SEAWHEAT COST ACTION – ANNUAL REPORTS
2022, 2023, 2024
WORKING GROUP LEADERS REPORTS (WG1–WG6)

2022 REPORT

WG1 Report

WG 1. Ulva biology: the collective knowledge on the different aspects of Ulva biology, including systematics, genetics, reproduction, microbiome, diseases, and ecology, will be shared and prioritised.

Ronan Sulpice (University of Galway, IE)

Co-leaders WG1: Olivier de Clerck, Thomas Wichard, Martina Orlando Bonaca

Membership: 32

Countries: 16

Summary of progresses and plans for next year.

Members of WG1 have been assigned to the three tasks part of WG1.

Task 1.1: The inconsistent systematics and phenotypic plasticity of the Ulva genus will be clarified. This task is linked to D 1.1.

This task is extremely wide in its scope and has been sub-divided.

Section 1. Develop a network of people collecting samples. Coordinates and abiotic environment will be documented. This work will also inform Task 1.3.

The leads are Olivier DeClerck. Sophie Steinagen, Florian Weinberger, Erik Jan Malta, AfCshwin Engelen, Celine Rebourg and Ronan Sulpice.

The methods deployed will include e-DNA technology (Florian Weinberger) and CAPS assays (Ronan Sulpice) in addition to “classical” barcoding. A citizen science approach will be investigated.

Section 2. “Clean” taxonomy.

A MS is on the way (Olivier De Clerck). We are considering for this group researchers outside cost countries.

Task 1.2: The microbiome (bacterial - seaweed interactions) will be identified at the molecular level; D 1.1.

LEADS: Aschwin Hillebrand Engelen, Thomas Wichard, Marcus McHale

This project will make use of the network of harvesters set up in Task 1.1. Thomas Wichard and his group will send kits to harvesters that they can collect water samples for analyses for the identification of morphogens (e.g. thalusin). Microbiome will also be analysed on some Ulva specimen, but budgetary issues for sequencing and chemical analyses still require to be sorted.

Task 1.3: The seasonal and environmental impact factors on Ulva reproduction, growth, biochemical composition, and productivity (including nutrients, light, temperature, etc.) will be identified; D 1.1.

LEADS: Erik Jan Malta. Sophie Steinagen, Rui Perreira, Martina Orlando-Bonaca and Sortiris Orfanidis

For this task, a meeting with WG2, WG3 and WG4 leaders is planned (first week of October 2022)

Deliverables:

Book on Ulva - Thomas Wichard will edit a book containing mini reviews about each deliverable of the COST action. Publication date: last year of the COST action.

A review is considered in 2023 roughly, and one is in revision at present (Simon, McHale and Sulpice, Applications of Ulva biomass and strategies to improve its yield and composition: a perspective for Ulva aquaculture, Biology).

WG1 is currently preparing a training school on “Ulva Biology”. Location Greece, tentative dates: 25-28 April 2023.

WG2 Report

WG2, Ulva in aquaculture - Progress report for year 1

During the first year of activity, WG02 increased the member participation from 52 members in the first trimester to 193 members in September 2022. These members include 27 countries, 9 of which are ITC countries, but also Cost Partners and International Partner countries. WG02 is being lead by Rui Pereira with the support of Ricardo Bermejo and Erik Malta.

The overall objective of the WG is to consolidate the collective knowledge on culture technologies (land and sea-based), strains selection, fertilization, seeding, biomass production, abiotic and biotic culture factors and harvesting of Ulva. To achieve this goal, 4 tasks were designed and 3 of them have very clear deliverables. A guideline of engineering concepts for large-scale cultivation in land and sea-based facilities (D2.1a); Cultivation protocol of spore collection and Ulva preservation, seeding, cultivation and harvesting in land and sea-based facilities (D2.1b) and a report on the potential impediments (biological, technological, economic and political) for Ulva biomass production (D2.1c). An initial questionnaire was sent around to all the members prior to the first general meeting, where a working methodology was discussed and accepted. To facilitate the organization, promote the networking and keep focus on final deliverables, 4 sub-groups were created each one with 2-4 Co-Leaders as follows: 1-2 Co-Leaders based on the results of the questionnaire; 1 Co-Leader from the 3 WG Leaders and 1 Co-Leader considered a Young Researcher. These non-official positions were assigned and sub-group activities started. During the rest of the year, each sub-group leaders worked on a proposition of contents for their respective deliverables and these will now be open to the entire WG02 membership. Despite the creation of these sub-groups, each WG02 member is free to be involved and contribute to several or all of the sub-groups.

During the 4th management committee meeting, in Sep 2022, it was discussed that Task 4 of WG02, dealing with bacterial-algal interaction should be merged with WG04 (Bioactive Chemicals and Ulva-associated microorganisms).

The leaders of WG02 were actively involved in the preparation of the 1st SeaWheat conference, in Cadiz, Spain, from 13 to 15th September to 2022 as well as in the activities of the SME platform and in attracting new SME members to the network. Still during this 1st year, WG02 started the preparation of the workshop and training schools planned for year 2. For year 2, planned activities include a Training School in cooperation with WG06 (to be held in Spain) and a Workhsop in cooperation with WG03 (to be held in

Portugal).

WG3 Report

Achievements for the first year 2022

- Kick-off WG meeting 27.01.2022 (41 participants), presenting members and COST Action.

- Followed by 2nd meeting on 27.04.22 (19 participants) , 3rd on 27.06.22 (25 participants), 4th is scheduled for 14.10.22.

- Setting up a members list with detailed interests and skills according to the results of a survey that was held among members.

- During the first 2 meetings, a need for redefinition of tasks was detected to better distinguish tasks between WG3 and WG4. Subjects beyond food and feed relate now to non-edible applications excluding extracts, which refer to WG4.

- In order to be able to better manage tasks, subgroups were formed and task leader groups assigned.

- As part of the deliverable 3.1, a minireview was initiated by a team of assigned coordinators (L. Hofmann, A. Meléndez, G. Göksen) in J. Comp. Rev. in Food Sci. and Food Safety. A TOC has been set up and a first draft is scheduled for end October 22.

- To fulfil the core task 3.1a 'Ulva nutritional analysis & food safety ', we are about to initiate a database with nutritional profiles from barcoded strains provided by samples from different interested Ulva producing members. Samples will be analysed in institutions of members from WG1 and WG3 (preferably completed by microbiome analysis). This will happen in close collaboration with WG1 (O. de Clerck/UGent, T.Wichard/UJena, A.Engelen/CCMAR) and WG3 (U.Ahsan, G.Göksen/MehmetAkif Univ.).

Further plans for 2023:

- Finding funding and publication of minireview "Ulva in food, feed and beyond"

- Task 3.1.a: Several meetings planned to generate sampling instructions, finding sample contributors, coordinate analysis and data mining. Solve regulatory issues regarding MTA. Start analysing Ulva samples.

- Organisation of Workshop "From Ulva aquaculture to food & feed production: state of the art, bottlenecks, risks and gaps" together with WG2 in 1st half of 2023 in Aveiro, Portugal.

WG4 Report

Report of Working group 4 "Bioactive Products"

by the working group leader Dr Thomas Wichard

This report covers the first funding period of the COST Action CA20106 in 2021/22. Due to the high popularity of the COST Action, the number of registered participants in WG4 has steadily increased, reaching more than 90 people in the first year. In three online meetings, the focus was, therefore, increasingly on communicating the organization, goals and benefits of the network that are necessary to achieve deliverables 4.1 and 4.2. A survey among the participants showed that the expertise in the group characterized by chemical issues is wide-ranging (chromatography, mass spectrometry, NMR, nutrient

analysis). However, many participants (> 50%) have not worked intensively with Ulva up to now. Indeed, this shows the great interest in Ulva among plant biologists and (food) chemists.

Even though only about 30% of the participants are active and take on tasks, it was possible to develop many steps and milestones together: (i) organization, (ii) compilation of the status quo of Ulva research in Europe, (iii) participation in STSMs, (iv) conference contributions (in Cádiz in Sept. 2023) (v) publications in scientific journals, and (vi) participation in coordinated research campaigns of WG2,3 and 4. (vi) future activities of orchestrated activities with other WGs of the COST Action.

The following individual activities are listed one by one:

(i) Organization: The first phase of our work in Working Group 4 was focused on organization and the distribution of tasks. Subgroups were introduced to achieve the milestones. Two participants (Wichard [Germany] and Zammit [Malta]) of WG4 belong to the Editorial Committee of the book project, preparing a monography about Ulva. One WG4 representative (Dilek Unal [Turkey]) was elected for the "dissemination team" of the COST Action.

(ii) Status quo of Ulva research: To compile the ongoing research on" Bioactive products in Ulva" the WG4 will provide and publish a review article in 2023 entitled "Ulva sp. metabolites as ingredients for agro-food, pharma, cosmetic and other industries." 11 participants are involved moderated by Wichard (Germany) and Meléndez Martínez (Spain).

(iii) COST Action events: The participants of WG4 have already participated in two STSMs with the topics entitled:

Isolation of bioactive compounds from xenic and axenic Ulva cultures applied in various bioassay-guided test systems (Gülçin Sevim; host: Thomas Wichard; March 1st 2022)

Biochemical characterization of Ulva biomass: looking for bioactive compounds, particularly healthy lipids, for food and feed applications (Sarah Schembri; host: Rosario Domingues; March 1st, 2022)

(vi) Conference: At the COST Action conference "From fundamental biology to aquaculture: state-of-the-art, bottlenecks and gaps "in Cádiz (Spain) in 2022, WG4 led a session with the contributions of its participants. Some of the presentations will be published in the Proceedings of the conference.

(v) Publications in scientific journals (in progress): WG4 is actively participating in the design of a monograph on Ulva, which is being compiled and developed across groups. The book project will accompany the group throughout the funding period of the COST Action.

Participants of Working Group 4 published three publications (Open Access). COST Action CA20106 is acknowledged in those publications (participants of the COST Action CA20106 are highlighted in bold).

Morales-Reyes, C., Ghaderiardakani, F., Wichard, T. (2022) Genome Sequence of Halomonas sp. strain MS1, a metallophore-producing, algal growth-promoting marine bacterium isolated from the sea seaweed Ulva mutabilis (Chlorophyta). Microbiology Resource Announcement in press.

Wichard, T. (2022) From model organism to application: Bacteria-induced growth and development of the green seaweed Ulva and the potential of microbe leveraging in algal aquaculture. Seminars in Cell & Developmental Biology in press.

Obolski, U.*, Wichard, T., Israel, A., Golberg, A., and Liberzon, A. (2022) Modeling the growth and sporulation dynamics of the macroalga Ulva in mixed-age populations in cultivation

and the formation of green tides. Biogeosciences 19, 2263–2271.

(vi) Future activities: It was suggested that a joint search for bioactive compounds (targeted analysis) would be carried out under the various conditions of the respective working groups in the COST Action to test the general validity of the methods used. Significantly, in an interdisciplinary campaign, WG4 will contribute

to a holistic picture of the distribution of Ulva, its microbiome and nutrients with various chemical analyses of the holobiont Ulva. In particular, specific algal and bacterial substances will be identified. It can be further complemented by elaborative approaches such as metabolomics.

The organization of a Training School in spring 2024 (date, location) on the topic "Ulva's bioactive metabolites (primary/secondary)" is still being discussed within the WG4 and the COST Action.

The collaboration with the SME platform should be intensified.

In summary, an active and motivated core group of the WG4 will work on the goals in the next funding period. One challenge is coordinating the synergies of the individual groups and the scientific work, considering the various funding situation. Notably, the COST Action inspired several participants to seek funding at the national and binational levels.

WG5 Report

Seawheat WG5 Ecosystem Services

Status

WG5 has 26 members, hereof 4 co-leads and one SC representative.

Three WG5 meetings are planned every three months. Three meetings have so far been held on-line with participation of approximately 10 members every time. One meeting has been cancelled.

Three to four members have been assigned to contribute to each of the sub-deliverables of WG5.

WG5 and Ulva Ecosystem Services has been represented at the 1st Seawheat Conference in Cadiz, with one keynote and four presentations, but no WG5 publications are planned as part of the 1st Seawheat conference proceedings.

Several WG5 members aim for contributing t the planned book on Ulva.

Plans have been made for cooperation with WG6 on joint workshop in 2024 in relation to the 13th Nordic Seaweed Conference in Grenaa, Denmark.

WG5 has had no STSM candidates or hosts, no SoMe posts, and no joint publications in the 1st grant period.

Plans for 2. grant period

In the 2nd Grant period, WG5 will be focusing in discussing possibilities and challenges in Ulva Ecosystem services, including methods for quantifying Ulva Ecosystem services, models and LCA. WG5 members aim for: members participating in workshops and training schools (WG5 will be organizing WS in 2024). WG5 deliverables are planned to be published as peer-reviewed mini review hereof potentially one in 2nd grant period. The potential for other joint WG5 publications will be explored. WG5 members aim for both hosting and going for STSM’s during the 2nd grant period, if possible in relation to associated SMEs, and aiming for outcomes as joint WG5 publications.

WG6 Report

The WG6 participants had 6 working meetings. During these meetings, national contact points (NPPs) were identified. These NPPs are responsible for conducting a policy survey in their country. This information will be further used to conduct a policy analyse at the European level. The NPPs also started to identify the stakeholders involved in the Ulva sector and are today fine tuning the list to define which stakeholders could potentially be consulted to collect more information about this sector. During the first 9 months of the project, the methodology used to prepare the two first deliverables of the WG were decided particularly to address the challenges generated by the regulatory framework and, to further be able to design the

recommendations that may facilitate the development of an Ulva industry in Europe. Two consultations were already conducted: one with the SMEs (consultation by email, results presented at the Rome`s meeting) and one with the whole SEAWHEAT network at the meeting in Rome to understand the level of awareness about RRI, CBD, ABS, and NP. Consultations of stakeholders will be conducted in 2023 and 2024 to address regulatory and markets issues. In the next months, NPPs in some of the major Ulva producing countries (Figure 1) would need to be identified and hopefully join in the WG6 work.

At the SeaWheat conference, a session on Legal, Social, and Regulation Aspects addressed specially the aspects of social responsibility, risks and environmental sustainability while developing new production systems. During the session, the knowledge build under another Cost Action (CA 18238 Ocean4Biotech) was transferred and results of the consultation conducted in Rome was presented to the Seawheat network.

Collaboration with the leaders of WG1, WG2 and WG3 were established to ensured implementation of the NP. Close collaborations with the leaders WG2 and WG5 resulting in drafting the future common Training Schools in 2023 and 2025.

Figure 1: Geographic distribution of the NPPs.

2023 REPORT

WG1 Report

CA20106 SEAWHEAT

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

WG1 – GP2 period

WG 1. Ulva biology: the collective knowledge on the different aspects of Ulva biology, including systematics, genetics, reproduction, microbiome, diseases, and ecology, will be shared and prioritised.

Ronan Sulpice (University of Galway, IE)

Co-leaders WG1: Olivier de Clerck, Thomas Wichard, Martina Orlando Bonaca

Membership: 48

Countries: 16

Summary of progresses and plans for next year.

Members of WG1 have been assigned to the three tasks part of WG1.

Task 1.1: The inconsistent systematics and phenotypic plasticity of the Ulva genus will be clarified. This task is linked to D 1.1.

A network of people collecting samples has been developed. Coordinates and abiotic environment are documented, as well as other parameters. Thomas Wichard has developed an excel spreadsheet that allows to document properly samples. This work will also inform Task 1.3 and is achieved in collaboration with WG3 and WG4.

The leads are Olivier DeClerck, Thomas Wichard, Sophie Steinhagen, Florian Weinberger, Erik Jan Malta, Afshwin Engelen, Celine Rebourg and Ronan Sulpice.

The sequencing is being made in Olivier DeClerck lab, with possible support of Ronan Sulpice lab is required (unlikely). A questionnaire has been sent to SEAWHEAT members by WG3 and labs interested in strated to send samples. Additional labs outside the Seawheat action are also participating.

Biochemical analyses are being made in Sulpice lab. Only a subset of samples being genotyped are going to be analysed for biochemical content due to the requirement of a freeze drier to preserve the samples.

Task 1.2: The microbiome (bacterial - seaweed interactions) will be identified at the molecular level; D 1.1.

LEADS: Aschwin Hillebrand Engelen, Thomas Wichard, Marcus McHale

This project is making use of the network of harvesters set up in Task 1.1. Thomas Wichard and Aschwin Hillebrand Engelen labs are sending kits to harvesters that they can collect microbiome samples.

Task 1.3: The seasonal and environmental impact factors on Ulva reproduction, growth, biochemical composition, and productivity (including nutrients, light, temperature, etc.) will be identified; D 1.1.
LEADS: Erik Jan Malta. Sophie Steinagen, Rui Perreira, Martina Orlando-Bonaca and Sortiris Orfanidis

This task will make use of STSM grants and appear difficult to implement. For seasonal effects, biochemical analyses in task 1.1 will help, but more efforts are required and will need to be planned in GP3.

Deliverables:
Book on Ulva - Thomas Wichard will edit a book containing mini reviews about each deliverable of the COST action. Publication date: last year of the COST action.

Simon, C., M. McHale and R. Sulpice (2022). "Applications of Ulva Biomass and Strategies to Improve Its Yield and Composition: A Perspective for Ulva Aquaculture." Biology (Basel) 11(11).

Training School In Kavala “Ulva Biology”. Location Greece, 25-28 April 2023. Organised locally by Dr. Sotiris Ofranidis

GP3:

Continue with sampling initiative to document Ulva species location, changes in biochemical content in response to environment / genetic, and microbiome variability

Meeting dedicated to task 1.3

WG2 Report

WG2: Ulva in Aquaculture
Progress report GP2

Chair: Dr. Rui Pereira

Co-chairs: Dr. Erik Malta, Dr. Ricardo Bermejo

WG3: 161 Members as of July 2023

The interest in the topic of WG2, Ulva in aquaculture is reflected by a constant increase in memberships to this Working Group. At the moment WG2 has 161 members, from 48 countries. From that total, 139 are members from ITC countries and a total of 22 ITC countries are represented. In terms of gender, the group is divided in 60% male and 40% female members. It is also interesting to note that 45% of the members are classified as Young Researchers.

Activities for GP2 / 2022-2023

Several regular meetings between the WG leaders to discuss updated of the different Deliveries and decide ways to go further

Meetings and discussions with the different Task Leaders

Tentative organization of a webinar series. A call was launched within the WG2 community seeking for volunteers for a series of short webinars during lunch time – “Seawheat for lunch”. This idea was dropped after realizing a similar initiative was being thought considering the entire Seawheat community.

One Short-Term Scientific Mission in Canary Islands, Spain. Young Researcher Viviana Pasquini, from the Univerisity of Cagliari (UNICA) was hosted by Banco Español de Algas (BEA) – University of Las Palmas de Gran Canaria (ULPGC), Prof. Juan Luis Gómez-Pinchetti , to carry out the planned tasks on “Intensive biomass production of Ulva in tanks, training and experimental trials”.

Co-organization of a Workshop in Lisbon, Portugal: ‘From Ulva aquaculture to food and feed production: state-of-the-art, bottlenecks, risks and gaps’, May 23-24, 2023

This workshop was a joint organization between the leaders of WG2 and WG3, “Ulva in Aquaculture” and “Ulva in feed and food”. The aim of this workshop was to bring together seaweed experts in the Ulva production and product development with experts in the food and feed sectors in general. Specific objectives were to identify bottlenecks and gaps, which were worked out in different chairing sessions and which were finally drafted in a common strategy document. It is relevant to note that 34% of the workshop participants were from SMEs, with relevant roles during the different sessions (both presenting their experiences and chairing sessions) and participating actively in the discussions, thus clearly contributing to reinforce the interaction between the commercial and the academia partners.

Co-organization of a Training School in Malaga, Spain: “Cultivation technologies of the land and sea-based system”, June 26-29, 2023. This Training School (TS) was a joint organization between WG2 and WG6, “Ulva in Aquaculture” and “Social Aspects & Security”, respectively. During this four-day TS, trainers with expertise in reproduction, aquaculture, and regulatory aspects of Ulva cultivation introduced the participants to theoretical and practical approaches to Ulva farming and consultation of industry stakeholders. The TS included a site visit and case studies in Almeria (i.e. a factory for biostimulant production from algae, and aquaculture facilities at the University of Almeria and IFAPA). There were 7 trainers and 4 assistants directly involved in the school, with the 17 trainees, selected from a total of 28 excellent applications received. The WG leaders strongly believe that the TS in Malaga was an enriching experience for all involved trainers, trainees, and hosts. In addition, this Training School helped “SeaWheat” COST Actions to create a network between SMEs and researchers that we are sure will contribute to knowledge transference from academia to the industry. A survey was developed after the TS sent to the trainees of TS1 and TS2 in order to use the information to improve the format of the future Training Schools organized in under the context of this Cost Action.

Further plans:

Task 2.1 and Deliverable 2.1a: with the finalization of a proposed table to contents and contribution from different experts, a call will be made to the entire WG2 community, seeking contributors for missing sections

Task 2.2 and Deliverable 2.1b: WG2 co-leader will intensify the involvement of the Task leaders, building up on the materials produced during this year’s TS in Malaga.

Task 2.3 and Deliverable 2.1c: a meeting of the core group of this task will be organized during the last trimester of 2023, to decide on ways to move forward with this task

A general meeting of WG2 will be organized by the end of 2023 or beginning of 2024, updating the entire group on progresses and needs for involvement.

Co-organization of a Workshop on “Ulva mass production and ecosystem services and economics” (led by WGs 2 and 5. End users, NGOs and decision-makers will also participate).

WG3 Report

WG3: Ulva as food, feed and beyond
Progress report GP2

Chair: Dr. Sylvia Strauss

WG3: 156 Members (August 2023)

An increased interest in Ulva as food and feed is reflected by a rise in memberships to this Working Group to 156, from 30 countries. However, there is only a group of about 30 members actively participating.

Activities for GP2 / 2022-2023

SeaWheat Conference Cadiz, September 13-15, 2022

4th WG3 members meeting online 14.10.2022 (20 participants)

5th WG3 members meeting online 14.02.2023 (35 participants)

Drafting and submitting a review:

As major part of the deliverable 3.1, a review manuscript was initiated in September 2022 by a team of assigned coordinators (L. Hofmann, A. Melendez, S. Strauss) and drafted during the following months. 27 authors and COST members have contributed to the manuscript

'Ulva in the sustainability and circular economy era: tomorrow's "wheat of the sea" in foods, feeds, nutrition, and biomaterials' , which after professional editing we are now about to submit to Critical Reviews in Food Science and Nutrition.

This extensive, multidisciplinary Ulva review features sections on taxonomy, production, nutritional composition, processing, food & food safety, feed and applications beyond such as biomaterials, biostimulants and biorefinery as well as recent patents and future directions.

Workshop Lisbon 'From Ulva aquaculture to food and feed production: state-of-the-art, bottlenecks, risks and gaps', May 23-24, 2023

This workshop was a joint organization between the leaders of WG2 and WG3, "Ulva in Aquaculture" and "Ulva in feed and food". The aim of this workshop was to bring together seaweed experts in the Ulva production and product development with experts in the food and feed sectors in general. Specific objectives were to identify bottlenecks and gaps, which were worked out in different chairing sessions and which were finally drafted in a common strategy document. 34% of the workshop participants were from SMEs, with relevant roles during the different sessions (both presenting their experiences and chairing sessions) and participating actively in the discussions, thus clearly contributing to reinforce the interaction between the commercial and the academia partners.

1 STSM hosted by The Seaweed Company/subsidiary TSC Red Turtle:

'Seasonal changes in the nutritional and quality profile of Ulva spp. in Nador region/Morocco' performed by Umair Ahsan, an early career researcher from Burdur Mehmet Akif Ersoy University, Turkey. Samples from different Ulva species as well as water probes and sediment were taken from several locations and different times during the growing season from April to June 2023 and submitted to nutritional and quality analysis as well as genotyping and microbiome analysis. Data analysis is still ongoing and results will also be incorporated in the Pan-European database of the SeaWheat sampling initiative.

Pan-European Ulva sampling project & building a database

To fulfil the core task 3.1a 'Ulva nutritional analysis & food safety ', in a close collaboration with members of WG1 and WG4, we set up a sampling initiative to collect Ulva species on a Pan-European scale that will allow us to create a database with nutritional profiles, biochemical data and microbiomes from barcoded strains of wild and cultivated Ulva. Several meetings among the coordinators took place in the winter 22/23 to define standards and communicate sampling, preservation and shipping instructions that were then sent to all members who indicated interest in contributing samples. Sample collection and shipping to the different labs started in spring 2023 and is still ongoing as another aim is to also collect data from different seasons.

Samples are being analysed in institutions of members from WG1 and WG3: species identification and barcoding is performed by Olivier de Clerck/UGent, biochemical analyses & nutritional profiles by Ronan Sulpice/ UGalway, and Umair Ahsan/MehmetAkif Univ., microbiome analysis by Thomas Wichard/UJena and Aschwin Engelen/CCMAR. The datamining and database will be set up thereafter combining all data. This step will also be supported by Lior Guttman/Univ. Haifa.

The expected outcome of this ambitious initiative will hopefully give us a more precise and better coverage of the existing Ulva species in the wild as well as an idea which strains are currently used as cultivars. The

combination with biochemical and nutritional data including the microbiome analysis of these species will ideally give us a biochemical characterisation of a taxonomically correctly identified species distribution, which can also allow for a potential selection of suitable strains for cultivation.

Further plans:

Task 3.1.a: continue sampling project, collecting further samples, datamining and data analysis.

Intensifying networks between SMEs and academia, discussing marketing strategies to increase consumer acceptance of Ulva in food and feed, such as in the SME & WG meeting to be held in October 2023 in Riga/Latvia.

WG4 Report

Report of Working Group 4 "Bioactive Products"

by the working group leader Dr Thomas Wichard

This report covers the second funding period of the COST Action CA20106 in Oct. 2022 – Sept. 2023. Due to the high popularity of the COST Action CA20106, the number of registered participants in WG4 has again increased, reaching more than 140 people (Sept. 2023).

In online and personal meetings, e.g., at the COST Action Workshop in Portugal and Training Schools in Spain and Greece, active members of WG4 have strengthened their collaboration within the COST Action in 2023, necessary to achieve deliverables 4.1 and 4.2. Therefore, even though only about 20 % of the registered participants are proactive and take on tasks, it was possible to develop further steps and milestones to achieve deliverables 4.1 and 4.2.:

The following individual activities are listed one by one:

(i) Organization: The first phase of our activities in Working Group 4 was focused on organisation and the distribution of tasks in 2022. Subgroups were introduced to achieve the milestones. Two participants (Wichard [Germany] and Zammit [Malta]) of WG4 belong to the Editorial Committee of the book project, preparing a monography about Ulva. One WG4 representative (Dilek Unal [Turkey]) was elected for the "dissemination team" of the COST Action.

(ii) Main Activities: After the first phase was filled with organisational tasks, in the second phase, overarching cooperations were formed, with a focus on chemical analyses of bioactive and morphogenetic substances.

In addition to the analysis of hormone-like, morphogenetic substances such as thallusin (FSU Jena, Germany), fatty acids (e.g., Universidade de Aveiro, Portugal), and nutrients (e.g., NUI Galway, Ireland) were determined in Ulva cultures. Surveys for Ulva-based sterols have also been started during this financing period. Furthermore, approaches for rapid biochemical survey (Palou et al. 2023) and targeted analysis (Ulrich et al. 2022) in Ulva's holobiont have been improved.

In those newly established multinational collaborations, researchers study Ulva and its associated bacteria in various habitats, for example, in aquacultures (in Portugal, Spain, Sweden and Israel), in lakes (Bear Lake, Romania) and the Baltic Sea (Kieler Bay, Germany).

Three highlights:

For instance, the COST Action CA20106 enabled a new partnership in 2023 between two different research groups in WG4 and a Romanian/Hungarian research group to examine Bear Lake in terms of the existing Ulva, its microbiome, and the production of thallusin in this rare ecosystem.

However, COST Action CA20106 has also brought together researchers of WG4 from Spain and Turkey to apply for joint exchange programs such as Erasmus Plus.

The Cost Action drew interest from outside Europe, and a Japanese-German collaboration on bioactive bacteria in green macroalgae is now part of WG4's work.

(iii) COST Action events: The participants of WG4 have participated in STSMs with the topics entitled:

Removal capability and stress response in Ulva aquacultures towards phenolic endocrine disruptors (Justus Hardegen; host: Alexander Golberg (Tel Aviv, Israel); April 2023)

(iv) Conference: At several national and international conferences, active members of the WG4 have presented their recent research, for example:

24th International Seaweed Symposium in Hobart (Australia)

20th Phycology Meeting in Jena (Germany)

8th European Phycologica Conference in Brest (France)

Theo Murphy meeting of The Royal Society Algal holobionts: Challenges and opportunities (UK)

(v) Publications in scientific journals (in progress): WG4 is actively participating in Special Issues dedicated to the COST Action, published in the Journal of Applied Phycology (in press) and Botanica Marina (in progress), which are being compiled and developed across all working groups of the COST Action. For example, participants of WG4 published several publications. COST Action CA20106 is acknowledged in those publications, selected examples:

Addis, P., Pasquini, V., Angioni, A. et al. Ulva as potential stimulant and attractant for a valuable sea urchin species: a chemosensory study. Journal of Applied Phycology 35, 1407–1415 (2023). https://doi.org/10.1007/s10811-023-02925-0

Blomme, J., Wichard, T., Jacobs, T. B., De Clerck, O. (2023). Ulva: An emerging green seaweed model for systems biology. Journal of Phycology, online. https://doi.org/10.1111/jpy.13341

Hardegen, J., Amend, G., Wichard, T. (2023) Lifecycle-dependent toxicity and removal of micropollutants in algal cultures of the green seaweed Ulva (Chlorophyta). Journal of Applied Phycology, https://link.springer.com/article/10.1007/s10811-023-02936-x

Hmani, I., Ghaderiardakani, F., Ktari, L., El Bour, M., Wichard, T. High-temperature stress induces bacteria-specific adverse and reversible effects on Ulva (Chlorophyta) growth and its chemosphere in a reductionist model system. Botanica Marina. Accepted, DOI: 10.1515/bot-2023-0053

Palou, A., Jiménez, P., Casals, J. et al. Evaluation of the Near Infrared Spectroscopy (NIRS) to predict chemical composition in Ulva ohnoi. Journal of Applied Phycology (2023).

Ulrich, J.F., Gräfe, M.S., Dhiman, S., Wienecke, P., Arndt, H.-D., Wichard, T. (2022) Thallusin quantification in marine bacteria and algae cultures. Marine Drugs 20: 690 https://doi.org/10.3390/md20110690

(vi) Ongoing activities in 2023 and 2024: In an interdisciplinary campaign, WG4 contributed to a holistic picture of the distribution of Ulva, its microbiome and nutrients with various chemical analyses of the holobiont Ulva. Laboratories in Portugal, Belgium, Ireland, and Germany mainly coordinate this activity [WGs 1,3,4]. Specific algal and bacterial substances will be identified. It might be further complemented by elaborative approaches such as metabolomics in various laboratories (Hmani et al. 2023).

Five highlights:

WG4 participates in the European survey of Ulva´s biodiversity (WG1,3) and accompanies it with chemical analysis in collaboration with the other WGs.

A joint search for bioactive compounds (targeted analysis) has been started under the various conditions of the respective working groups in the COST Action to test the general validity of the methods used (Ulrich et al. 2022).

A Workshop and Training School on the topic "Ulva's bioactive metabolites (primary/secondary)" will take place in Aveiro (Portugal) in June 2024. An organization committee has been set up, and the first call will be circulated in November 2023

To compile the ongoing research on" Bioactive products in Ulva" the WG4 will provide and submit a review article in 2023 entitled "Ulva sp. metabolites as ingredients for agro-food, pharma, cosmetic and other industries." 11 participants are involved moderated by Wichard (Germany) and Meléndez Martínez (Spain).

The collaboration with the SME platform will be intensified through, for example, the SME workshop in Riga in 2023. For instance, AlgaPlus will eb in involved in the Training School in Aveiro in 2024.

(vii) In summary, an active and motivated core group of the WG4 will work on the goals in the next funding period. One challenge is coordinating the synergies of the individual groups and the scientific work, considering the various funding situations. Notably, the COST Action inspired several participants to seek funding at the national and binational levels. Particularly encouraging are the activities of young scientists in Training Schools (Malaga, Spain), which then lead to the completion of scientific papers and their publication (e.g., Hmani et al. 2023)

WG5 Report

Seawheat WG5 Ecosystem Services

Status

WG5 members and meetings: The number of participants in WG5 has increased from 26 to 30 members, hereof 4 co-leads and one SC representative.

WG5 meetings are planned every three months. In GP2, three meetings have been held on-line with participation of approximately 10 members every time. Brief meeting minutes are kept at the Teams platform.

Deliverables and milestones: Three to four members have been assigned to contribute to each of the sub-deliverables of WG5.

Planning and organisation: 1) Plans are progressing on a joint WG5+WG6 workshop and training school 22nd to 26th of April 2024 in Riga, involving WG lead, co-lead and SMEs. 2) 13th Nordic Seaweed Conference in Grenaa, Denmark, has been planned with focus on Ulva ecosystem services: Keynotes professors Muki Sphigel and Marianne Thomsen (SeaWheat), other talks: Esben Rimi Christiansen. https://algecenterdanmark.com/nordic-seaweed-conference/

Presentations: WG5, Ulva Ecosystem Services, and science industrial cooperation has been represented at the 8th European Phycological Congress in Brest, France by SME partner PureAlgae (CEO Esben Rimi Christiansen), and at the Danish AquaNet Symposium 13.4.2023.

STSMs: WG5 has had one STSM candidate (Kristoffer Larsen Ledet, Aarhus University) visiting Olivier de Clerkcs lab (WG2).

Publications: The outcome of the STMS (Above) will be input to 1-2 joint publications. In addition, we have initiated a joint publications (mini-review) in the 2nd GP to be submitted in the 3rd GP. Several WG5 members aim for contributing to the planned book on Ulva.

Plans for 3. grant period

WS/TS in Riga: WG5 will in cooperation with WG6 carry out a combined WS (2 days) and TS (3 days) in Riga hosted by Georg Martin, at University of Tartu, Estonian Marine Institute, Latvia. Scientific responsible is Annette Bruhn (WG5 lead). The WS7TS 5 will be focusing in discussing possibilities and challenges in Ulva Ecosystem services, including methods for quantifying Ulva Ecosystem services, models and LCA.

STSMs: WG5 members aim for both hosting and sending young scientists for more STSMs in GP3, increasing after the Nordic Seaweed Conference in Denmark, and the WS/TS in Riga, where networking across cooperators will be more intense.

Publications: One joint mini-review is in progress (D5.1-4). The WS/TS in Riga will be planned so that outcome hereof also will be 1-2 joint papers. Joint papers across cooperating labs is in progress as outcomes of i.e. the recent STSM between Aarhus and Ghent Universities. More joint papers as outcomes of future STSMs are expected.

Presentations: 13th Nordic Seaweed Conference in Grenaa, Denmark will be taking place on the 11+12th of October with focus on Ulva ecosystem services: Keynotes professors Muki Sphigel and Marianne Thomsen (SeaWheat), other talks: Esben Rimi Christiansen, and 5 other Ulva and SeaWheat relevant talks: https://algecenterdanmark.com/nordic-seaweed-conference/

WG6 Report

Aim

The Action will examine the regulatory framework relevant to Ulva production at a large scale, investigate the acceptance of Ulva products by society, and the economic aspects, including risk assessment in different scenarios and geographical locations. This WG will fulfill the Cost Action challenge “e” about enhancing acceptance of Ulva as food, feed and source of bioactive products by society, and drawing recommendations for amendment or reinforcement of the current regulatory framework for Ulva-based industries.

Tasks

Task 6.1 The Eu regulations on Ulva mass production (land- and sea-based) and algae marketing as food, feed and a source of valuable secondary metabolites (safety regulations and food quality control for different uses of Ulva) will be reviewed and analyzed to identify the gaps in the regulatory framework. The implementation of these EU regulations by members states will be investigated by national contact points and consultations of SMEs across Europe; D 6.1.

Task 6.2 The impact of Ulva cultivation, processing and marketing on various communities concernig social aspects (social acceptance of Ulva as food, improved nutrition in the communities as well as job creation, increasing community income and education) will be evaluated; D 6.1.

Task 6.3 Conflicts of interest regarding space for large scale production of Ulva vs. other maricultured organisms and with other users of the sea (shipping, fishing, leisure, etc.) will be tackled and the information will be transferred to related governments with suggestions for possible amendments; D 6.1.

Task 6.4 The economics of Ulva farming (land and sea-based), processing (e.g., biorefineries), marketing including risk assessment in different systems or scenarios will be analysed; D 6.1.

Remark: Task6.1 was modified to fit what can be done; D6.2 to 1.4 are still under development and may be also adapted once WG6 has better define the details of the work that would need to be done.

Deliverable

Deliverable D6.1: (a, b,c). Report on the following topics

a. Recommendations for amendment or reinforcement of the current EU regulations about Ulva culture in regard to environmental issues and conflicts of interest (e.g., on land space).

b. Recommendations for amendment or reinforcement of the current EU regulations on Ulva mass production and its use as products in the food, feed and valuable secondary metabolites sectors.

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

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

Remark: D6.1 a &b are modified from the applications to fit what can be done; D6.1 c & d are still to better understood in order to be able to define the work that would need to be done

Workshop 4: Social and regulation aspects of Ulva production; consumer acceptance, food security, risk assessment and wellbeing, etc.". End users, NGO and decision makers will also be invited.

Training School

TS 1 (WG2&6): Cultivation technologies of land and sea-based systems was conducted In Malaga Spain in June 2023 (for more detailed see report send by Riccardo Bermejo).

TS 4 (WG5&6): Drafting legislation and holistic framing of social, legal, and regulatory aspects of sustainable seaweed mass production organised with the workshop in Estonia at the end of April 2024. Organisation still in progress.

STSM: Hosts and participants in future STSM calls

Dissemination: Coordination by Karina Balina with WP7

Joint review paper: list of potential papers and leads under construction.

Policy analyses under progress: the National report have been received for 9 countries. The main producers' countries have not yet sent their reports (e.g. Portugal, Israel, France, Ireland, Spain, Germany, UK)

Risk assessment: is led by Avi Griffel. The methodology is under progress.

Conference contributions – presentations and posters at SEAWHEAT conferences and conferences attended by WG6 participants:

March 2022 SEAWHEEAT working meeting: SME survey

September 2022 conference:

"Marine biotechnology: ABS, Convention of Biological Diversity, Nagoya protocol, (Access and Benefit sharing Clearing house – ABSCH), RRI" Celine Rebours

"Use of waste streams for culture of Ulva as food and feed products: Norwegian and European regulatory framework" Celine Rebours

Collaboration with other WGs

WG6 brought support to WG1 about Nagoya Protocol, Convention of Biological Diversity and RRI

WG6 will work closely with WG2 to define the value chain to be investigated.

WG6&5 will collaborate to make the regulatory framework review. Participants from the WP5 are invited to join WP6 on the tasks about the environmental issues.

2024 REPORT

WG1 Report

Report of Working group 4 "Bioactive Products"

by the working group leader Dr Thomas Wichard

This report covers the first funding period of the COST Action CA20106 in 2021/22. Due to the high popularity of the COST Action, the number of registered participants in WG4 has steadily increased, reaching more than 90 people in the first year. In three online meetings, the focus was, therefore, increasingly on communicating the organization, goals and benefits of the network that are necessary to achieve deliverables 4.1 and 4.2. A survey among the participants showed that the expertise in the group characterized by chemical issues is wide-ranging (chromatography, mass spectrometry, NMR, nutrient analysis). However, many participants (> 50%) have not worked intensively with Ulva up to now. Indeed, this shows the great interest in Ulva among plant biologists and (food) chemists.

Even though only about 30% of the participants are active and take on tasks, it was possible to develop many steps and milestones together: (i) organization, (ii) compilation of the status quo of Ulva research in Europe, (iii) participation in STSMs, (iv) conference contributions (in Cádiz in Sept. 2023) (v) publications in scientific journals, and (vi) participation in coordinated research campaigns of WG2,3 and 4. (vi) future activities of orchestrated activities with other WGs of the COST Action.

The following individual activities are listed one by one:

(i) Organization: The first phase of our work in Working Group 4 was focused on organization and the distribution of tasks. Subgroups were introduced to achieve the milestones. Two participants (Wichard [Germany] and Zammit [Malta]) of WG4 belong to the Editorial Committee of the book project, preparing a monography about Ulva. One WG4 representative (Dilek Unal [Turkey]) was elected for the “dissemination team” of the COST Action.

(ii) Status quo of Ulva research: To compile the ongoing research on" Bioactive products in Ulva" the WG4 will provide and publish a review article in 2023 entitled "Ulva sp. metabolites as ingredients for agro-food, pharma, cosmetic and other industries." 11 participants are involved moderated by Wichard (Germany) and Meléndez Martínez (Spain).

(iii) COST Action events: The participants of WG4 have already participated in two STSMs with the topics entitled:

Isolation of bioactive compounds from xenic and axenic Ulva cultures applied in various bioassay-guided test systems (Gülçin Sevim; host: Thomas Wichard; March 1st 2022)

Biochemical characterization of Ulva biomass: looking for bioactive compounds, particularly healthy lipids, for food and feed applications (Sarah Schembri; host: Rosario Domingues; March 1st, 2022)

(vi) Conference: At the COST Action conference "From fundamental biology to aquaculture: state-of-the-art, bottlenecks and gaps "in Cádiz (Spain) in 2022, WG4 led a session with the contributions of its participants. Some of the presentations will be published in the Proceedings of the conference.

(v) Publications in scientific journals (in progress): WG4 is actively participating in the design of a monograph on Ulva, which is being compiled and developed across groups. The book project will accompany the group throughout the funding period of the COST Action.

Participants of Working Group 4 published three publications (Open Access). COST Action CA20106 is acknowledged in those publications (participants of the COST Action CA20106 are highlighted in bold).

Morales-Reyes, C., Ghaderiardakani, F., Wichard, T. (2022) Genome Sequence of Halomonas sp. strain MS1, a metallophore-producing, algal growth-promoting marine bacterium isolated from the sea seaweed Ulva mutabilis (Chlorophyta). Microbiology Resource Announcement in press.

Wichard, T. (2022) From model organism to application: Bacteria-induced growth and development of the green seaweed Ulva and the potential of microbe leveraging in algal aquaculture. Seminars in Cell & Developmental Biology in press.

Obolski, U.*, Wichard, T., Israel, A., Golberg, A., and Liberzon, A. (2022) Modeling the growth and sporulation dynamics of the macroalga Ulva in mixed-age populations in cultivation

and the formation of green tides. Biogeosciences 19, 2263–2271.

(vi) Future activities: It was suggested that a joint search for bioactive compounds (targeted analysis) would be carried out under the various conditions of the respective working groups in the COST Action to test the general validity of the methods used. Significantly, in an interdisciplinary campaign, WG4 will contribute to a holistic picture of the distribution of Ulva, its microbiome and nutrients with various chemical analyses of the holobiont Ulva. In particular, specific algal and bacterial substances will be identified. It can be further complemented by elaborative approaches such as metabolomics.

The organization of a Training School in spring 2024 (date, location) on the topic "Ulva's bioactive metabolites (primary/secondary)" is still being discussed within the WG4 and the COST Action.

The collaboration with the SME platform should be intensified.

In summary, an active and motivated core group of the WG4 will work on the goals in the next funding period. One challenge is coordinating the synergies of the individual groups and the scientific work, considering the various funding situation. Notably, the COST Action inspired several participants to seek funding at the national and binational levels.

WG2 Report

WG3 Report

WG3 members & meetings: The # of members is still increasing to currently around 200, from 38 countries, thereof 18 SMEs (but not necessarily Ulva production related). The last working group meeting (online) was held in April 2024 with 20 participants, giving update on review and a renewal of call for sample contribution.

Publication of review: After an extensive revision process, our manuscript was accepted and finally published for open access in July 2024:

Laurie C. Hofmann, Sylvia Strauss, Muki Shpigel, Lior Guttman, Dagmar B. Stengel, Céline Rebours, Natasha Gjorgovska, Gamze Turan, Karina Balina, Gabrielle Zammit, Jessica M. M. Adams, Umair Ahsan, Angela G. Bartolo, John J. Bolton, Rosário Domingues, Ömerhan Dürrani, Orhan Tufan Eroldogan, Andreia Freitas, Alexander Golberg, Kira I. Kremer, Francisca Marques, Massimo Milia, Sophie Steinhagen, Ekin Sucu, Liliana Vargas-Murga, Shiri Zemah-Shamir, Ziv Zemah-Shamir & Antonio J. Meléndez-Martínez (09 Jul 2024):

The green seaweed Ulva: tomorrow’s “wheat of the sea” in foods, feeds, nutrition, and biomaterials. Critical Reviews in Food Science and Nutrition, 1-36.

27 authors contributed to this extensive, multidisciplinary paper which includes sections on taxonomy, production, nutritional composition, processing, food & food safety, feed and applications beyond such as biomaterials, bio stimulants and biorefinery as well as recent patents and future directions. This review therefore covers all aspects and fulfils deliverable D 3.1 (a, b).

European Ulva sampling initiative: Jointly organised by WG1, WG3 and WG4, we renewed calls for samples in spring 2024 in order to increase the number of total samples but also the number of geographical locations. For the genotyping part, more than 3700 samples from > 120 collectors have been sent to the lab of Olivier de Clerck in Gent, while microbiome analyses on Ulva samples are performed by Aschwin Engelen in Portugal. Parallel sample numbers for biochemistry analysis (performed by Ronan Sulpice, Univ. Galway) occurred much less due to technically more demanding sample preparation. The majority of samples derived from the wild, however, only few contributions from cultivated strains of SMEs were sent in so far.

Plans for GP4:

WG1 and WG4 will finalize sample analyses after which datamining and a database will jointly be organised by the respective groups, followed by preparation of publication(s).

WG4 Report

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In summary, an active and motivated core group of the WG4 will work on the goals in the next funding period. One challenge is coordinating the synergies of the individual groups and the scientific work, considering the various funding situation. Notably, the COST Action inspired several participants to seek funding at the national and binational levels.

WG5 Report

Seawheat WG5 Ecosystem Services

Status

WG5 has 26 members, hereof 4 co-leads and one SC representative.

Three WG5 meetings are planned every three months. Three meetings have so far been held on-line with participation of approximately 10 members every time. One meeting has been cancelled.

Three to four members have been assigned to contribute to each of the sub-deliverables of WG5.

WG5 and Ulva Ecosystem Services has been represented at the 1st Seawheat Conference in Cadiz, with one keynote and four presentations, but no WG5 publications are planned as part of the 1st Seawheat conference proceedings.

Several WG5 members aim for contributing t the planned book on Ulva.

Plans have been made for cooperation with WG6 on joint workshop in 2024 in relation to the 13th Nordic Seaweed Conference in Grenaa, Denmark.

WG5 has had no STSM candidates or hosts, no SoMe posts, and no joint publications in the 1st grant period.

Plans for 2. grant period

In the 2nd Grant period, WG5 will be focusing in discussing possibilities and challenges in Ulva Ecosystem services, including methods for quantifying Ulva Ecosystem services, models and LCA. WG5 members aim for: members participating in workshops and training schools (WG5 will be organizing WS in 2024). WG5 deliverables are planned to be published as peer-reviewed mini review hereof potentially one in 2nd grant period. The potential for other joint WG5 publications will be explored. WG5 members aim for both hosting and going for STSM's during the 2nd grant period, if possible in relation to associated SMEs, and aiming for outcomes as joint WG5 publications.

WG6 Report

WG 6

The WG6 participants had 6 working meetings. During these meetings, national contact points (NPPs) were identified. These NPPs are responsible for conducting a policy survey in their country. This information will be further used to conduct a policy analyse at the European level. The NPPs also started to identify the stakeholders involved in the Ulva sector and are today fine tuning the list to define which stakeholders could potentially be consulted to collect more information about this sector. During the first 9 months of the project, the methodology used to prepare the two first deliverables of the WG were decided particularly to address the challenges generated by the regulatory framework and, to further be able to design the recommendations that may facilitate the development of an Ulva industry in Europe. Two consultations were already conducted: one with the SMEs (consultation by email, results presented at the Rome`s meeting) and one with the whole SEAWHEAT network at the meeting in Rome to understand the level of awareness about RRI, CBD, ABS, and NP. Consultations of stakeholders will be conducted in 2023 and 2024 to address regulatory and markets issues. In the next months, NPPs in some of the major Ulva producing countries would need to be identified and hopefully join in the WG6 work.

At the SeaWheat conference, a session on Legal, Social, and Regulation Aspects addressed specially the aspects of social responsibility, risks and environmental sustainability while developing new production systems. During the session, the knowledge build under another Cost Action (CA 18238 Ocean4Biotech) was transferred and results of the consultation conducted in Rome was presented to the Seawheat network.

Collaboration with the leaders of WG1, WG2 and WG3 were established to ensured implementation of the NP. Close collaborations with the leaders WG2 and WG5 resulting in drafting the future common Training Schools in 2023 and 2025.