Conference Abstract

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ParAqua Conference Abtracts Booklet

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Abstracts Booklet

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Investigations of plankton dynamics in the open South Adriatic in the last decades

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ABSTRACT

Over the past two decades, the open South Adriatic plankton community has been subject to continuous investigation by scientists from the Institute for Marine and Coastal Research at the University of Dubrovnik. The open South Adriatic represents a dynamic ecosystem influenced by diverse hydro-meteorological conditions. One such factor is the strong, cold, and dry winter wind known as the "bura," which triggers deep winter convection in February, enriching the surface layer with nutrients. This nutrient infusion sets the stage for phytoplankton bloom in March, followed by zooplankton bloom. Another significant phenomenon affecting the South Adriatic is the BiOS mechanism (Bimodal Oscillating System) (Civitarese et al., 2023). BiOS theory postulates that there is a feedback mechanism between Ionian and Adriatic Sea which causes changes in the rotation direction of the North Ionian Gyre (NIG) on the decadal scale. Depending on its direction, either cyclonic or anticyclonic, the NIG brings water of different properties into the Adriatic. During the cyclonic phase, the gyre brings nutrient-poorer, saltier, and warmer eastern Mediterranean waters into the Adriatic. Conversely, during the anticyclonic phase, the gyre transports nutrient-richer, colder, and less salty waters from the western Mediterranean, thereby influencing the productivity of the open South Adriatic. Shortly after the theory's postulation, it was demonstrated that the BiOS phase directly affects the origin of non-indigenous species entering the Adriatic (from the eastern Mediterranean/Indo-Pacific or western Mediterranean/Atlantic) as well as the abundance of already established non-indigenous species (Batistić et al., 2014). In addition to seasonal and decadal changes, there is a persistent upward trend in salinity and temperature in the Adriatic, attributed to climate change, resulting in a warmer and saltier Adriatic. This trend appears to have accelerated since 2011. As the Adriatic is becoming increasingly warmer and saltier, it is also becoming more hospitable to warm and warm/temperate species. Over the last two decades, more than 20 non-indigenous plankton species have been recorded for the first time in the Adriatic, with five of them being new to science. Currently, three active projects funded by the Croatian Science Foundation are investigating the open South Adriatic. Two projects are led by the Institute for Marine and Coastal Research, while one is led by the Center for Marine Research (Ruđer Bošković Institute). These projects are exploring the diel vertical migration of zooplankton (DiVMaD), zooplankton distribution and diversity using a metabarcoding approach (SpaTeGen), and phytoplankton diversity and physiological responses in different environments (ADRILife).

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The physiology and ecoanthropology of algal pathogens in a rapidly changing world

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ABSTRACT

Collectively, algae account for nearly half of Earth's primary production; increasingly, their interactions with parasites are recognised as key to the dynamics and resilience of marine and freshwater food webs. In temperate and cold coastal areas, macroalgae are ecosystem engineers and provide habitat for fish and other animals. For the last two decades, omics-enabled studies in particular have unveiled a diversity and prevalence of algal pathogens – including but not limited to zoosporic parasites – far greater than had previously been appreciated.

In parallel, algal cultivation has been growing exponentially for the last 50 years, in the wider context of intensifying use and increasing dependency of mankind on the oceans, as well as heightening pressures on freshwater resources. In marine coastal areas, the intensification of seaweed aquaculture represents a comparable mutation to introducing the cultivation of land plants since the Neolithic. Since then, the domestication of plants has fundamentally transformed human societies and terrestrial ecosystems, with agriculture shaping landscapes, cultures, societal values and governance, including our trade rules and conservation policies. Today, the health of land plants dictates agricultural yields as well as ecosystem health; plant pathogens co-evolve with domesticated species, whilst intensive farming favours outbreaks as well as the emergence of novel pathogens. Alarmingly, reports of diseased or declining wild macroalgal populations, as well as significant losses in seaweed farms accumulate worldwide. This disease pressure is compounded by a faster rate of climate change in the sea compared to terrestrial systems. This suggests that man-driven changes potentially occur at a vastly higher pace in marine ecosystems than they did historically on land, calling for the inception of suitable mitigation strategies to underpin the ecological sustainability of seaweed (and more generally algal) cultivation[1,2].

In this talk, I will brush upon the work performed in my group towards accelerating the discovery of algal pathogens, as well as their phylogenetic and physiological characterization[3]. This will lead to some pilot work on disease monitoring and management in algal production facilities, as well as developing proof of concepts of enhancing resistance through microbiome engineering or breeding. Finally, I will report on capacity and capability building initiatives to support disease management, as well as inform the development of conservation and biosecurity policies worldwide.

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The role of microalgae biotechnology within EU Mission Restore Our Ocean and Waters

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ABSTRACT

In September 2021, the EU Commission launched five Missions: Restore our Ocean and Waters, Adaptation to Climate Change, Cancer, 100 Climate-Neutral and Smart Cities, and A Soil Deal for Europe. All Missions represent a new way of bringing concrete solutions to some of our biggest challenges, with ambitious goals to achieve concrete results by 2030. Their main aim is to bring together the necessary resources in terms of funding programmes, policies, and regulations, as well as other activities. They also aim to mobilize and activate public and private actors, such as EU Member States, regional and local authorities, research institutes, entrepreneurs and investors to create real and lasting impact.

The EU Mission "Restore our Ocean and Waters" aims to protect and restore the health of our ocean and waters through research and innovation, citizen engagement and blue investments. It supports regional engagement and cooperation through area-based “lighthouses” in major sea/river basins: Atlantic-Arctic, Mediterranean Sea, Baltic-North Sea, and Danube-Black Sea. Mission lighthouses are places for piloting, demonstration, development and implementation of Mission activities in EU seas and river basins, but there are also cross-basin projects/actions. The main objectives of this Mission are to (1) protect and restore marine and freshwaters ecosystems and biodiversity (2) prevent and eliminate pollution of our oceans, seas and waters, and (3) make the blue economy carbon-neutral and circular. Related to the objectives, the targets are to (1) protect at least 30% and strictly protect 10% EU’s sea areas, (2) restore 25.000 km free-flowing rivers and marine nature restoration targets (including degraded sea beds, coastal ecosystems, etc.), (3) reduce by at least 50% plastic litter, (4) reduce by at least 30% microplastics, (5) reduce by at least 50% nutrient losses and chemical pollutants, (6) net zero maritime emissions, (7) zero-carbon aquaculture and (8) multi-purpose use of low-carbon marine space.

Algaculture (algae production), although it was used for aquaculture purposes from the past century [1], in last two decade has become an increasingly broad field of research that aims to use the biological properties of algae for the development of products and solutions for different sectors, from food and energy to medicines and cosmetics [2]. By June, 2023, a Portfolio Analysis Report published by European Commission identified over 800 EU-funded projects, contributing to the objectives of the EU Mission “Restore our Ocean and Waters”. Many of these projects related to algae biotechnology, such as: microalgae production pilots based on wastewater and side streams, microalgae and seaweed biorefinery processing pilots, extraction of bioactive compounds from algae, wastewater treatment by algae production, development of bioplastic materials from brown algae, etc[3]. Although many projects have been funded, there is still a large amount of opportunities, which is presented in this study.

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Microalgae screening in brewery effluents

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ABSTRACT

In Europe, the total beer production in 2022 was 399,403 hectoliters[1]. To produce 1 L of beer 2 to 8 L of water are required[2]. In large-scale breweries, the generated effluent is treated in their production facility before discharging it into the sewage. This effluent must comply with local regulations in the limitation of Nitrogen (N), Phosphorus (P), chemical oxygen demand (COD), and total suspended solids (TSS). Microalgae-bacteria systems are an alternative to water treatment as they upcycle N and P into valuable biomass. This work is part of the PROALGAE project (PID2022-140240OB-C21) which aims to revalorize agri-food effluent into protein-enriched microalgae biomass. The objective was to select the optimal combination of effluent and microalgae strain for nutrient removal and microalgae biomass production. A screening test was performed with different effluents of the brewery industry and microalgae strains. The effluent was taken from the outlet of different steps of the water treatment: raw wastewater (1), pre-acidification (2), anaerobic treatment (3), and dissolved air flotation (4). Three microalgae strains were tested: *Scenedesmus* sp., *Chlorella* sp., and *Spirulina* sp. To assess microalgae growth optical density (OD) was measured daily and dry weight (g/L) was calculated based on a calibration curve. The maximum biomass concentration was found in the effluent 2 after 4 days of cultivation being 1.02 g/L for *Scenedesmus* sp., 1.26 g/L for *Chlorella* sp., and 0.95 g/L for *Spirulina* sp. Effluent 1 performed worse than the other effluents the difference between effluent 1 and 2 was the pH being 4 and 7.5, respectively. COD was significantly reduced in effluent 2 by 49, 36, and 27% for *Scenedesmus* sp. *Chlorella* sp. and *Spirulina* sp, respectively. For the other effluents, the COD was increased by an average of 297% and 37% in effluent 4 and 3, respectively. Further analyses will be performed in the filtered media to evaluate N and P removal by microalgae. These results show that effluent 2 is optimal for growing *Scenedesmus* sp. and *Chlorella* sp. Further studies with the selected effluent and microalgae will focus on the characterization of nutrient removal in a semi-continuous system.

This project closely relates to Paraqua's aim to connect knowledge from natural ecosystems and industrial microalgae facilities. For the feasible industrial application of microalgae as wastewater treatment, knowledge is needed to monitor and prevent zoosporic infections, microbiome composition, and parasites. Developing protocols for monitoring contaminants in the early research stage must be achieved together with experts in natural ecosystems. Moreover, the PROALGAE project focuses on protein-enriched microalgae production for human consumption thus the establishment of monitoring protocols for hazardous contaminants needs to be developed.

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Management and protocols for commercial microalgae production

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ABSTRACT

When growing microalgae, in an industrial facility as well as in the laboratory, there are several main factors that need to be optimized and maintained: (1) physical factors such as light, water temperature, pH and other abiotic factors, (2) microalgae species and biochemical profile (nutrient factors), (3) stages of algal growth to harvest algae in their optimal condition, (4) steps in the production of the microalgae starter culture, (5) cultivation methods (e.g. batch, continuous, semi-continuous, indoor or outdoor), and all this will contribute to successful and continuous production. Of utmost importance to the operation is the proper utilization of sterile techniques in every step of the process from the first stages of maintaining or accepting stock cultures to the harvest. The purpose of the Microalgae Cultivation Protocol is to serve as a step-by-step guide for technicians in industrial production facilities as well as in the microalgae laboratory1. Sterile techniques, cleaning and preparation of the culture vessel, preparation of nutrients and media, inoculation and maintenance of the culture area should be described in detail.

Several generic protocols are available, according to which each production plant should develop its own protocol regarding its specific conditions. Production protocol, its detail and comprehensiveness will depend on a number of factors, the most important of which are the production technology, equipment and sophistication of the facility, and the education of its employees. If it is a plant that is modernly equipped and includes sophisticated equipment, it is necessary to describe the way the equipment functions and its use, while in less equipped plants, simpler and less sophisticated methods of sterilization, inoculation, manipulation, and transfer of cultures should be described in more detail, simpler and more comprehensible. The example of transfer of intermediate cultures in a flow laminar versus transfer in a room or laboratory "in the open" with repeated sterilization of vessels is a good example. A simple and detailed description of the activities during the different stages of the process is easier to follow and does not allow any improvisation or "cutting corners" to facilitate or speed up a particular activity. The fact that one mistake, i.e. one failure to adhere to the protocol, threatens the entire production process should be especially emphasized and that the old rule that a process is only as efficient as its weakest link. The protocol is usually subject to constant changes caused by accumulated experience, the acquisition of new equipment or the inclusion of new technology. The suggestions of everyone involved in the production process should be considered, but their possible inclusion in the protocol should be enabled only after repeated experiments at the laboratory level and at the mass production level, and finally after the approval of the competent technologist.

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Case study: Structure of experimental microalgae culture community assessed using V4 region of 18S rRNA as a biomarker

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ABSTRACT

Algal cultures kept in open systems are in direct contact with external environment and are therefore more exposed to contaminants that include grazers, bacteria, fungi, photosynthetic organisms, and viruses[1]. Zoosporic parasitic fungi and other contaminants can cause crashes in algal cultures and significant losses in profit[2]. That is why it is important to recognize their appearance as soon as possible and understand the biotic and abiotic factors that are responsible for their presence. For that reason, the aim of this research was to check if V4 region of 18S rRNA is a suitable biomarker to screen for zoosporic parasites and other contaminants in algal cultures. Algal cultures were experimentally kept in unhygienic conditions in two different salinities, 25 and 35 ppt, to simulate inappropriate open pond culturing conditions. Sampling was performed three times over the course of three months using an automatic micropipette with a sterile tip. Before each sampling, physiochemical water parameters were measured using a multiparameter probe. From each culture, 0.5 mL was taken, stored into a 2 ml cryotube, and preserved by adding 1,5 ml of absolute ethanol. The samples were stored at – 80 °C until further analysis. DNA was extracted using Dneasy Power Soil Pro Kit (Qiagen, Germany) according to the manufacturer's instructions. An additional step of lysis by lysozyme (0.5 mg/mL) for 30 min at 37°C was included after homogenization. The purity and concentration of DNA were quantified. After the DNA extraction, the samples were sent to Novogene for commercial NGS analysis. A specific region of the V4 18S rRNA of the fungal primer pair 528F (GCGGTAATTCCAGCTCCAA) and 706R (AATCCRAGATTTCACCTCT) was used for amplification. PCR reactions were performed with 15 μl of Phusion High Fidelity PCR Master Mix (New England Biolabs), 6 μM primers and 10 ng genomic DNA in final volume 30 μl. For thermal cycling, an initial denaturation was performed at 98°C for 1 minute followed by 30 cycles: denaturation at 98°C for 10 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 30 seconds. Final elongation step was performed at 72°C for 5 minutes. As negative control ddH20 was used. PCR products and equal volumes of 1x loading buffer were mixed and checked in 2% agarose gel using gel electrophoresis. For library construction, TruSeq DNA PCR-free sample preparation kit (Illumina, USA) was used. Quantification of constructed library was done by Qubit 3.0 Fluorimeter and an Agilent Bioanalyzer 2100 system. Illumina NovaSeq6000 was used for machine sequencing. DNA of algae, fungi, protists, plants, invertebrates, and vertebrates was identified. Their abundance changed over time in cultures on both salinities. A large proportion of grazers was detected in the first sampling, and it drastically reduced in the second and third sampling. In the third sampling a dominance of fungi was recorded. Among the recorded fungi, two genera that contain parasitic zoosporic fungi were recorded: Aplanochytrium and Paraphysoderma.

This method proved to be useful for identification and quantification of genera and higher taxonomic groups of zoosporic parasites and other contaminants that are present in algal cultures. Linking changes that are observed in community composition and presence of parasites over time to their biotic and abiotic drivers could be useful to predict dynamics and potential crashes of the cultivated algae.

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Ammonia-based method to control the predator *Poterioochromonas malhamensis* on *Chlorella vulgaris* massive cultures

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ABSTRACT

Poterioochromonas malhamensis is a mixotrophic flagellate (golden-brown alga) known for rapidly causing the collapse of microalgal mass cultivation within a short period. In this study, we explored a method to control and prevent contamination by *P. malhamensis* on large-scale *Chlorella vulgaris* cultures. The method proposed here is based on the use of ammonia as a toxic agent to the predator while assuring a low toxicity to *C. vulgaris*. Our results demonstrated that the three ammonia concentrations tested (23.03, 45.68, and 69.17 mg/L) within 24 hours were effective to eliminate *P. malhamensis* while causing from none to affordable toxicity effects on C. vulgaris. This contamination control method has been validated in co-cultures of both species at laboratory scale and the method was adapted to semi-industrial scale with successful results. In this sense,180 L photobioreactors working with *C. vulgaris* under industrial production conditions were inoculated with P. malhamensis and were maintained under regular ammonia inputs without *Chlorella*-crash events. The ammonia-control method has been demonstrated as feasible and simple strategy to minimize the impact of *P. malhamensis* predatory effects on *Chlorella* massive cultures.

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Occurrence of parasitic fungi in French Peri-Alpine lakes

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ABSTRACT

Fungi are key players in ecosystem functioning, though overlooked and udersampled in lakes. In pelagic environments, fungi and fungus-like protists (such as oomycetes, labyrinthulids, thraustochytrid) are mainly recognized as parasites infecting a wide variety of hosts, among which major importance target is phytoplankton. Despite their recognized ubiquity, there is a general lack of knowledge and data regarding fungal parasites occurrence and diversity in lakes, notably at different depths and across seasons.

During one year we conducted a regular seasonal sampling on four deep peri alpine lakes located in France (Léman, Annecy, Bourget and Aiguebelette) at three different depths (surface, metalimnion and bottom). Samples were stored on 0.22 µm Sterivex filter units (Millipore) and the taxonomic diversity was analysed using DNA metabarcoding and high-throughput sequencing.

This presentation will explore the detected fungal taxonomic diversity, the co-occurrence with other potentially relevant compartments of the planktonic community and their ecological interactions (mainly parasitism on phytoplankton) to bring new insights on how undiscovered biodiversity structures the biotic interactions in large lakes. This work will contribute to WG1 objectives by elucidating the occurrence of fungal parasites in lakes ecosystem and to WG2 objectives by investigating the relation of detected fungal parasites with physical drivers and biological drivers.

Exploring the occurrence of zoosporic parasites and their hosts in coastal marine systems

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ABSTRACT

In coastal environments, marine phytoplankton recurrently produces blooms driven by nutrient availability and low hydrodynamism. These blooms represent conditions of high biomass and low diversity, creating optimal circumstances for the occurrence and transmission of parasitic interactions. In this study, we explore the diversity and ecology of zoosporic parasites infecting dinoflagellates across different coastal habitats along the Catalan coast (NW Mediterranean Sea), including the water column, sediments, and epiphytic communities. Employing a combination of metabarcoding, microscopy, and cultivation we determine the nature of theses parasitic interactions. Generally, the parasites exhibit clear host preferences, being consistently detected when their preferred hosts bloom (1, 2). However, parasitic species from different taxonomic groups co-occur during blooms, and their infection dynamics do not always coincide with bloom development, suggesting potential competitive interaction among parasites. The diversity of these parasites in marine systems is still poorly understood. Our findings indicate that the composition of parasites differs between the water column and the benthos, with a generally higher diversity in the sediments (2). This suggests that sediments in coastal environments may serve as a diversity reservoir. When conditions are optimal for their growth, i.e., high biomass of preferred hosts in the water column, parasites present in the sediments can spread and proliferate into the water column. Furthermore, benthic substrates, including macroalgae, provide suitable conditions for the colonization of protists. Such colonization includes parasites (3), becoming highly diverse during the late phases of biofilm formation. This suggests that parasites are a key component of mature and well-structured microbial communities. As such, parasitic interactions must be better understood for a comprehensive understanding of marine food webs.

The ParAqua project aims to advance in knowledge and expertise on zoosporic parasites and the relation with their hosts in natural ecosystems and industrial algal biotech production. This study provides new insights into the diversity and behavior of zoosporic parasites in marine systems. In this regard, this study is linked to WG1, devoted to determine the occurrence and early detection of zoosporic parasites, and WG2, devoted to elucidate drivers underlying the dynamics of zoosporic parasites. Finally, algal blooms and commercial cultures represent similar cases (high biomass and low diversity). Therefore, results presented are of interest for WG3, devoted to the management and control of zoosporic parasites in industrial production.

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Overview of studies on parasitic aquatic fungi in Bulgaria

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ABSTRACT

The presentation shows the results of the reference search in the Bulgarian literature on aquatic zoosporic parasites and their hosts issued in the period 1929-2007. The investigations started with the works of prominent Bulgarian hydrobiologist, Prof. Alexandar Valkanov, and covering chytridiomycetous and oomycetous parasites of rotifers, pseudofungi and algae from natural freshwater habitats in lowland and mountain regions. In the period 1929-1964 he published eight papers, describing two new genera (*Dangeardiana* Valkanov and *Hydatinophagus* Valkanov), six new species (*Aphanomyces hydatinae* Valkanov, *Dangeardiana eudorinae* Valkanov, *Hydatinophagus apsteinii* Valkanov, *Hypochytrium hydrodictii* Valkanov, *Polyphagus asymmetricus* Valkanov and *Rhizophydium pyriforme* Valkanov) and one new variety (*Rhizophidium pollinis* var. *pirinicum* Valkanow). Prof. Valkanov also reported findings of 18 parasites of the rotifer *Hydatina senta*, of *Saprolegnia* spesies, and of green algae, streptophytes, euglenophytes, diatoms and yellow-green algae from the genera *Chlamydomonas*, *Eudorina*, *Hydrodictyon*, *Oedogonium*, *Euglena*, *Mougeotia*, *Spirogyra*, *Zygnema*, *Epithemia* and *Vaucheria*. Much later, since the mid-1980s until the beginning of the 21st century, Assoc. Prof. Irina Puneva from the Laboratory of Experimental Algology of Bulgarian Academy of Sciences and her co-workers studied chytrid zoosporic parasites of the genus *Tetradesmus* (syn. *Scenedesmus* p.p.) in laboratory algal cultures and in wastewaters from cellulose factories. Her works were focused on the findings and life cycle of *Phlyctidium scenedesmi* and on the influence of fungicides, as well.

The presented review contributes to the knowledge on occurrences of aquatic zoosporic parasites found both in natural waterbodies and in artificial habitats in Bulgaria, which is one of the scopes of the ParAqua project.

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Parasitic aquatic fungi found in freshwater phytoplankton in Bulgaria in the last 20 years

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ABSTRACT

The presentation presents our findings of aquatic zoosporic parasites in phytoplankton samples from different natural Bulgarian wetlands collected during the last 45 years. Although the number of collected samples and visited sites is quite big, exceeding 5000 samples and more than 600 water bodies of different types starting from coastal freshwater and mesohaline natural lakes, hyperhaline basines of salines, river mouths and reservoirs, lowland reservoirs, lakes, rivers and canals, passing through the same types of waterbodies in kettles and mountains, and finishing with high alpine glacial lakes, peatbogs and streams, we can state that findings of zoosporic parasites in algae were quite rare. The observed material was mainly from Chytridiomycota, found mostly in single specimens in desmid algae from the genus *Euastrum* in a high-alpine peatbog in Pirin Mts (unpubl. data) and in the blue-green algae (known also as cyanoprokaryotes or cyanobacteria) from the genus *Sphaerospermopsis* in a lowland microreservoir reservoir Studena (Stoyneva-Gärtner et al. 2023). Once parasites from Oomycota, found in stage of oospores, were observed in the cells of the filamentous streptophyte alga *Spirogyra* in a lowland swamp on the Danube shore (unpubl. data). In addition, working on phytoplankton samples from the alpine lake Popovo Ezero in Pirin Mts, we found the oomycete *Ducellieria chodatii* considered previously to be a green alga. The findings of this pollen parasite in different development stages allowed us to contribute to the knowledge on its life cycle, taxonomy and ecology (Stoyneva et al. 2013).

The presented review provides additional data to the knowledge on spread of oomycetous and chytrid zoosporic parasites found in different wetlands of Bulgaria on conformity with the aims of the ParAqua project and in particular on its focus on collection of data on occurrences of these organisms in aquatic habitats.

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FunAqua: a collaborative approach to understanding aquatic fungal communities

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ABSTRACT

Aquatic fungi have historically received less attention compared to their terrestrial counterparts, leading to a limited understanding of their diversity and function on a global scale, hindered by inconsistent sampling methods and molecular approaches. Recent advancements in DNA metabarcoding using environmental DNA (eDNA) offer a standardized approach to exploring the taxonomic, phylogenetic, and functional diversity of aquatic fungi worldwide.

In 2019, a collaborative effort between the Estonian University of Life Sciences and Tartu University initiated the FunAqua project, aiming to establish the first comprehensive DNA-based inventory of aquatic fungi in freshwater habitats. This initiative has engaged researchers and citizen scientists across 80 countries, resulting in the collection of over 1,500 water and sediment samples from diverse aquatic ecosystems. The extensive dataset achieved by FunAqua not only contributes to ParAqua's aim of creating a comprehensive database but also facilitates seamless data integration across different regions. Moreover, FunAqua's standardized eDNA metabarcoding approach provides the framework for developing early detection methods for fungal threats to algal systems in the future.

This presentation provides a comprehensive overview of the current stage of the FunAqua project, highlighting achievements in data collection and analysis, and emphasizing ongoing efforts. Additionally, it explores future perspectives, offering insights into potential applications and implications of the project's findings.

Keywords: Aquatic fungi, FunAqua, eDNA metabarcoding, Global Diversity, ParAqua

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Monitoring fungi and fungal-like parasites occurrence throughout Europe: synergies with Biodiversa+ FUNACTION and MoSTFun projects

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ABSTRACT

Aquatic fungi (AF) and fungal-like organisms are ecologically diverse and play key functions in aquatic ecosystems and their food webs. Among AF, parasitic species are particularly important actors in the bioindustrial microalgal sector, as they affect production of food ingredients, biofuels, pharmaceuticals and nutraceuticals. Understanding and monitoring AF diversity is an ambitious, but urgently required task that is receiving increasing interest.

Capitalizing on the expertise and knowledge of the FunAqua project, we started two transnational projects within the Biodiversa+ European Biodiversity Partnership: FUNACTION and MoSTFun.

FUNACTION aims at filling knowledge gaps and developing conservation guidelines based on a low-resolution sampling campaign across Europe and a high-resolution one in eight European rivers. Through modeling, it aims at identifying pan-European drivers of AF diversity. Moreover, in light of the EU Biodiversity Strategy for 2030, it aims at the identification of priority conservation areas that maximize total aquatic eukaryotic diversity. The project also initiated the establishment of the IUCN SSC Aquatic Fungi Specialist Group of the IUCN Species Survival Commission.

MoSTFun will leverage on existing biomonitoring programs and networks to reanalyze existing samples and evaluate procedures for their use in routine biomonitoring of AF biodiversity. It will field-test novel technologies and approaches, especially -omics technologies, Earth Observation, GIS, and modeling. We will also test their integration into practicable monitoring procedures based on Essential Biodiversity Variables (EBVs) and open-accessibility of sequencing and environmental data in FAIR databases.

Both projects aim at harmonizing data and expertise with existing initiatives to create a dynamic network of relevant stakeholders.

As the main objectives of the COST Action CA20125 ParAqua consortium are to compile and integrate existing knowledge on zoosporic parasites across Europe, and to design monitoring campaigns for fungi and fungal-like parasites (including to explore the potential of naturally occurring parasites as metrics for novel monitoring strategy in lakes and reservoirs), we discuss how synergies among these initiatives can lead to increased capacity (both in term of data and knowledge exchange) to integrate and magnify these project results.

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A review on the occurrence of parasites in microalgae culture

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ABSTRACT

Nowadays, microalgae are exploited in various fields such as, cosmeceuticals, nutraceuticals, organic fertilizers, human food and animal feed supplements, etc. Due to different ways of use, their production is increasing rapidly every day. Depending on their final application, they can be grown in various cultivation systems. Microalgae are more often cultivated in open systems due the low costs of these systems, while an increase in production is also expected in the closed cultivation systems, mainly photobioreactors[1].

For biotechnological applications, large volumes of microalgae are required to produce considerable amounts of biomass, which increases the possibility of contaminations. It is more likely to occur in open systems, then in closed systems where environmental parameters (temperature, light, pH, oxygen, turbidity, etc.) and nutrients can be controlled, and kept at optimal levels. The presence of contaminants can often cause a reduction in biomass production or massive death of microalgae cultures. There are many biological constraints on mass algae production in the form of grazers, pathogens and parasites [2]. Parasites have been recognized as important drivers of algae population regulation in nature. For example, in fresh water environments,zoosporic fungi *Chytridomycota* and fungi-like organisms (including oomycetes, labyrinthulids, thraustochytrids and phagomyxids) are wellknown to parasitize microalgae. Members of the *Chytridiomycota* are extremely common fungal parasites in freshwater systems that prey on algae. Their host range can be narrow or wide, depending on the parasite species. Recently, a number of important algal parasites that belong to phyla *Endomyxa*, *Cryptomycota*, *Blastocladiomycota*, *Chytridiomycota*, *Labyrinthulomycota*, *Oomycota* and *Alveolata*[2,3] have been identified in algal mass culture systems. This number is sure to grow as the number of commercial algae facilities increases. In this study, the identified parasite species and their hosts in microalgae mass production systems are presented.

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Environmental refuges from disease

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ABSTRACT

Organisms show differences in their physiological performance depending on their environmental context, resulting in performance–response curves along environmental gradients. Since parasites generally have shorter generation times and hence faster adaptation than their larger hosts, parasite performance–response curves are generally expected to be broader than those of their hosts. However, certain environmental conditions may limit parasite performance more than that of the host, thereby providing an environmental refuge from disease. Thermal disease refuges have been extensively studied in response to climate warming, but other environmental factors may also provide environmental disease refuges which, in turn, respond to global change. In this presentation, we want to (1) showcase laboratory and natural examples of refuges from parasites along various environmental gradients, and (2) provide hypotheses on how global environmental change may affect these refuges[1]. We strive to synthesize knowledge on potential environmental disease refuges along different environmental gradients including salinity and nutrients, in both natural and food-production systems. Although scaling-up from single host–parasite relationships along one environmental gradient to their interaction outcome in the full complexity of natural environments remains difficult, integrating host and parasite performance–response can serve to formulate testable hypotheses about the variability in parasitism outcomes and the occurrence of environmental disease refuges under current and future environmental conditions.

This work proposes a framework for environmental refuges from disease with some examples from zoosporic infections but also including a wider scope. It links to the objectives of WG2 on drivers of zoosporic infections, and tangentially to the objectives of WG3 on control of zoosporic infections in production systems.

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Fungal parasites infecting benthic diatoms in the Arctic and Antarctic coastal waters

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ABSTRACT

Fungal parasites have been recognized as critical and abundant components of every ecosystem. They have the potential to regulate host populations, mediate interspecific competition between hosts and other species, and affect community structure. However, parasite diversity is still poorly known, particularly for fungal parasites in aquatic ecosystems, and only rough estimates exist on total species diversity and abundance. Few recent studies in polar regions indicate that parasitic species, in particular of the early fungal lineages, are highly abundant in both Antarctic and Arctic aquatic ecosystems, yet their diversity and ecological roles are still poorly understood. Therefore, we aimed to address these gaps and investigate the relevance of fungal parasites for microphytobenthic communities, which form the basis of coastal food webs and are key components for trophic interactions in polar ecosystems. In our study, we describe the fungal diversity and identify host-parasite interactions in marine, brackish and freshwater benthic habitats of Kongsfjorden (Svalbard) and Potter Cove (King George Island, Antarctic Peninsula) using high-throughput sequencing and microscopical observations[1]. We show that fungal parasitic taxa are present in these habitats in high abundances, whereby their correlations with benthic diatoms indicate potential parasitic interactions. Moreover, we show that salinity is a major driver of fungal diversity and community composition and thus emphasize the need for further research, considering the effects of increased inputs of glacial meltwater caused by increasingly severe climate change. Our study, extent the habitat-range of fungal parasites on phytobenthos and reveals that at even low temperatures (<4°C) fungal parasites are active and have the potential to control primary production of polar food webs. These findings are in the core of ParAqua addressing the occurrence and ecological function of zoosporic fungal parasites as well as their drivers.

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How does environmental temperature impact the ability of *Daphnia* to resist a novel fungal parasite?

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ABSTRACT

Increasing lake temperatures, associated with global warming, are fundamentally changing the structure of aquatic communities and these factors may increase the susceptibility of *Daphnia* species to certain parasites. We aimed to test how the ability of *Daphnia* to resist a fungal parasite (*Metschnikowia bicuspidata*) had changed in response to 60 years of artificial heating of natural lakes, by dumping a hot water from a nearby power plant (the average water temperature difference between heated lakes and nearby control lakes corresponds to ca. 4 °C). The increased physiological demands of living in a warmer climate may necessitate more resource investment into temperature tolerance and less into immunity. We hypothesised that *Daphnia* isolated from artificially heated lakes would be more susceptible to parasitic infections. Twelve *Daphnia* clonal lineages (isolated from two heated and two control lakes) were exposed to *Metschnikowia* spores or to placebo solution, and exposures took place under 18 °C (control) or 22 °C (elevated) temperatures. By keeping *Daphnia* from heated and control lakes at two different lab temperatures we aimed to test whether differences in resistance and fitness are due to evolution caused by long-term exposure to elevated temperature or short-term phenotypic plasticity to lab thermal environment. By comparing the intensity of infection and host fitness of *Daphnia* from different temperature environments we hope to determine to what extent temperature affects the ability of *Daphnia* to resist parasitism. This project focuses on the relationship between *Daphnia*, a keystone species in limnological systems, and *Metschnikowia*, an important fungal parasite of a wide range of aquatic crustaceans. The host-parasite pair used in this research has the potential to become a model system and utilised to gain a broader understanding of eco-evolutionary dynamics in aquatic environments.

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Experimental evolution of a fungal parasite in response to elevated temperature

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ABSTRACT

It is commonly believed that global warming will result in a “sicker world”, with infectious diseases increasing in prevalence and virulence. However, these predictions are mainly based on short-term experiments that did not allow a parasite to evolve, and thus overlook the predisposition of parasites to evolve thermal adaptation rapidly. Elevated temperatures can enhance parasite performance in reproduction, infectivity, and virulence. To investigate the adaptive response of parasites to elevated temperatures, we conducted an experimental evolution assay in a model system of the parasite *Metschnikowia bicuspidata*, a fungal parasite of *Daphnia magna*, were maintained at either elevated (24°C) or control (20°C) temperatures over six generations. The performance of each parasite line was subsequently assessed under both elevated and control temperature conditions. Reciprocal infection test revealed that parasite fitness was diminished in lines evolving at higher temperatures across both test temperature conditions. These findings suggest that, contrary to expectations, the “sicker world” hypothesis may not hold true for the Daphnia-Metschnikowia system.

While our model system is not exclusively designed for zoosporic parasites, it is applicable to various host-parasite systems that involve a free-living stage, including chytrids. Thus, it has the potential to contribute to our understanding of interactions between zoosporic aquatic fungi and their hosts.

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Increasing winter temperature effect on diatom-chytrids' dynamic and carbon fluxes in lakes

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ABSTRACT

Our project aims to investigate the impact of rising water temperatures, on the parasitic relationship between the Diatom *Asterionella formosa* and the Chytrid *Zygorhizidium planktonicum* in two lakes: Switzerland's Lake Geneva and the Netherlands' Lake Maarsseveen. This exploration will help understand how temperature fluctuations influence the dynamics of this host-parasite relationship, and carbon fluxes to the lakes' sediment. Indeed, uninfected *Asterionella* drives substantial carbon fluxes to lake sediment during spring blooms. However, higher winter temperatures and changes in infection rates could affect *Asterionella*'s spring bloom formation, in turn reshaping spring bloom communities, impacting lakes' food webs and carbon fluxes[1]. We will develop a fundamental theoretical and empirical understanding of the temperature dependence of host-parasite interactions, while addressing the consequences of environmental change for ecosystem processes. To achieve this, we will use a combination of high frequency monitoring, laboratory assays and mechanistic models parameterized with our field sampling and experiment results.

As such, this project will support the work done in ParAqua WG1 and WG2 and contribute directly to the Action RCO2 (integrate information on environmental drivers of parasite epidemic development) and RCO5 (setting up monitoring schemes). Specifically, this collaboration will allow to add new data to a 60-year long data history on chytrid parasitism within Lake Maarsseveen. This comprehensive dataset would hold significant promise in comprehending the impact of climate change through increasing water temperatures on host-parasites' interactions' dynamics. Moreover, the resulting data will be shared and added to the Paraqua's databases on zoosporic parasites and help determine parasitism effects on lake ecosystems across Europe, through their impact on processes such as carbon fluxes. We aim to develop new automated processes for monitoring parasitic infection in-situ, which represents one of the most important challenges for the study of zoosporic parasites at a bigger scale. Presently, the assessment of parasitic infection relies predominantly on time-consuming methods involving Utermöhl chambers and conventional inverted microscopes. By developing more efficient automated monitoring systems, we aspire to catalyse a more widespread monitoring of parasitic infections in lakes or algal biotech production systems, thereby facilitating large-scale data collection throughout Europe, by sharing and publishing these new methods in the Paraqua Method Handbook. This project will also support the aims of ParAqua WG3 with the creation of mechanistic models of our host-parasite system and its contribution to carbon fluxes to the lake sediment.

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Microscopic techniques for the detection of the parasite in microalgae culture

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ABSTRACT

Microscopic techniques are essential in the detection and monitoring of microalgae cultures due to their ability to provide detailed information about cell morphology, growth, and health. This abstract presents an overview of the various microscopic techniques commonly used for the detection and analysis of microalgae in culture systems.
One of the most commonly employed techniques for the detection of parasites in microalgae in culture is brightfield microscopy. Fluorescence microscopy is another powerful tool that enables the specific labeling and visualization of parasites. These techniques can provide valuable insights into the physiological status of the parasites.
Confocal microscopy is a high-resolution imaging technique that allows for the three-dimensional visualization of microalgae cells and provides detailed information about cell structure and organization. This technique is particularly useful for studying cell- cell interactions and spatial distribution of cellular components within the culture system.
Furthermore, transmission electron microscopy (TEM) and scanning electron microscopy (SEM) are advanced microscopic techniques that provide detailed information about the ultrastructure of microalgae cells and can help in the identification of intracellular organelles, lipid bodies, and other key cellular components.
Overall, microscopic techniques play a crucial role in the detection of parasites in microalgae cultures, providing valuable information about the physiological status of the cells and facilitating the optimization of culture conditions for enhanced biomass production and biofuel generation. By employing a combination of these microscopy techniques, researchers can gain a comprehensive understanding of microalgae growth dynamics and make informed decisions to improve the efficiency and sustainability of microalgae culture systems.

Keywords: microalgae cells, parasites, microscopic techniques, TEM, SEM

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Scaling Up algae cultivation on waste substrates: a sustainable approach

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ABSTRACT

Large-scale algae systems represent a promising approach supporting circular bioeconomy principles adopted by EU programmes like Green Deal and Farm-to-Fork. Algae cultivation in waste substrates (wastewater, anaerobic digestate) as a parallel bioremediation and biomass production presents an interesting industrial ecology model. Nutrients, organic carbon and minerals that would otherwise be lost by the discharge into environment, are recovered by algae for their growth. In wastewater cultivation, the large-scale production can be done without consuming large volumes of quality water and expensive commercial growth media. The produced biomass can be exploited for a variety of products, from low-added-value biofuels, organic fertilizers, or biomaterials to the high-added-value compounds for pharmacy, cosmetics, and agriculture (biostimulants, biopesticides).

Algae-bacteria community that establishes itself in the waste substrate has a symbiotic relationship: algae produce oxygen that aerobic bacteria use for the degradation of organic matter, while bacteria provide the nutrients and organic carbon for the algal growth. The produced oxygen considerably reduces costs of the energy-demanding technological oxygenation in the wastewater treatment process. Carbon dioxide is consumed by photosynthesis and the odors are significantly reduced.

The large-scale cultivation of macroalgae monocultures in the waste substrate predominantly occurs in the open pond systems: raceways ponds with paddlewheels for water circulation, maintaining the algae in the suspension. To accomplish good algae performance, the system has to be optimized to specific goal. For biomass production, nutrient composition of the substrate, light availability and absence of infections are crucial. Waste substrate is problematic in this aspect as it's a highly variable, sometimes colored, medium with changing chemical and microbiological composition.

Waste substrate generally promotes growth of a mixed culture with alternating strain composition, making the running of continuous culture a special challenge. Decision support tools can help mitigating some of the challenges, but for infections that rapidly destroy the culture, they are not sufficient. Especially in large ponds, improper design or mixing can result in so called "dead zones" where bacteria or fungi can thrive and eventually infect the culture. We need deeper understanding of drivers that cause the culture collapse that will enable early detection of the infection to enable uninterrupted production of quality biomass and make the large-scale algae production on waste substrates a viable and economical option.

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Microbeco and ParAqua: enhancing the communication on your past activities for a more impactful future of the ParAqua network

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ABSTRACT

Microbeco is a non-profit scientific organization dedicated to promote the understanding and appreciation of microbes, and to support the development of new knowledge and applications that can benefit society. The association is active on various fronts: from writing articles, to managing social media, to participating in festivals. It also annually organizes the International Course in Microbial Ecology (ICME) for 20-30 participants all over the world to learn and discuss the latest methods and techniques used in the field of microbial ecology. Its role within the COST- Paraqua- COST action was mainly focused on the early creation of the webpage and its maintenance. Now is the time to develop new actions, create infographics, and promote messages that summarize the project's research and results in a clear and concise way and to tailor content for different social media platforms, adapting tone and language to the target audience, mainly going beyond academy and opening to industrial stakeholders. Indeed, implementing communication channels would serve not only to expand the project's networks but also to build a clear and recognizable identity. Starting from 2024, the objective of Microbeco's actions are: 1) increase traffic to the COST-Paraqua website and social media channels; 2) increase visibility of the project and its activities among the scientific community and the general public; 3) increased number of downloads of project publications and materials; 4) organizing citizen science events that include an interested generalist audience. To measure and evaluate the results, the communication channels (website visits, social media followers, etc.) will be monitored. It is essential to have a continuous commitment, from now until the end of the project, to highlight the work done so far, which has seen the full involvement of some colleagues and which deserves to be adequately valued.

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In-vivo detection of zoosporic parasites in aquatic systems using Digital Holographic Microscopy

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ABSTRACT

Detecting microparticles, microorganisms, counting various cell species in liquids to ensure quality is paramount across multiple industries, including microalgae culturing, food production, water utilities, and beyond. A wide range of technologies and methodologies are available to measure critical parameters of these processes. However, many of these solutions can be labor-intensive, costly, and challenging to integrate into existing industrial workflows. Our team specializes in creating innovative solutions that utilize a range of advanced technologies. These include digital holographic microscopy (DHM), fluorescent microscopy, and cutting-edge artificial intelligence. The DHM devices developed by our team alleviate the need to use manual methods for cytometry, offering a significant advantage in scaling up operations in microalgae farming, food processing operations or ensuring water safety for water utilities. Automation in quality control is crucial for cost efficiency and maintaining consistent quality. Our devices are customizable to meet the specific needs of various species, microparticles, and can flexibly integrate with a wide range of industrial equipment.The developed instruments feature measurement cuvettes with depths ranging from 100 to 10,000 microns to accommodate different magnification needs. Leveraging the power of digital holographic microscopy, Holodetect captures the entire sample volume information in a single image, eliminating the need for focus. This technology allows for reconstructing all sample objects from the recorded hologram with minimal sample preparation. It also supports flow-through operations for analyzing large volumes of samples. Analysis of reconstructed images is powered by advanced artificial intelligence. These AI models are highly adaptable and can be trained to identify various species and objects within the sample accurately. Additionally, our holographic microscope can be integrated with fluorescent microscopy to enhance classification precision and conduct a variety of fluorescence-based measurements.

Detecting and managing zoosporic parasites in microalgae cultures is essential for maintaining the health and productivity of these cultures and one of the important objectives of ParAqua. Digital holographic microscopy offers a powerful, non-invasive, in-vivo method for detecting and analyzing zoosporic parasites in microalgae cultures. By combining it with fluorescent microscopy (both labeled and label free) it's possible to implement inline, quasi real-time monitoring allowing for the early identification of parasitic infections.

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AI-powered Microfluidic Microscope: a new automated cell-counter

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ABSTRACT

Microalgae and cyanobacteria growth are usually monitored by conventional analytical techniques such as microscopy, gravimetry, or spectrophotometry measurements – flow cytometry is not a reliable option for cyanobacteria. Among them, manual cell counting remains the reference method to access the cells concentration. As opposed to other traditional techniques, manual counting is less sensitive to interferences such as presence of suspended solids or self-absorbance of the liquid media such as wastewater and agro-industrial effluents. Despite these advantages, manual counting is a time consuming and labor-intensive technique, which often limits its utilization in most of research laboratory and industrial facilities.

Microdeep™ is an automated cell counter based on microscopy using artificial intelligence technology to continuously monitor the presence of microscopic objects. The liquid is pumped through a microfluidic single channel flow cell, microscope images are captured and analyzed on-device by means of an object detector deep neural network. The objective of this study was to test the Microdeep™ and compare its performances with traditional analytical techniques. Consequently, a concentrated solution of *Arthrospira platensis* was diluted with demineralized water (DW) to prepare various solutions ranging from 90 to 400 kcells/mL. The cyanobacteria concentration was evaluated by measuring: the total suspended solids concentration, the turbidity, the absorbance at 680 nm and the cell concentration (Microdeep™).

The experimental results indicated that the Microdeep™ counter was perfectly comparable with conventional techniques when analyzing the concentration of *A. platensis* cells in solutions diluted with DW. All the calibration curves showed a linear relationship with coefficient of determinations (R^2) higher than 0.99. Besides counting, valuable information in terms of growth was obtained from the morphology of filaments.

Importantly, the Microdeep™ counter is extendable to other types of microscopic objects: the tool was designed to be extremely versatile, in allowing other models to be developed and integrated quickly. We will outline the workflow of capturing, treating new data and training new models to fit more ambitious goals than counting and determining morphology of microalgae. In particular, we will examine the relevance of the equipment to characterizing zoosporic parasite contents of algae solutions. We will conclude with a possible path towards this solution.

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Zoosporic parasites, foes of microalgae production

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ABSTRACT

A4F - Algae for Future is a leading biotechnology company based in Portugal, specialist in algae production and research. With over 15 years of experience, A4F excels in designing, building, operating, and transferring both algae production and biorefining units. Known for its capacity to develop large scale algae facilities and its strong connections with major research institutions, A4F stands as the preferred partner for commercial-scale algae endeavors, integrating the best technologies and best practices to meet industry standards. Moreover, A4F also provide consulting services to algae producers based on expertise and experience.

One of the greatest difficulties for algae production are parasitic diseases. Many of zoosporic parasites infect and feed on microalgae for survival, causing severe host mortality and, consequently, a huge economic impact in algae biotechnology industry. Therefore, A4F joined ParAqua COST Action innovative and dynamic network for the development of methods for monitoring and early detection of these parasites and the implementation of prevention, management and control strategies to mitigate parasitic infections.

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Controlling the protozoa *Poterioochromonas* in large scale raceway open ponds

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ABSTRACT

The protozoan flagellate *Poterioochromonas malhamensis* is a well know contaminant for many algae species. Several studies reported the presence of this parasite in various algae cultivation systems, different water types and environments (Ma et al., 2023). In our experience, *Poterioochromonas* is a ubiquitous organism that appears in *Nannochloropsis* algae culture in very short time. The presence of such predator can burden on the algae culture and explode till complete devastation of the ponds.

Several approaches were studied to monitor the presence of such organisms and to anticipate on its blooming. Parameters, as oxygen level in the water, were closely monitored to detect any suspicious activity that could indicate the presence of parasites and to rescue the algae culture before reaching an anoxic condition that would lead to a crash.

As well as few protocols were established to keep the contamination down. *Poterioochromonas* count was kept in control using chemical agents as peroxide and ammonia (He et al., 2021). Use of peroxide shown positive results in controlling *Poterioochromonas* explosion but only if the organic load in the algae culture was low. Peroxide effect on culture running for more than a month is not efficient since peroxide breaks down on organic matter before having any impact on *Poterioochromonas*. The use of ammonia was also effective in controlling the contaminant. On the other hand, the downside of using this method is that it's difficult to control and requires high pH level in the culture to avoid that the ammonia is rapidly converted into ammonium. High pH can lead to cell precipitation and, in case of salty and hard water, to scaling and carbonate formation.

The experience gained during this study and the amount of data collected in a large-scale facility can be a gateway to learn more about this parasite and its occurrence in algae ponds. The study fits well the objective and the scope of the ParAqua project and can provide a good insight into the life cycle of this organism under different conditions. Moreover, a correlation between the microbiome and the presence of *Poterioochromonas* is crucial to understand the triggering of this contamination and to find a way to control it working more on a microbiological approach than a chemical repression.

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An SME dedicated to the microalgae production

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ABSTRACT

Algaria is a SME based in northern Italy that develops research and innovation in the field of microalgae production. As one of the pioneer *Spirulina* producers in Italy.

Algaria is active in the soil-less production of food ingredients and products based on a specific microalga (*Spirulina*), with the brand: https://spireat.it/. Algaria's brand is already one of the leading ones in Italy and EU. Different innovative *Spirulina*-based food products with improved nutritional values are on the market, such as snack bars, risotto. Recently, Algaria succeeded in the EU-contest Ecotrophelia for innovative-sustainable food, in partnership with the University of Milan, launching a new chips snacks protein-rich based on Spirulina.

Algaria's productive and experimental site is located in Casalbuttano (CR, Italy). The technology includes the integration to a biogas co-generation unit, which allows recovering heat and producing continuously, over wintertime in rigid climates. An innovative membrane- based extraction unit allows the co-production of food-grade Phycocyanin pigment from *Spirulina*, used in the food industry as blue-dye with antioxidant and anti-tumoral properties. Algaria has also recently developed an innovative biomimetic technology based on microbial electrochemistry: the e-Soil (www.e-soil.net/). This technology behaves like an artificial soil, by stimulating microbial mineralization of organic compounds, using electroactive microbial population and conductive materials. This technology would allow the production of soil-less food, using fully-recycled nutrients sources, such as secondary products, process water from food transformation industries and food waste. Algaria's objective is to bring this technology to full-scales applications in microalgae production and to apply it to other types of soil-less farming, such as hydroponic horticulture.

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Scale-up, biorefineries and sustainability of microalgae production

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ABSTRACT

The industrial production of microalgal biomass, coupled with the mitigation of CO_2 and recycling of nutrients from wastewaters, using natural sources like sunlight, has been considered one of the most promising sustainable approaches for different biotechnological applications (e.g. food, feed, pharmaceuticals, fertilizers, cosmetics, and biofuels).

Mass culture of microalgae can be achieved in open (e.g., open ponds or raceways) or closed (e.g., vertical, planar, tubular photobioreactors) production systems. Open ponds are the system chosen by most companies producing microalgae at an industrial scale due to the low capital and operational costs[1]. However, cultures are directly exposed to the atmosphere, and the water and CO_2 losses, and the probability of contamination are the main barriers to open production systems. In addition, the strict control of temperature and other culture parameters is challenging. On the other hand, closed systems display lower CO_2 and H_2O losses, reduce the probability of contamination, and allow a tighter control of growth[1].

To meet the full potential of microalgal biomass, selecting robust and fast-growing strains is crucial for stable and sustainable microalgae cultivation[1]. Several microalgae ventures have been established in recent years, but the implementation of industrial biomass production is still at an infant stage[2]. In addition, microalgae-based bioproducts remain expensive, due the three major segments that need to be considered for economic microalgae biorefinery, which is low-cost nutrient source, efficient harvesting methods, and production of by-products with high market value. By using a biorefinery platform and circularity towards zero-waste, all the biomass components can be separated and used, producing multiple products, maximizing the value of the feedstock, preventing resource loss and environmental impacts, and improving the overall economic feasibility[3].

In Keynote, the scale-up of *Tetraselmis* sp. CTP4 will be shown from an agar plate to 100-m^3 industrial photobioreactors. This microalga was isolated and considered as a robust, euryhaline, lipid-rich able to grow both in standard growth media, as well as in urban wastewater efuents[1]. I will be also present its own biorefinery and other examples, as well as the circularity and sustainability of different systems with other microalgae. An interesting work called “from Pig to Wheat” will also be exhibited, demonstrating the total circularity and sustainability of these systems using microalgae.

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Challenges in parasite detection: old and new approaches revisited.

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ABSTRACT

The importance of microalgae as a staple feedstock for products ranging from feed to pharmaceutical applications is known by both the industry and the academy. However, there are still challenges that make the production of biomass expensive and not viable in most cases. One of these challenges is the appearance of predators, competitors and parasites that can harm industrially produced algae. There have been cases where the accompanying microbiota associated with the culture of microalgae being produced has been cited as not harmful [1]. However, specific amoebae-like microorganisms and fungi, such as chytrids, can collapse a culture on a full production scale in a matter of days [2]. The methods used to detect these biological contaminants can be separated into two main classes: optical and molecular. Optical methods can include microscopical daily observation and taxonomical targeted analysis of the contaminants; employ continuous flow sampling and detection via changes in turbidity of the culture and flow cytometry. The specific method to be used depends on the species of algae and contaminant targeted, as each type of organism may respond to different optical methods due to their different auto- or epifluorescence characteristics. Molecular methods comprise metagenomic analysis and targeted (q)PCR. Despite their higher specificity, the latter methods require highly skilled personnel and equipment that might not be as practical as the former type of methods. This presentation brings together an overview of the ever-running struggle of identifying and mitigating the microalgal production contaminants throughout the production pipeline, presenting a practical perspective on how to improve the currently available methods. Under the Paraqua COST action, this effort will undoubtedly shed new light on the future detection of zoosporic parasites on microalgal production. Moreover, it will highlight the necessity of developing new, faster, easier, and cheaper methods for molecular analysis and detection of these organisms, considering current projects being executed on this specific topic. It will also briefly discuss the existence of alternatives for mitigating these biological contaminants that can be used by either small or large algae producers.

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Strategies to mitigate the impact of parasitic fungi in *Nannochloropsis sp.* industrial cultures

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ABSTRACT

Microalgae cultivation has emerged as a promising solution to address challenges related to climate change and the increased human population since these microorganisms offer sustainable solutions by treating wastewater, sequestering CO_2, and providing biomass for diverse applications in food, feed, nutraceuticals, and agriculture. However, industrial production faces significant challenges, with biological contaminants, reducing microalgal biomass productivity within a few hours upon detection, resulting in some cases in the complete culture collapse and, thus, significant economic losses [1].

Culture collapse can be triggered by various contaminants, including competitors (e.g., photosynthetic microorganisms), parasites (e.g., fungi, viruses), and grazers (e.g., amoebas, ciliates, flagellates). This research aims to investigate the biological contaminants responsible for the collapse of *Nannochloropsis* sp. cultures, focusing on identifying, isolating, and developing strategies to mitigate this issue. Industrial culture samples experiencing collapse were analysed through DNA extraction and next-generation sequencing (NGS) targeting the 18S regions [2]. Bioinformatic analysis revealed a parasitic microscopic fungus as responsible for the collapse of *Nannochloropsis* cultures. Further lab-scale investigations involved studying the identified contaminant and testing mitigation strategies. Chemical agents, such as sodium dodecyl sulphate (SDS) and sodium dodecyl benzenesulfonate (SDBS) were used, proving to be effective in controlling the contamination without harming the microalgae culture [3], when concentrations as low as 25 mg/L and 2 mg/L were added in 100-mL Erlenmeyer flasks, respectively.

Future work includes biochemical analysis to understand the impact of mitigation on final biomass quality, along with the development of monitoring and early detection methods using contaminant-specific primers. Implementing these findings in large-scale production will empower the microalgae industry to effectively control zoosporic parasites and comprehend their relationship with the host microalgae.

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Preliminary biological risk assessment of microalgae biomass cultivated in wastewater

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ABSTRACT

Microalgae cultivation is in line to circular economy principles due to their capacity to recover nutrients from wastewater, absorb carbon dioxide and produce valuable biomass that can be used to biofuels or biofertilisers. However, wide implementation of microalgae biotechnology is still limited, and significant barriers have been detected in the use of microalgae bio-products (Acién et al., 2023). There exists significant concerns on possible health and environmental risks in the use of microalgae bio-fertilisers. Microalgae can get infected by numerous organisms such as pathogenic bacteria, cyanobacteria and/or zoosporic parasites (Fiolka et al., 2021). This can limit the commercialisation of microalgae-based biofertilisers for not accomplishing legal requirements. It can also make the consumers mistrust in their health assurance of these biofertilisers. Consequently, innovative tools are needed to provide reliable and transparent information that assures the safe production and use of microalgae bio-products. To do so, the risks of using wastewater borne-microalgae biomass must be identified and evaluated.

Description of the key approaches and primary results: A green microalgae consortium formed by *Chlamydomonas reinhardtii*, *Auxenochlorella protothecoides, and Tetradesmus obliquus* was grown in controlled lab-conditions and acclimated to urban wastewater from Jesi's urban wastewater treatment plant (WWTP) (Ancona, Italy). This consortium was then cultivated in a pilot-scale high-rate algal pond (HRAP) of 1.25 m^2 and 10 cm deep to treat urban wastewater and produce microalgae biomass for potential biofertiliser production. After 10 weeks of outdoor cultivation, preliminary microscopic observations showed that the consortium evolved to mixed culture composed (mainly) of: *desmidiales*, *tetradesmus* (two strains), *diatoms*, and *chlorella*-like microalgae. Zoosporic parasites on the cultivated microalgae were not observed, but more detailed analysis using stains such as the Calcofluor white fluorochrome (CW) (Fiolka et al., 2021) would be needed to confirm these results. Moreover, LC-MS/MS chemical analysis of microcistins revealed that their amounts were under detectable levels of the methodology. With respect to E. coli, analyses under lab-scale conditions showed significant amounts of bacteria within microalgae biomass (data not shown). However, outdoor cultivation is expected to reduce them significantly thanks to the disinfection capacity of solar ultraviolet irradiation. This must be confirmed with experimental data from the pilot reactor.

Link to ParAqua main objectives and scope: This work focuses on the preliminary identification of biological contaminants of the microalgae biomass, specifically pathogenic bacteria (e. coli), cyanobacteria and zoosporic parasites. The preliminary results (to be completed) will provide information on implementing pollution control systems to obtain high-quality microalgae biomass that can be used for biofertiliser production, as well as on the analysis of their potential risks for the consumers. It is highly aligned with the objectives of ParAqua, especially with WG3.

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Visual, early & fast detection kit for algae biological contaminants. Would companies buy this kit or not?

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ABSTRACT

Biotic contaminations are a major economic hurdle in industrial-scale cultivation of photoautotrophic microalgae. In fact, prior visual, empiric and personal communication tell us that these microorganisms could be Amoeba or other protozoan, fungus or bacteria are known to collapse microalgae cultures within a short time period (24-78 hours) if not detected and controlled on time. In this context, the present work focused on applying Next-Generation Sequencing (NGS) technologies, to study contaminated cultures and develop an early detection kit to be used at industrial level. Loop-mediated isothermal amplification (LAMP), a gene amplification method combines rapidity, simplicity, and high specificity. Therefore, the development of a LAMP kit intended to detect contaminant in cultures using a simple and visual method allows producers to apply mitigation strategies on time. We observed a contaminated *Tisochrysis lutea* culture and studied the life cycle of the contaminant causing microalgae collapse. Using FACS we successfully isolated and identified the organism Chrysophyceae *Paraphysomonas* sp., finally a LAMP kit was developed to facilitate the identification in early stages of contaminated cultures. In conclusion, further research on these controlled ecosystems and microorganisms at industrial scale is necessary to avoid economic losses of microalgae ventures. Improvements in early detection/mitigation strategies are crucial to minimize biological contamination and enhance biomass productivity, ensuring the future sustainability of the algae production sector. Zoosporic parasites and other biological contaminants are a big problem in microalgae cultivation, non-aseptic microalgae cultures are a complex ecosystem when in production and scale-up steps need to be followed and understood carefully. Molecular and phylogenetic analyses performed in parallel with persistent microscopic observation of contaminated cultures are powerful tools to clearly identify harmful contaminants and evaluate their life cycle under controlled conditions. Identification and early detection techniques, for example LAMP kits, are crucial steps in the microalgae cultivation field, by providing easy-to-use tools in the daily routine that can help to prevent losses and improve production.

Keywords: LAMP, biological contaminants, early detection, industrial-scale production

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First Molecular occurence of *Cryptosporidim* spp in fish from Algeria

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ABSTRACT

Microbial eukaryotes of the genus *Cryptosporidium* are apicomplexan parasites that infect mainly the digestive tract of humans and a broad range of domestic and wild animals, including mammals, birds, fish, reptiles and amphibians. The present study aimed to estimate the prevalence and molecular characterization of *Cryptosporidium* spp. in six different fish species both from marine and freshwater environments. During a period of 2 years (2018–2020), a total of 415 fecal samples and 565 intestinal scrapings were collected in seven provinces from the central and eastern Algeria. From those, 860 fish belonged to six different species, two of which are cultured marine and four are wild freshwater fish. All samples were screened for *Cryptosporidium* spp. presence using molecular techniques. Nested PCR approach was performed to amplify partial sequences of the small subunit ribosomal RNA (SSU rRNA) and 60-kDa glycoprotein (GP60) genes for *Cryptosporidium* genotyping and subtyping. Detailed statistical analysis was performed to assess the prevalence variation of *Cryptosporidium* infection according to different risk factors. Nested PCR analysis of SSU gene revealed 173 *Cryptosporidium* positive fish, giving an overall prevalence of 20.11% (17.5–23.0). Cryptosporidium spp. was detected in 8.93% (42/470) of cultured marine fish and 33.58% (131/390) of wild freshwater fish. Overall, the prevalence was affected by all studied risk factors, except the gender. Molecular characterization and subtyping of *Cryptosporidium* isolates showed occurrence of IIaA16G2R1 and IIaA17G2R1 subtypes of *C. parvum* in the fish species *Sparus aurata*. The present study provides the first epidemiological data on the prevalence and associated risk factors of *Cryptosporidium* spp. in farmed marine and wild freshwater fish and the first molecular data on the occurrence of zoonotic *C. parvum* in fish from North Africa (Algeria).

Key words: *Cryptosporidium* spp. · Prevalence · Molecular characterization · Algeria

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Exploring on-site diagnostic markers for aqua systems: a fish iridovirus model

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ABSTRACT

Lymphocystis disease, stemming from the iridovirus lymphocystis disease virus (LCDV), poses a significant challenge across diverse marine and freshwater fish species worldwide, often exacerbated by environmental stressors common in aquaculture settings. The gilthead sea bream (*Sparus aurata*), a cornerstone of Mediterranean aquaculture, notably harbours a distinctive strain of LCDV, necessitating further characterization.

In our study, LC-MS/MS proteomic analysis was employed to assess the presence of pathogenic signals within complex biological systems. Our findings shed light on the major protein markers associated with lymphocystis lesions in *Sparus aurata*, revealing the dominance of LCDV-Sa signals alongside other viral indicators. Leveraging this knowledge, we propose the development of an aquaculture diagnostic kit, facilitating rapid on-site disease detection and intervention based on identified protein markers.

An aquaculture diagnostic kit is an affordable solution possibly designed for early disease detection and prompt intervention using detected major protein markers related to pathogens. It will enable self-testing, allowing the aquaculture industry to perform the diagnostic test on-site in a few minutes. Using cost-effective and on-site diagnostic approaches will be the most pressing knowledge gap in algal production systems in biotech settings. These should not be limited to DNA detection methods; hence, the active involvement of pathogens needs additional omics proof, especially protein level, to support their involvement in algal production dynamics. It is not always the case that DNA detection does not reflect any pathogenesis, so it can misdirect culture maintenance precautions. Simplistic and on-site applicable monitoring protocols or tools (POC – Point of care) need to be envisioned.

This approach not only addresses the immediate need for efficient disease management but also underscores the importance of incorporating proteomic insights into diagnostic protocols, complementing traditional DNA-based methods. By bridging this gap, we aim to enhance the sustainability and resilience of aquaculture systems, paving the way for proactive monitoring and intervention strategies tailored to the dynamic challenges of algal production in biotech settings.

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Novel insights on freshwater oomycete ecology, monitoring, and sustainable control

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ABSTRACT

Oomycete pathogens of freshwater animals cause considerable ecological and economic losses. Among them, *Saprolegnia parasitica* causes saprolegniosis with negative effects on freshwater aquaculture. *Aphanomyces astaci*, the causative agent of crayfish plague, is listed among the 100 worst invasive species in the world as it decimates populations of native European freshwater crayfish species. I will present the results of our work on these pathogens, which are closely related to the themes of the ParAQUA working groups.

Firstly, we have developed non-invasive monitoring methods to track the oomycetes in freshwater ecosystems. We have developed a novel, non-destructive approach for monitoring *A. astaci* based on the detection of the pathogen in the microbial community on the cuticle of individual live crayfish. The results are consistent with traditional destructive sampling and the method has already been used to monitor *A. astaci* in naturally infected but viable native crayfish populations. In addition, we have developed a rapid, sensitive and specific digital droplet PCR assay for *S. parasitica* and demonstrated its applicability for the detection and quantification of the pathogen in environmental samples, i.e. in swab DNA (trout skin, surface of eggs, crayfish cuticle) and in eDNA extracted from water.

We have identified some of the drivers that promote the spread of saprolegniosis and crayfish plague. We have shown that trout farms can serve as a source for the spread of *Saprolegnia* species in the environment, with potentially negative effects on wild fish populations. Furthermore, we have shown that some water parameters are positively correlated with the load and sporulation intensity of oomycete pathogens, characterising the way in which water chemistry can promote disease outbreaks. For example, *S. parasitica* load in water is positively correlated with some site-specific abiotic parameters such as electrical conductivity and calcium, while organic matter in water, particularly its aromatic components, promotes sporulation of this pathogen. These results are a first step towards defining freshwater habitats with an increased likelihood of disease outbreaks.

Finally, we are developing environmentally friendly control strategies for oomycete diseases in crayfish and salmonid aquaculture to reduce the use of toxic chemicals. We have shown that the application of bioactive plant products such as propolis and sage essential oil can inhibit the mycelium and zoospores of *A. astaci* and *S. parasitica*, while commensal bacterial isolates from the cuticle of crayfish and the skin of trout can inhibit their mycelial growth. We are currently investigating the mechanistic basis of these effects and the potential for *in vivo* applications for the prevention and/or treatment of crayfish plague and saprolegniosis in aquaculture.

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Zoonotic parasites in freshwater fish of north Macedonia: implications for public health and fisheries management

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ABSTRACT

This study examines zoonotic parasites infecting freshwater fish populations across North Macedonia, with a specific emphasis on Lake Dojran. The research evaluates the prevalence, diversity, and implications of these parasites for both public health and fisheries management. A primary focus is placed on *Eustrongylus* spp., a prevalent parasite known to infect various predatory fish species within the region[1]. Furthermore, the study addresses the presence of *Diphyllobothrium latum*, which was previously diagnosed but lacks recent reports, which does not preclude its ongoing existence[2]. Additionally, the suspected presence of *Opisthorchis felineus*, a known zoonotic parasite reported in neighboring regions but not yet identified in North Macedonia, highlights the need for enhanced vigilance and further investigation[3,4]. The findings underscore the critical importance of understanding and mitigating the threats posed by zoonotic parasites in freshwater ecosystems. They stress the urgent need for sustained surveillance programs and robust management strategies to protect human health and the ecological equilibrium of aquatic environments in North Macedonia. Through elucidating the prevalence and implications of these parasites, this research contributes vital insights to guide evidence-based policies aimed at minimizing the risk of zoonotic disease transmission and ensuring the long-term sustainability of fisheries resources in the region.

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World Cafe on developing a Gender Equality Plan for ParAqua

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ABSTRACT

The ParAqua Moderators aim to work with the participant in the Dubrovnik meeting on developing a Gender Equality Plan (GEP) for our Action. We mean to gather the collective opinions and suggestions by setting up a World Cafe. Overall, the aim of a gender equality plan for a COST Action is to create a more equitable, inclusive, and impactful research environment that harnesses the full potential of all members, regardless of gender, and contributes to advancing knowledge and addressing societal challenges. A World Café is a participatory process and methodology used for hosting large group discussions in which participants engage in conversations around a set of questions or topics. The World Café typically involves setting up multiple small tables or discussion areas, each with its own topic or question. Participants rotate between these tables in timed intervals, contributing their ideas, insights, and perspectives to the conversation at each table. As they move between tables, participants build upon the ideas shared by others and gain a broader understanding of the topics under discussion. We will use guiding questions for the World Cafe along these headlines : (i) Gender Representation and Leadership, (ii) Access to Resources and Opportunities, (iii) Work-Life Balance, (iv) Integration of Gender Perspectives in Research, (v) Addressing Unconscious Bias, (vi) Planning and Implementation of the GEP

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