green macroalga *Ulva* (Chlorophyta, Ulvales) coexists with a diverse microbiome. Many *Ulva* species proliferate in nature and form green tides, which can occur when nutrient-rich wastewater is flushed into the sea. Bacteria are necessary for the adhesion of *Ulva* to its substrate, its growth, 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.



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Oral Presenters Abstracts



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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 off-shore cultivation have completely different production lines, therefore the environmental impact





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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.



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State of knowledge regarding the potential of macroalgae cultivation in providing climate-related and other ecosystem services

Authors: Bermejo R^{*1}, Buschmann A², Capuzzo E³, Cottier-Cook E⁴, Fricke A⁵, Hernández I⁶, Hofmann LC⁷, Pereira R⁸, van der Burg S⁹.

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⁷ Alfred Wegener Institute Helmholtz Center for Polar and Marine Research, Germany

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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





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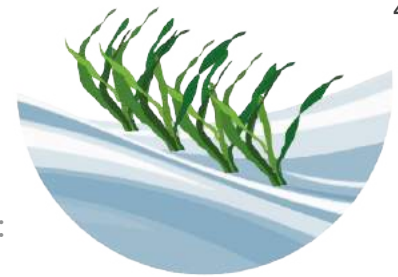
TOMORROW'S 'WHEAT OF THE SEA': ULVA, A MODEL FOR AN INNOVATIVE MARICULTURE

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.



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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





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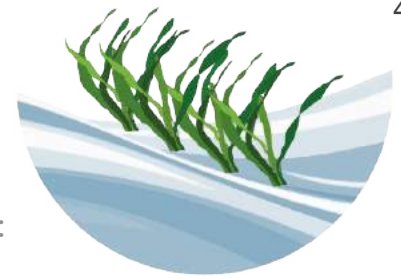
TOMORROW'S 'WHEAT OF THE SEA': *ULVA*, A MODEL FOR AN INNOVATIVE MARICULTURE

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.



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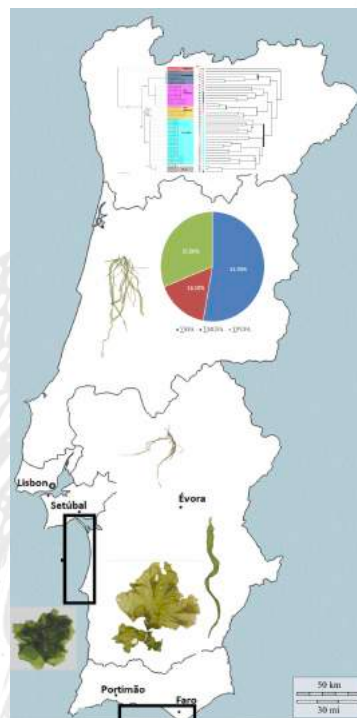
Ulva microbial contrasts between lagoon and open coast habitats in southern Portugal

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Graphical abstract:





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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 non-indigenous 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.



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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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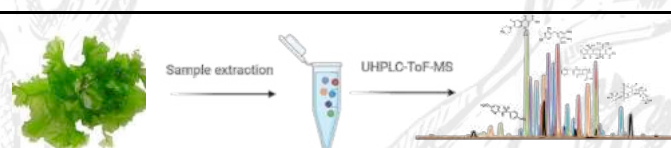
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The increasing use of macroalgae in animal feed, including in the aquaculture industry, has several advantages, one of them their high nutritional properties. Another interesting benefit is related with their ability to function as biofilters, with 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





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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.



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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 (gluco- 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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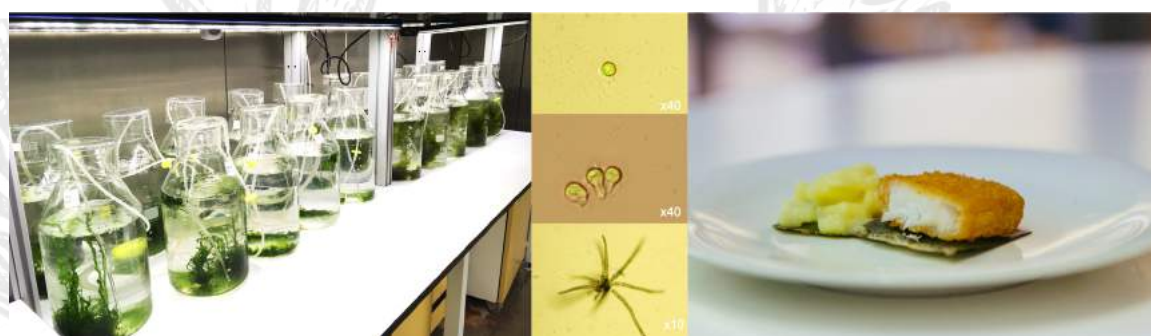
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The use of single-use packaging materials has increased dramatically in recent decades in parallel with increasing trends in convenience and fast-food. Most of these packaging materials are made of non-biodegradable, petroleum-based polymers that have degradative impacts on the environment and contribute to the global plastic pollution crisis. Finding alternative packaging materials is an important step towards building a bio-based circular economy. Sustainable land-based 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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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?





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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.

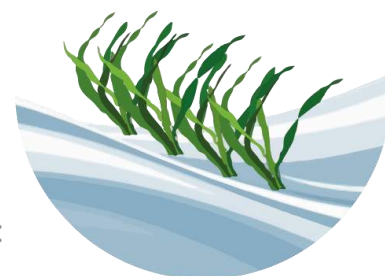
The Azores islands appears to be the most suitable location, because the temperatures are warm but moderate. These weather and climate conditions give the best results regarding nutrient, water and energy use efficiency and economic revenue. Ulva lactuca is the highest-potential crop species due to the 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 especially supports fish farmers with their farm close to saltwater sources and who are interested in sustainable farming approaches for people, profit and planet.



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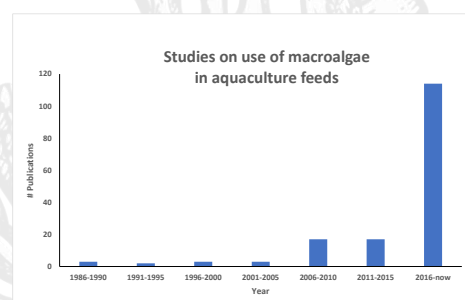


State of the art of Applications of *Ulva* and its extracts in fish and invertebrate aquaculture

Authors: E.-j. Malta

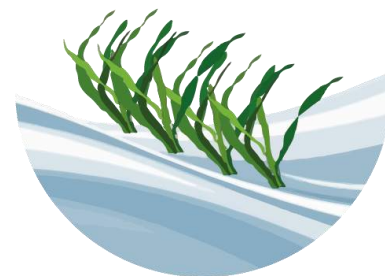
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To be able to feed the ever-growing world human population, animal and plant marine aquaculture is considered as one of the most promising strategies. As it does not rely on fresh water and can be carried out at sea or in transitional areas on saline 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).





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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.



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SeaWheat COST Action Conference

“*Ulva*: from fundamental biology to aquaculture:
state of the art, bottlenecks and gaps”



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Cultivation of *Ulva intestinalis* in fish farm effluents – experience from the NE Baltic Sea

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Intensive finfish aquaculture damages the environment by releasing large quantities of nutrients which drives eutrophication in aquatic ecosystems like the Baltic Sea. Level of eutrophication is the main obstacle for licensing finfish aquaculture in NE Baltic Sea as countries have committed to reduce nutrient flow to the Baltic environment. Macroalgae are efficient in uptaking nutrients as they grow with past studies suggesting their integration into aquaculture systems as a means to improve wastewater quality. This study was designed to assess the feasibility of using macroalgae as a biological filtration system for the removal of dissolved nutrients found in finfish farm wastewater. To test this, an experimental fish farm and mesocosm system was established on the northern coast of Saaremaa island, West Estonian archipelago. The green algae *Ulva intestinalis* was selected as a good candidate to assess the efficacy of a macroalgae biofiltration system to uptake nutrients. The results obtained show at best a 18–25% reduction in waste water nutrient concentrations for the nitrogenous compounds nitrite and nitrate for mesocosms stocked with macroalgae compared with the control. The system experienced an average 60% reduction in nitrogen and phosphorus concentrations in wastewater outflow compared to concentrations present within the finfish mesocosm. Additionally, the subsequent biomass gain of the incubated macroalgae species *Ulva intestinalis* is reported to be 4% per day at its maximum rate. The results obtained in this study indicate that *Ulva intestinalis* can be integrated into aquaculture systems as a nitrogen biofilter making possible to increase the amount of finfish aquaculture otherwise eutrophicated Baltic Sea. In addition, the macroalgae biomass produced may offer aquaculture operations an additional income stream improving farm economics.



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Carotenoids as versatile compounds for the sustainable production of health-promoting products

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Carotenoids are isoprenoids compounds of great 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 health-promoting 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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SeaWheat COST Action CA20106

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

revealed fastest growth and α -amylase activity in 3% salinity at 30°C temperature. Finally, we have inserted of a synthetic plasmid of pUC19 which contain the loci of enzymes glycoside hydrolase 16 and carbohydrate binding modules CBM 4 and 6, as in the genome of *Alkalihalobacillus* sp., into competent cells of *E. coli* dh5 alpha. This transformation was successful in terms of the degradation of laminarin by *E. coli* while on-going trials aims at examination of ulvan degradation by the competent *E. coli* and the extraction and characterization of the relevant proteins.



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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 (Kützinger) 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= $20.22^{\circ}\text{C} \pm 7.2\text{SD}$), salinity (36.74 ± 0.89), suspended solids (17.65 ± 10.32 mg/l), total dissolved inorganic nitrogen ($\text{N-NO}_3 = 9.15 \pm 6.45$ $\mu\text{mol/l}$), and dissolved reactive phosphorus (0.53 ± 0.21 $\mu\text{mol/l}$) 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,





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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 , $\Phi PSII$, $rETR_{max}$, E_k), 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 29 2019 that lowered the salinity (34.4) and accumulated nitrogen (11.28 $\mu\text{mol/l}$) and suspended sediment (41.11 mg/l) reduced the photosynthetic efficiency (α) 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 DIO/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: 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.



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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 than $100 \text{ g DW m}^{-2} \text{ d}^{-1}$, 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.





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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.



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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* spp. play an important role both in the morphogenesis and in the reproduction of *Ulva*, it is possible that bacteria also play a role in *Ulva* spp. adaptation to light. Moreover, *Ulva* laminar thallus provides an important niche for biofilm-forming 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* spp., was demonstrated in experimental infections with *V. anguillarum*. Those results paved the path to the possible manipulation of *Ulva*-associated bacterial communities as strategy for *Vibrio* control in fish-*Ulva* IMTA-RAS cultures.





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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 developed in a pilot-scale system with effluents from sole (*S. senegalensis*) cultures.

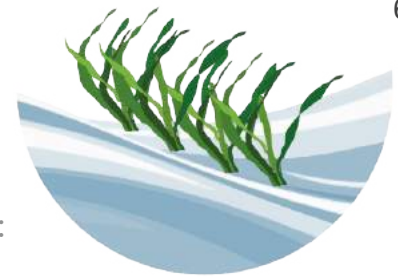
Aknowledgements

The UlvaQuo Project funded by the Spanish Ministry of Science, Innovation and Universities MICINN (Refs. RTI2018-095062-A-C21 and RTI2018-095062-A-C22)



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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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Sustainable large-scale aquaculture of the northern hemisphere Sea Lettuc: *Ulva fenestrata*, in an off-shore seafarm





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TOMORROW'S 'WHEAT' OF THE SEA: *ULVA*, A MODEL FOR AN INNOVATIVE MARICULTURE

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.



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The seaweed *Ulva lactuca* as a nutritional ‘tool’ to prevent Chronic Degenerative welfare Diseases (CDDs) in the human population.

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Introduction: 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, NAFLD, atherosclerosis, mental disorders, and some cancers.

M&M and Results: 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





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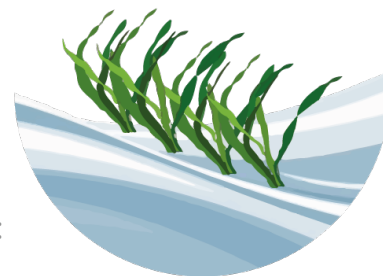
ω 3 'fish oil' LC-PUFAs EPA(C20:5) & DHA(C22:6) also operating via eicosanoids in an anti-inflammatory way. An important observation is that the two rate-limiting enzyme systems of Δ -6 desaturase: ω 6: from LA \Rightarrow γ -Linolenic acid (C18:3) & ω 3: ALA \Rightarrow Stearidonic acid (C18:4) are both present. Striking, the rare Δ -17 desaturase, catalysing conversion of ω 6 ARA(C20:4) to ω 3 'fish-oil' EPA(C20:5) is present.

Conclusions & Perspectives: *Ulva lactuca* is a good and virtually inexhaustible source for PUFAs with an ω 6/ ω 3 ratio of $\approx 1.0 \Rightarrow$ presently $\approx 25-50!$ 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.



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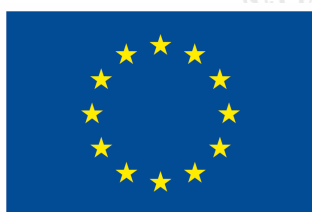
Ulva, seagrasses and ecosystem services: a tale of love and coldness

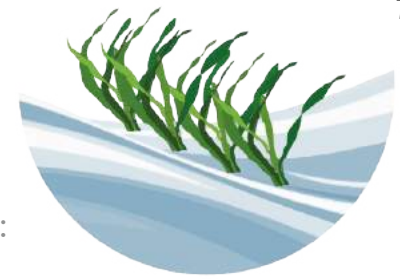
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It is well known that eutrophication causes negative effects on shallow coastal ecosystems and affects the ecosystem services they provide. Often these effects trigger massive proliferations of ephemerophytes such as *Ulva* (green tides), which can harm seagrass meadows due to shading by dense overgrowing mats. In addition, seagrass development can be undermined by direct ammonium toxicity, as 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, 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 macroalga, 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* spp. germlings for bioprocessing

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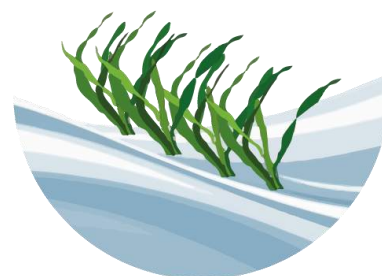


Ulva species have cryptic morphologies with few distinctive features, as well as significant intraspecific variation. This often renders them difficult to identify. This study reports the sustained growth of *Ulva* spp. germlings in culture and their identification via morphological characters and DNA 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* spp. 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* spp. that are being grown in culture, *U. compressa*, *U. torta* and *U. californica* have been successfully barcoded so far.





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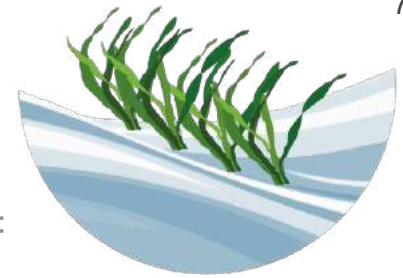
TOMORROW'S 'WHEAT' OF THE SEA: ULVA, A MODEL FOR AN INNOVATIVE MARICULTURE

Poster Abstracts



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Enriching *Ulva* sp. with nutritional compounds by exploiting IMTA

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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.





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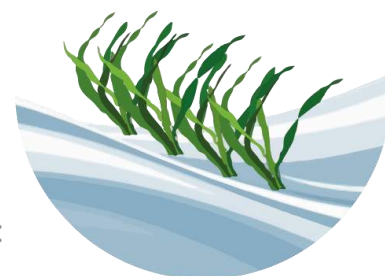
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The seaweeds were then exposed to short-term abiotic stressors (i.e., high irradiance, nutrient starvation, and high salinity) to stimulate synthesis of desired compounds in their tissues. Our methodology enabled high growth rates (25% biomass increase per day), with significant enhancements in the amount of protein, starch, and minerals. Moreover, the seaweeds removed up to 90% of the Ammonia, NO_3 and PO_4 . 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.



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Influence of abiotic factors on valuable compounds in natural and cultivated biomass of *Ulva* spp.

C.L. Cara, D.B. Stengel

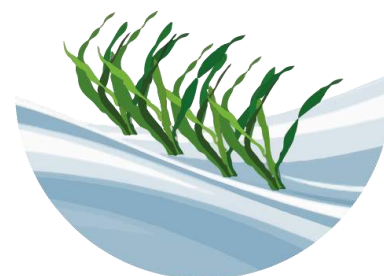
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The genus *Ulva* is globally highly abundant and widely distributed within coastal marine macroalgal communities. In addition to its ecological significance as an important primary producer, its commercial value is increasingly recognised. As the world's population continues to rise, it is imperative to focus on the sustainable production of new food sources. *Ulva* spp. (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, water movement, 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.



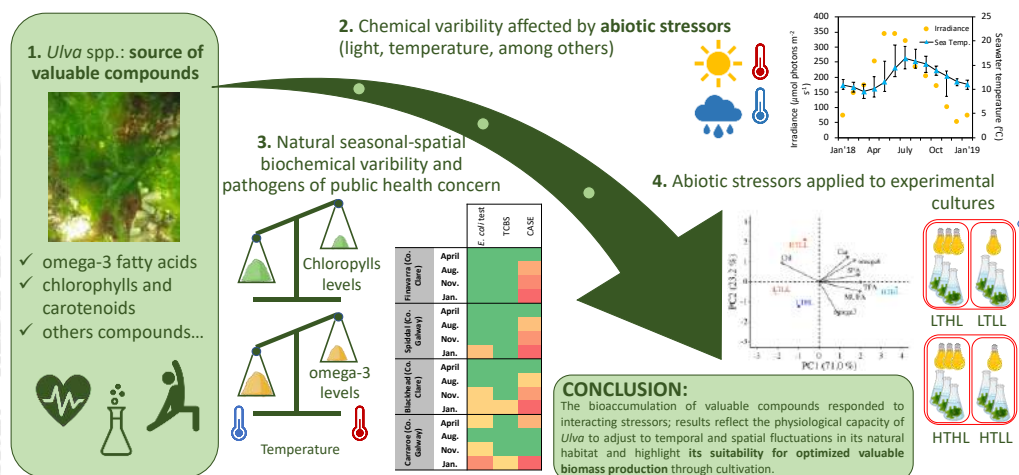


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TOMORROW'S 'WHEAT OF THE SEA': ULVA, A MODEL FOR AN INNOVATIVE MARICULTURE

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.

Influence of abiotic factors on valuable compounds in natural and cultivated biomass of *Ulva* spp.





Salinity as a key factor for strain selection and optimization of *Ulva* spp. for land-based cultivation

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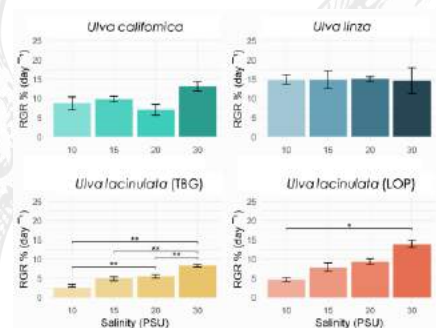
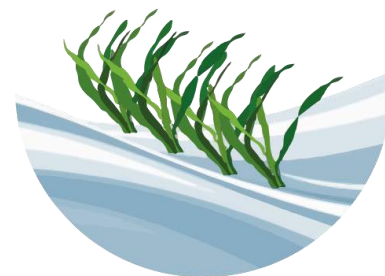


Figure 1 - Relative Growth Rate (in % day⁻¹) of *Ulva*'s germlings after 3 weeks under different salinity conditions and statistical comparison between treatments (pvalue ≤ 0.05).

The green seaweed *Ulva* spp. 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





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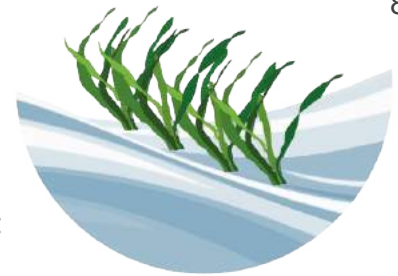
TOMORROW'S 'WHEAT OF THE SEA': *ULVA*, A MODEL FOR AN INNOVATIVE MARICULTURE

material and to sustainably scale-up the production of seaweed, including *Ulva* spp., 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 land-based production. At the same time, the data presented serve as a baseline for further work with *Ulva* under different cultivation conditions.



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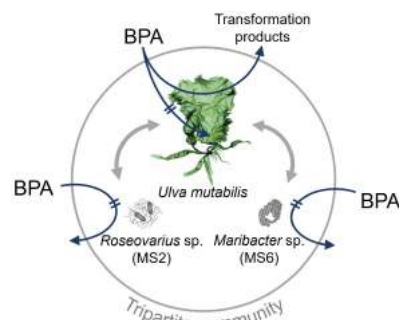


Ulva mutabilis removes the endocrine disruptor bisphenol A without bacterial support

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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.





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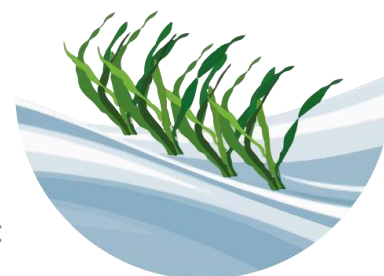
TOMORROW'S 'WHEAT OF THE SEA': *ULVA*, A MODEL FOR AN INNOVATIVE MARICULTURE

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.



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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.





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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 - $28.68 \pm 0.15\%$), 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 - $49.73 \pm 7.24\%$ 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 ALGApplus, Ltd.



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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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Abstract

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





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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

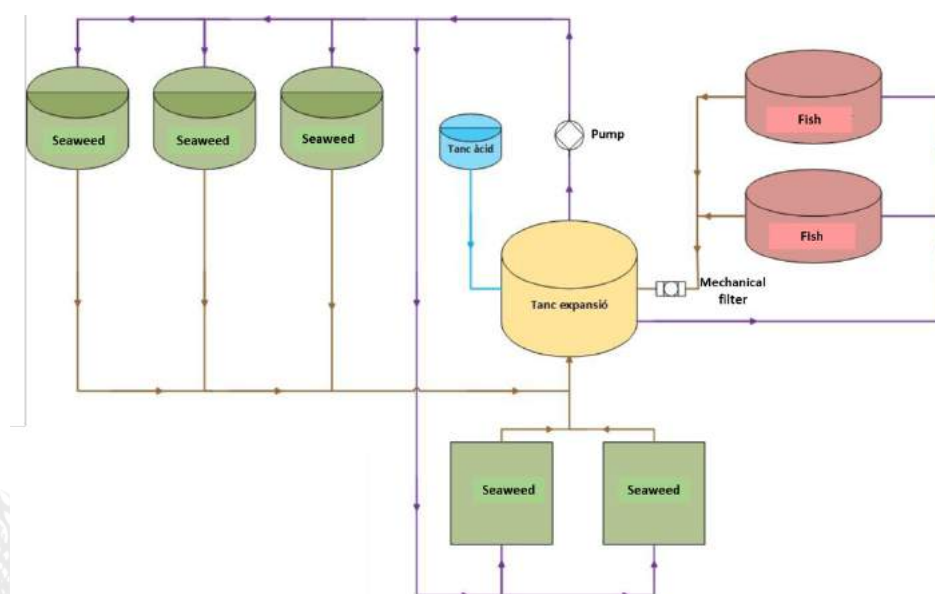


Fig 1. IMTA-RAS system (Sole-*U. ohnoi*)

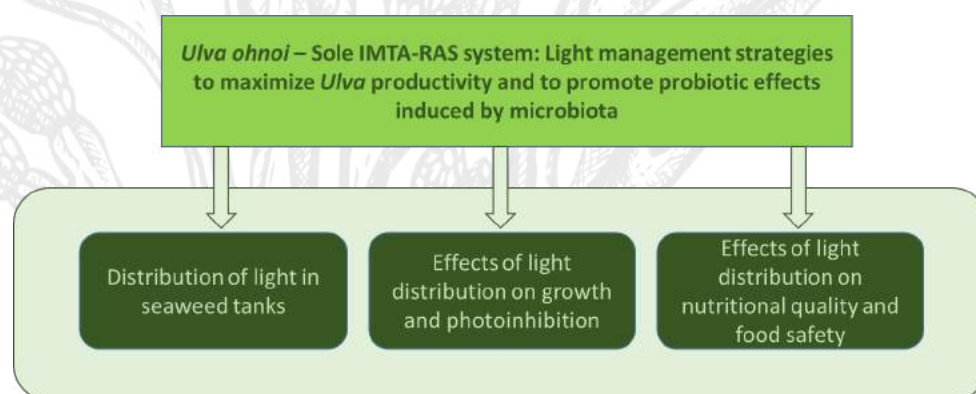


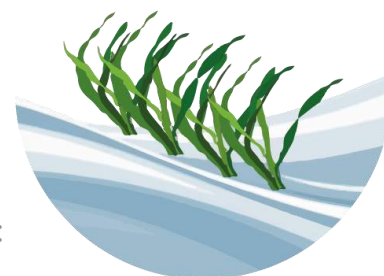
Fig. 2: Main points of the UlvaPro project

Acknowledgments: The UlvaQuo Project is funded by the Spanish Ministry of Science, Innovation and Universities MICINN (Refs. RTI2018-095062-A-C21 and RTI2018-095062-A-C22)



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

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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





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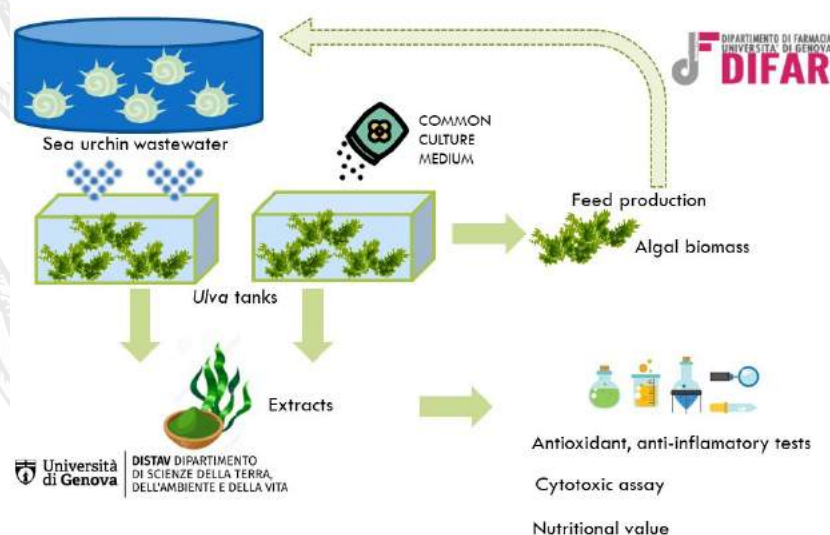
TOMORROW'S 'WHEAT' OF THE SEA: ULVA, A MODEL FOR AN INNOVATIVE MARICULTURE

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 bio-depurators and as a source of substances of commercial interest in a circular economy perspective.

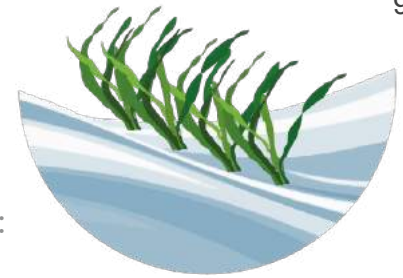
This experimentation complements a project that aims to reuse sea urchin waste from the food industry (BRITEs project, <https://www.ricicliamo.it/brites/>) to produce high-valuable materials, as marine collagen (from sea urchin peristome) and bioactive calcium-rich flour (grounding sea urchin test). 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.

Graphical abstract:



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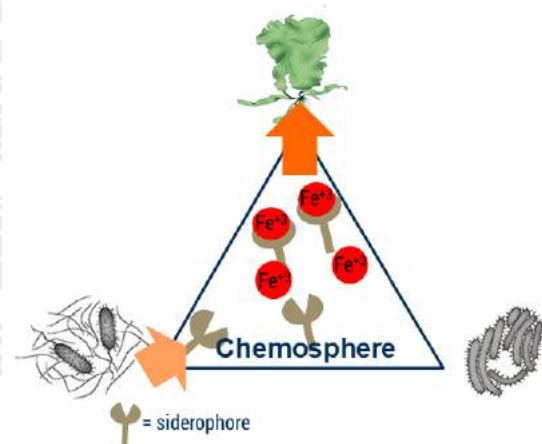


Macroalgal-bacteria interactions: screening for siderophores and their ecophysiological role in the chemosphere of the green tide forming macroalga *Ulva* (Chlorophyta)

Cristina F. Morales-Reyes^{*1}, Michael Deicke¹, Christian Paetz², and Thomas Wichard¹

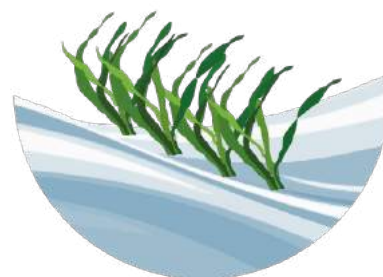
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Ulva spp. life is tightly dependent on its microbiome. Bacteria produce algal growth and morphogenesis promoting factors (AGMPF) and contribute to *Ulva*'s growth and development. Recently, in chemical ecology, *Ulva mutabilis* has been considered 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.





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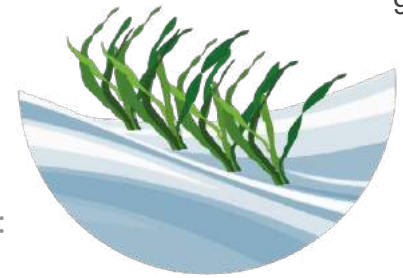
Ulva and its associated bacteria require iron to manage essential biological processes like most organisms. However, due to low iron bioavailability in marine ecosystems, organisms have developed several recruitment strategies. For example, bacteria release siderophores to acquire iron from the environment. 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. $\text{Fe}^{(III)}$ -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 cross-kingdom interactions potentially applied in the maintenance of algal aquacultures.



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Developing a protocol for the effective application of *Phaeobacter* in *Ulva* cultures for pathogen control in IMTA-RAS

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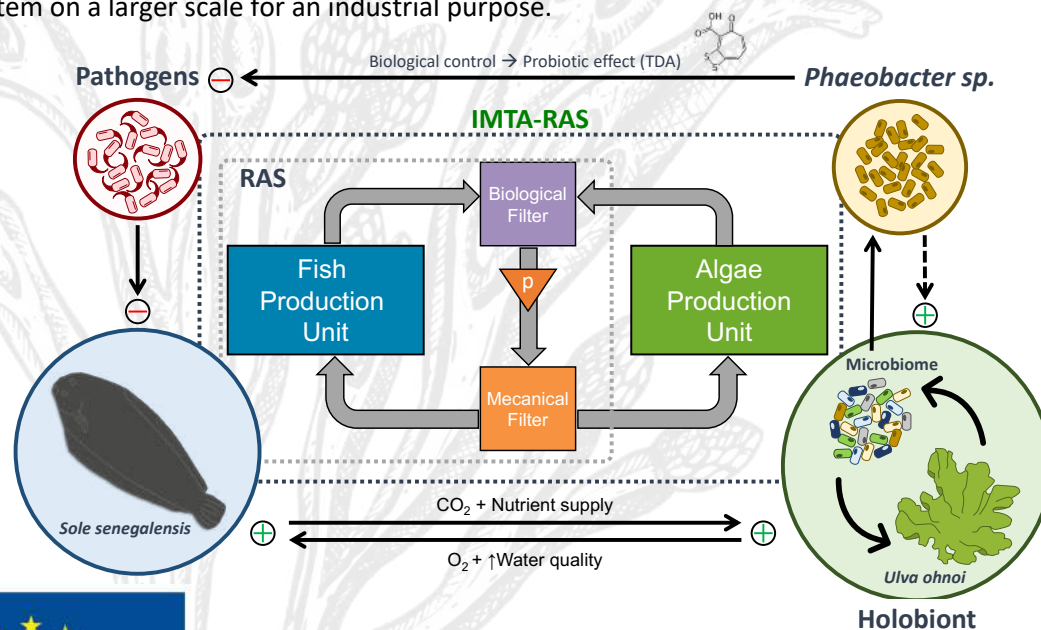




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The co-culture of *Solea senegalensis* and *Ulva ohnoi* in an integrated multi-trophic aquaculture recirculation systems (IMTA-RAS), is one of the most promising possibilities for the development of a more sustainable aquaculture. Moreover, *Ulva* not only improves water quality by removing nutrient-excess 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 influenced 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.





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* spp. is an economical relevant genus with several applications in the industry, including aquaculture. Thanks to *Ulva* spp. high growth rate and physiological plasticity, *Ulva* spp. 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* spp. 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* spp. surface, and its concomitant production of TDA.





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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.



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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 environmental 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 cosmetic 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* fluorescence 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 °C. Effective quantum yield range from 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 of cultivation under natural conditions, with chemical fertilizers and without the addition of 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 its photosynthesis is not inhibited by changes in pH (due to the change of carbon forms) and reducing the risk of the microalgae growth in the *Ulva* ponds.





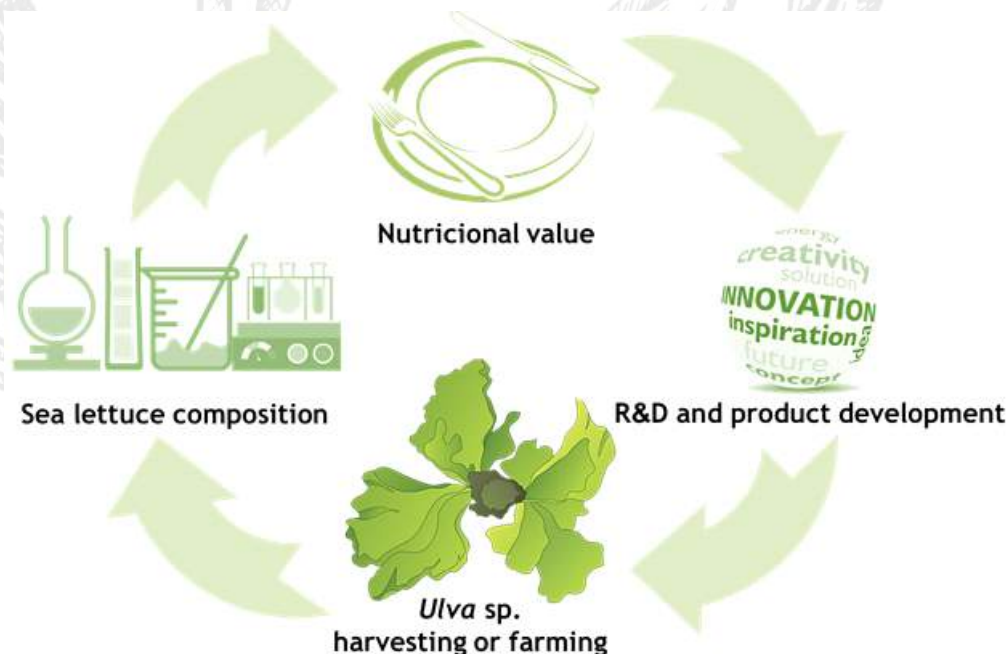
Earthen pond to fork: the journey of the sea lettuce from the bay of Cádiz to the consumer's plate

Authors: Fini Sánchez-García ^{1,*}, Ignacio Hernández ², Víctor M. Palacios ¹ and Ana M. Roldán ¹

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TOMORROW'S "WHEAT OF THE SEA": *ULVA*, A MODEL FOR AN INNOVATIVE MARICULTURE

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* spp. 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.



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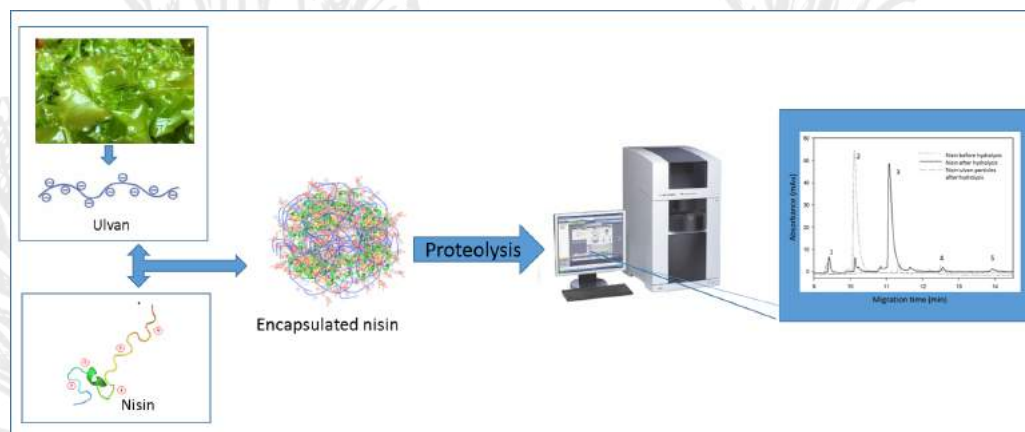


The application of ulvan for the proteolytic stabilization of nisin

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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-





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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.

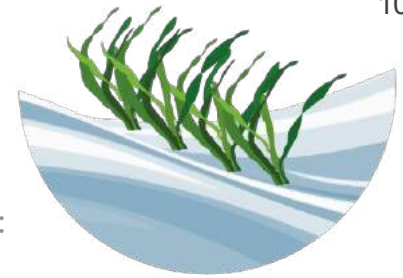
The particles were prepared in the pH range of 4.0-7.0 and at the final ulvan and nisin concentrations of 0.4 and 0.2-0.5 mg/mL, respectively. For the determination of proteolytic stability, two types of proteases with different pH optimum and cleavage specificity were used, i.e. the protease Type XIII 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*.



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A rapid methodology for the selection of *Ulva* elite strains tailored to specific growth conditions

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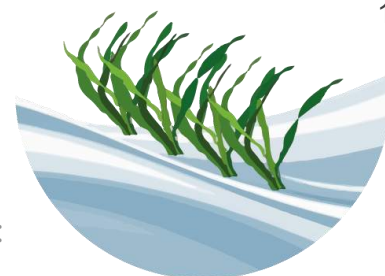
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Sea lettuce (*Ulva* spp.) is recognised for its potential in food, pharmaceutical, nutraceutical, biorefinery and bioremediation industries and is increasingly being cultivated in aquaculture. The demands of industrial applications vary widely in terms of biomass composition and cultivation requirements. To screen a large diversity of wild-isolates, 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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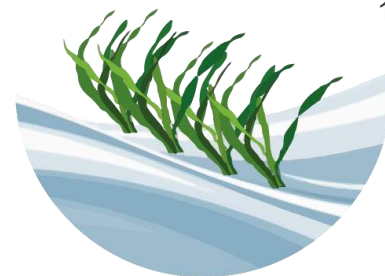
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Growth and development of the sea lettuce *Ulva* spp. (Chlorophyta) depend on growth- and morphogenesis-promoting factors released by associated bacteria. Hereby, the morphogen thallusin induces the rhizoid and cell wall formation in *Ulva* at a very low concentration of 11 pmol L⁻¹. It is mainly produced by various species of *Maribacter* spp. and *Zobellia* spp. (Bacteroidetes). 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 sterile-filtered 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-HR-MS). Before the analysis, a derivatization step of the carboxylic acid groups was crucial to avoid iron-complexing, 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.





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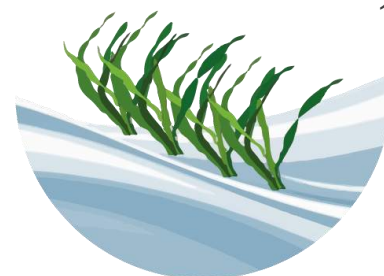
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The bacterial thallusin concentration was measured at selected growth phases with values of 0.16 ± 0.01 amol cell⁻¹ (turning point of the exponential growth), of 0.27 ± 0.05 amol cell⁻¹ (end of exponential growth) and of 0.86 ± 0.13 amol cell⁻¹ (late 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.



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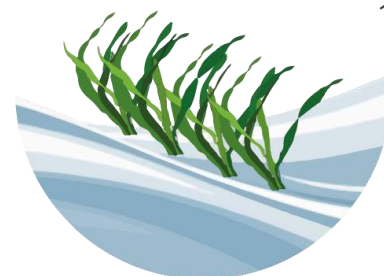
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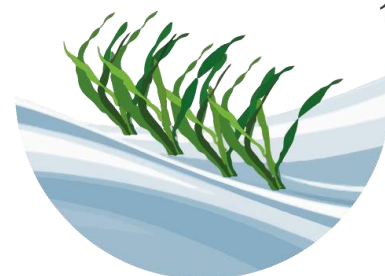
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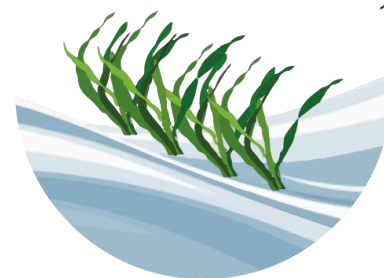
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