ORAL COMMUNICATIONS
S.1 - CLIMATE SMART STRATEGIES

OC A1- THE COMBINED EFFECT OF PH AND TEMPERATURE ON THE PHYSIOLOGICAL PERFORMANCE OF JUVENILES OF PACIFIC OYSTER, MAGALLANA GIGAS.

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The combined effect of pH and temperature on the physiological performance of juveniles of Pacific oyster, *Magallana gigas*.

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Climate changes are predicted to involve increases in temperature, sea level, ocean acidification and salinity fluctuations. Ocean warming and acidification are key anthropogenic stressors that negatively affect marine biological and physiological processes, consequently, impacting bivalve production. Most studies have focused on these stressors individually, although, in the natural environment, organisms are affected by several combined factors. Considering the worst scenario predicted by the Intergovernmental Panel on Climate Change (IPPC), which forecasts a 4°C increase in temperature and a decrease in pH to levels below 7.7, this study addressed the combined effects of temperature and pH on the physiological and growth performance of *Magallana gigas* juveniles.
Four treatments combining two pH levels (pH 7.6 and pH 8.2) and two temperatures (22 ± 1ºC; 26 ± 1ºC) were tested in triplicate. The pH 8.2 and 22ºC treatment was considered as the control group, reflecting the baseline conditions. Juveniles of *M. gigas* (9.29 ± 0.58 mm shell length; 86.63 ± 11.12 mg total weight) were separated into twelve groups of 200 individuals and placed into experimental tanks for 30 days. Oysters were fed daily with a mixed microalgae diet at a ratio of 6% of oyster dry weight (g) in algal dry weight (mg). Mortality and abiotic parameters (temperature, pH, salinity) were registered daily. Biometric measurements, condition index, and biochemical composition (proteins, total lipids and glycogen content) were evaluated every 15 days.
Survival and shell growth were not affected by any of the experimental conditions. However, after 30 days of exposure, soft tissue weights and the condition index were significantly lower in oysters exposed to higher temperature (26°C), regardless of the pH, with the control group (pH 8.2; 22ºC) showing the best condition. Principal component analysis (PCA) revealed that temperature was negatively correlated with soft tissue weight and condition index. Surprisingly, pH showed no significant correlation with any other variable. Biochemical composition did not show significant differences among treatments.
This study highlights that the physiological performance of bivalves is more affected by ocean warming than ocean acidification. Consequently, increasing of heatwaves frequency and intensity may have severe implications for bivalve aquaculture, mainly during the recruitment stage.

Keywords: Bivalves, Ocean warming, Acidification, Physiological condition, Growth

OC A2 - INCORPORATING INSECT MEAL AND SPIRULINA IN DIETS FOR NILE TILAPIA: OPPORTUNITIES AND CHALLENGES FOR AFRICAN AQUACULTURE

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African aquaculture is a rapidly growing industry that provides a sustainable source of protein while supporting job creation, economic stability, and equal opportunities across communities. To sustain this growth, innovative protein sources are crucial for advancing the aquaculture and aquafeed sectors. The INNOECOFOOD project aims to strengthen fish farms by evaluating protein sources produced locally within ECOHUBS, including spirulina and black soldier fly (*Hermetia illucens*) larvae meal (BSFLM). This initiative promotes efficient aquaculture practices while supporting the production of certified, marketable food and feed products. Nile tilapia (*Oreochromis niloticus*) is a key species in African aquaculture, valued for its fast growth and high yield. As demand for sustainable and cost-effective fish production rises, diversifying feed ingredients is crucial. Therefore, the objective of this study was to evaluate the incorporation of Spirulina and BSFLM in diets for Nile tilapia juveniles. Five practical diets were formulated (35% crude protein, and 8% crude lipids). A control diet contained 5% fishmeal (FM), while for the other experimental diets, FM-protein was partially (25%) replaced by either BSFLM, or spirulina, or totally (100%) by a combination of 75% BSFLM and 25% spirulina, or 100% BSFLM. Diets were tested in triplicate with groups of 20 Nile tilapia juveniles (initial body weight 8.2 ± 0.03 g) and fish were hand-fed to apparent visual satiation for 71 days. At the end of the trial, growth performance and feed utilization showed no differences among treatments. Final body weight averaged 88.7 g, daily growth index 3.43% day^{-1}, and feed conversion ratio 1.14. Diet's nutrient digestibility, retention, and gain remained unaffected by the experimental diets. The histomorphological evaluation revealed no significant effects on the intestinal integrity of fish fed these diets. Overall, the present results demonstrate the feasibility of replacing FM with spirulina and BSFLM as innovative feed sources in diets for Nile tilapia, without compromising growth, feed efficiency, or digestive health. The impact of the diets on the nutritional quality of tilapia fillets is still under study. These findings highlight the potential for locally-produced alternative proteins to enhance the sustainability and self-sufficiency of African aquaculture systems while reducing dependency on imported fishmeal.

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OC A3 - CITIES OF HOPE: CREATING A CLIMATE RESILIENT FUTURE - CLIMATE WATERFRONT BUILDING WITH NATURE WITH SAFETY, BIODIVERSITY PROMOTION AND HABITAT RESTAURATION RESILIENT LAND AND SEA. – URBAN DRANAGE PLAN

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Climate change is a problem in aquaculture and for the entire marine habitat for global quality. Water scarcity, habitat destruction, the construction of poor quality infrastructures, and the lack of information in societies intensify the entry of pollutants into the sea from wastewater and rainwater itself.

According to the 'UN Atlas of the World', eight of the world's ten largest cities are located on the coast and 44% of the world's population lives in vulnerable coastal areas. The Resilience of coastal cities and their inhabitants to climate change is becoming increasingly important (Abadie, de Murieta, and Galarraga 2016).

The importance of adaptation measures is thereby implicitly important. Adaptive measures were taken in the most vulnerable coasts and coastal cities to increase the resilience and safety of the oceans (Sanders & Oliveira 2023).

According to the six IPCC reports, from the first report from 1990 and including the most recent report from 2022, it is becoming clear that the initial focus on mitigation measures should be broadened to include adaptation measures, with the vulnerability of coastal cities logically receiving increasing attention (GCA2020 2020) (CAS2021 2021) (IPCC 2022).

It has also become clear that cities on the coast are experiencing the most problems from climate change, with the largest cities in the world located on the coast, and that these cities are growing the fastest in terms of population, a development that seems to continue to strengthen. This situation of large cities, and with it the situation of most of our planet's growing population, makes the effects of climate change the main concern of coastal cities (McKinseyGI 2021) and their effect on oceans and the quality of the water.

Bioavailability (Brito, P.; M.Caetano, 2020) and geochemical fractionation play a crucial role in determining the mobility and ecological impact of metals in marine environments. Sediment and water samples from various coastal regions produce important data to analyze the level of water quality. The results indicate that metals such as cadmium and lead are predominantly associated with reducible and oxidizable fractions, affecting their bioavailability to marine organisms. Furthermore, environmental factors such as pH, salinity, and organic matter content significantly influence metal partitioning and bioavailability. These findings highlight the importance of developing strategies for marine pollution mitigation and it is coming from the urban coast, can we promote better places to result in better water?

OC A4 - LAMINARIA DIGITATA AS AN IMMUNOSTIMULANT TO COUNTERACT THE EFFECTS OF MARINE HEATWAVES AND DISEASE OUTBREAKS IN FARMED FISH

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Abstract

Climate-related stressors are placing unprecedented pressure on marine aquaculture, with the intensification of extreme weather events — such as marine heatwaves (MHWs) — emerging as one of the most challenging phenomena. These prolonged periods of unusually high sea surface temperatures disrupt environmental stability and compromise the performance of farmed species, particularly in outdoor systems. Moreover, MHWs create favorable conditions for the proliferation and increased virulence of opportunistic pathogens, such as *Vibrio harveyi*, which is often associated with high mortality rates. This is of particular concern for commercially valuable species such as gilthead seabream (*Sparus aurata*), one of the most widely farmed finfish in the Mediterranean.

Addressing the need to implement sustainable strategies to enhance fish resilience to climate-related stressors, our study investigated the immunostimulatory potential of *Laminaria digitata*, a brown macroalga, as a sustainable dietary supplement for juvenile gilthead seabream. Fish (*n* = 243) were exposed to simulated MHW conditions (25.7 °C) and experimentally challenged with *V. harveyi* (4.9 × 10^8 CFU mL^{-1}), while being fed diets supplemented with either *L. digitata* powder (0.3% and 1.5%) or extract (0.3%). A multidisciplinary approach was employed, including flow cytometry (for viability of different leukocytes populations), hematological profiling (red blood cells (RBCs), white blood cells (WBCs), hemoglobin (Hb), hematocrit (Ht), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCHC)), and key humoral immune parameters (antiprotease activity, immunoglobulin M (IgM), and peroxidase activity).

The results showed that both 1.5% powder (P1.5%) and 0.3% extract (EXT0.3%) formulations significantly improved fish survival, reducing cumulative mortality to 7.3% and 7.7%, respectively, compared to 32.5% in the control group (CTR, no supplementation). These formulations also improved leukocyte viability, increased granulocyte proportions, and

stabilized hematological parameters, while reducing peroxidase activity and IgM levels, indicating a more efficient and balanced immune response. Notably, P1.5% supplementation emerged as the most cost-effective strategy, making it more suitable for industrial or large-scale application.
Overall, these findings support the potential of *L. digitata* as a climate-smart and eco-innovative feed supplement to strengthen the immune defenses of farmed seabream against the dual threat of heat stress and disease outbreaks. As climate change continues to intensify, such functional feed additives offer a promising path towards more robust, sustainable, and resilient aquaculture practices.

Keywords: aquaculture, extreme weather events, functional feeds, macroalgae, disease outbreaks, immune response

OC A5 - DIGITAL TOOLS FOR AQUACULTURE NUTRITION: FROM FEED PLANNING TO DATA-DRIVEN TRIALS

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SPAROS has developed a set of digital tools to support aquaculture nutrition through web-based applications tailored to key production species. These include a Virtual Nutrition Lab based on a nutrient-based mechanistic model, allowing users to explore and optimize feeding strategies for species such as gilthead seabream, European seabass, Atlantic salmon, and others. SPAROS also provides Customized Production Plans that use actual farm data—such as observed growth and feed intake—to align nutritional strategies with real-world performance. To support research and innovation, a digital trial management solution streamlines the planning, monitoring, and reporting of feeding trials, improving efficiency and accelerating insights. Additionally, SPAROS offers personalized services, including farm data analysis, custom model development, and machine learning tools. These solutions can be integrated via API and are designed for exclusive client use, ensuring flexibility and scalability.

S.2 NEW TOOLS FOR ANIMAL WELFARE

OC A6 - AQUAPONICS FOR CLIMATE-SMART AND SAFE AQUACULTURE: INSIGHTS FROM THE AQUALLIANCE AND AQUAPONIC 4.0 INITIATIVES

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We gratefully acknowledge the support and contributions of all individuals and institutions that made the development and implementation of the AQUALLIANCE and Aquaponic 4.0 initiatives possible.
First and foremost, we thank **EIT Food** for their strategic and financial support through the Collaborative Missions Programmes, which enabled the launch of the AQUALLIANCE initiative. Their commitment to fostering sustainable food innovation across Europe has been instrumental in creating the foundations for policy advocacy, farmer training, and stakeholder engagement surrounding aquaponics.
We would also like to acknowledge our partners from the **AQUALLIANCE consortium**, including academic institutions, non-profits, and private sector actors whose expertise and collaboration have contributed significantly to the production of educational materials, the completion of a market study on aquaponics in Europe, and the drafting of a white paper addressing regulatory gaps.
Special thanks are extended to the **University of Exeter** and the **Future17 Sustainable Development Goals Challenge Program**, whose interdisciplinary and multicultural framework provided the platform for the Aquaponic 4.0 project. This initiative brought together passionate student researchers from the University of Exeter, Stellenbosch University, Satbayev University, The American University in Cairo, and others. Their dedication to exploring consumer perceptions and willingness to pay for aquaponically grown products across multiple continents enriched the scientific and social dimensions of our work.
We would like to express sincere appreciation to **Fresh Water Farms Europe's extended stakeholder network**, which includes farmers, policymakers, environmental NGOs, educators, and investors, for their openness to engagement and feedback throughout the projects.
Additionally, we recognize the technical and design contributions of those who helped create the survey instruments, animated educational video, and visual communication materials that supported our outreach and dissemination efforts.
Finally, our deepest gratitude goes to the broader **aquaponics and sustainable food systems community** in Europe and beyond, whose continued advocacy, experimentation, and passion drive this movement forward. This work is the result of a shared vision for a healthier, fairer, and more resilient future.

As the global food system faces increasing pressure from climate change, population growth, and resource scarcity, aquaponics emerges as a promising solution that integrates aquaculture and hydroponics into a closed-loop, sustainable system. This presentation shares the experience of two strategic initiatives, AQUALLIANCE and Aquaponic 4.0—designed to promote aquaponics as a viable, resilient, and publicly accepted method of food production.
AQUALLIANCE, led by Fresh Water Farms Europe in collaboration with European partners, seeks to establish a policy and education ecosystem favorable to the adoption of aquaponics. The project delivers concrete outputs such as training materials, a European market study, a policy-oriented white paper, and impact evaluation tools. Its main ambition is to engage decision-makers and industry stakeholders to build long-term conditions for the uptake of aquaponics in urban and peri-urban areas.
In parallel, Aquaponic 4.0, developed under the Future17 program with international student researchers, investigated public perceptions of aquaponic systems across nine countries. The

project found significant knowledge gaps but a high willingness to engage with sustainable food technologies, especially among younger consumers. Educational tools, such as an animated video infographic, were developed and tested to enhance public awareness.

The presentation will discuss how the two initiatives complement each other—one through systemic advocacy, and the other through social research and engagement. It will also cover next steps, including a pilot urban aquaponic farm in Portugal, and recommendations for harmonizing policy frameworks to support this climate-smart approach to food production.

Keywords: aquaponics, sustainable food systems, circular economy, public awareness, urban

OC A7 - DEVELOPMENT OF AN IN VITRO GASTROINTESTINAL MODEL FOR GILTHEAD SEABREAM (SPARUS AURATA) TO ASSESS THE EFFECTS OF MICROPLASTICS CO-INGESTION ON FEED DIGESTIBILITY

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Marine litter has been growing worldwide due to the increase in industrial production over the years. This increase is largely driven by the continuous population growth, and all the needs that plastics-derived products provide in the daily basis of human lives. As a result, environmental and food safety problems are emerging because the small particles that breakdown from plastics (i.e. the so called microplastics) are able to enter, settle and contaminate ecosystems and organisms. Their persistence in the environment and bioavailability in organisms have been raising growing concerns among the industry and scientific communities, and the general public. Aquaculture plays a key role in providing fish, shellfish, and other seafood in a sustainable way, helping to reduce the pressure on wild fish populations from overfishing. However, this industry is not free from microplastics contamination, especially through fish feeds. Numerous studies have identified microplastics presence originating from feed ingredients and even from secondary sources, such as synthetic clothing worn by personnel during feed preparation or handling. These contaminants raise concerns, not only about animal health and potentially growth performance but also a risk for humans though the food chain.

In vitro methods have been emerging as more ethical (less use of live animals), reliable, less time and resources consuming strategy to replace traditional *in vivo* experiments, despite the fact they are physiological relevant and provide reliable and realistic data. However, there is still a lack of studies devoted to the optimization of *in vitro* methods. This loophole is due to the fact that there is a great variability in fish species, habitat and feeding regime, making it difficult to standardize a universal method. With this, optimizing *in vitro* models is crucial not only to enhance reproducibility but also to ensure their applicability across diverse species without the need to use live organisms.

Within this context, the present study aimed to optimize an *in vitro* gastrointestinal model to simulate the digestion process of a marine carnivorous fish (*Sparus aurata*), with the goal of investigating whether the co-ingestion of microplastics affect protein digestibility.

Keywords: fish welfare, bioaccessibility, fish feeds, marine plastics

OC A8 - OPTIMIZATION OF A RAINBOW TROUT INTESTINAL CELL MODEL FOR MYCOTOXIN INTESTINAL ABSORPTION AND TOXICITY STUDIES

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The aquaculture sector has been progressively incorporating plant-based proteins into feed production, which has resulted in exposure to contaminants like mycotoxins (1). These harmful fungal metabolites can impair fish intestinal function and nutrient absorption, thereby adversely affecting aquaculture productivity (2).
To assess the impact of mycotoxin exposure in fishes, *in vivo* studies, owing to their high physiological relevance, have led to the evaluation of different diets and formulations. However, their use is limited by ethical concerns, high cost, and logistical constraints. *Ex vivo* systems, such as gut-sac and Ussing-chambers applications, have been adapted to study fish intestinal epithelial barrier and have significantly advanced the understanding of intestinal absorption. Nonetheless, their use is limited due to interindividual variability, tissue viability, and restricted scalability (3). In this context, *in vitro* studies emerge with great advantages, such as elimination of ethical concerns, low cost and the possibility of high throughput.
This work presents the first application of RTgutGC intestinal epithelial cell line, derived from Rainbow trout, to study the bioavailability and intestinal toxicity of mycotoxins. Methodological optimization of subculturing RTgutGC cells is detailed, including assessment of population doubling time, cell seeding densities, measurement of transepithelial electrical resistance (TEER), and permeability evaluation (Lucifer yellow (LY)). To define sub-cytotoxic exposure levels, RTgutGC cells were exposed to four mycotoxins, aflatoxin B1 (AFB1), fumonisin B1 (FB1), enniatin B (ENNB) and enniatin B1 (ENNB1), individually and in mixture, in a crescent range of concentrations (0-2500 µg/kg), during 24 and 48 hours. Cytotoxicity and lysosomal function were evaluated by neutral red assay. Polarized intestinal monolayers of RTgutGC cells were prepared in permeable tissue culture inserts, enabling their use in transport assays of mycotoxins (4), both individually and in mixture, at a concentration of 400 µg/kg, simulating realistic multi-contaminant scenarios found in fishfeeds. Further assessments, including quantifications of transported mycotoxins and gene expression analysis will provide data to elucidate the effects of these compounds on the fish intestinal barrier.
This study demonstrates in vitro models as a valuable alternative to traditional systems and introduces a novel tool for aquaculture toxicology and feed safety assessment.

References

1. doi: 10.1007/s12550-019-00368-6
2. doi: 10.3390/toxins12030160
3. doi: 10.1111/j.2042-7158.2011.01391.x
4. doi: 10.1111/j.1365-2095.2010.00757.x

Keywords: In vitro, RTgutGC, mycotoxins, intestinal barrier, absorption

OC A9 - BIOMARKERS FOR WELFARE ASSESSMENT IN AQUACULTURE

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In fish farming, it is recognised that appropriate welfare conditions are crucial, with a clear correlation between good aquaculture practices and animal health. Nevertheless, aquatic animal welfare is still a complex concept, existing several proposed definitions and, therefore, existing no consensus on how it should be defined and measured.
The central idea is that a healthy organism should grow well, have a functional immune system, be free of disease, reproduce actively, and have low-stress levels. However, the welfare of aquatic animals is less well-characterised when compared to terrestrial/laboratory animals. Furthermore, classical welfare assessment strategies used for terrestrial animals are not adequate for either characterising fish well-being or establishing good farming practices.
Understanding the stress fish experience is essential to unveil how its well-being and performance are influenced not only by its anthropogenic interactions (e.g. recreational, farming, ornamental) but also by the effect of external factors (e.g. meteorological events). Therefore, stress indicators are essential for assessing how external, internal, and environmental changes affect fish physiology and behaviour.
The quantification of stress in fish has been extensively studied, with numerous stressors identified and used as markers of stress responses. Traditionally, these assessments have relied on physiological and behavioural indicators. However, recent advances—particularly in omics technologies such as genomics, transcriptomics, proteomics, and metabolomics—have opened new avenues for a deeper and more precise understanding of stress mechanisms in fish. In parallel, there is a growing demand for non-invasive procedures of stress assessment, reflecting an increasing concern for animal welfare in aquaculture and research settings. These approaches not only reduce the impact on the animals but also allow for repeated measurements over time, improving the quality and reliability of data.
In this review, we present an overview of the existing biomarkers used to study stress in reared fish. We explore stress indicators across multiple biological levels, including molecular and cellular markers, primary and secondary physiological responses, and whole-organism indicators. Furthermore, we differentiate between invasive and non-invasive assessment techniques, highlighting their respective advantages and applications.

Keywords: Stress, Fish, Omics technologies, Physiological indicators, Non-invasive methods, Animal welfare

OC A10 - LIVING ON THE EDGE: HOW TEMPERATURE AND SALINITY SHAPE PERFORMANCE OF SHALLOW-WATER TEMPERATE CARIDEAN SHRIMP

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Phenotypes are plastic and can shift within certain limits when organisms are exposed to environmental changes. These adjustments occur over short timescales without involving genetic modifications, serving as an immediate coping mechanism.
Understanding phenotypic plasticity to environmental changes may improve conservation and enhance aquaculture efficiency by optimizing resource use within species' tolerance limits.
The temperate caridean shrimp *Palaemon varians* Leach, 1814 was used as a model species due to its economic significance and natural adaptability to highly variable environments, such as those of saltpans, where temperature and salinity fluctuate significantly. Shrimps were exposed to temperature (10, 15, 20, and 30 °C) and salinity (10, 20, 30, and 40) gradients for 15 days. Performance curves were generated for physiological parameters including Critical Thermal maximum (CTmax), Routine Metabolic Rate (RMR), Cumulative Survival (CS), and Cellular Stress Response (CSR) biomarkers, distinguishing between males and females.
Results showed that salinity had minimal impact on shrimp physiology, with CTmax, RMR, and CSR remaining stable across treatments and survival near 100%. However, males had higher Total Protein content and Superoxide Dismutase levels than females, suggesting distinct cellular responses. Temperature, in contrast, had a significant effect, with higher temperatures reducing survival. Shrimp exposed to 30 °C had a CTmax 5.4% higher than those at 10 °C, indicating some acclimation capacity. CSR biomarkers also varied with temperature, with shrimp exposed to 30 °C showing significantly higher levels of antioxidant proteins than those at lower temperatures (10 and 15 °C), suggesting that high temperatures cause oxidative damage. Males consistently exhibited higher Total Protein levels than females, likely reflecting differences in reproductive energy demands. Neither salinity nor temperature significantly affected shrimp weight.
Our results suggest that this species can acclimate to varying conditions. This would allow producers that use this species for integrated multitrophic aquaculture to optimize farming practices without compromising survival or growth. Shrimp exposed to 30 °C had an average CTmax of 38.8 °C, while the maximum recorded temperature in their habitat was 35.5 °C, leaving a thermal safety margin of approximately 3.5 °C in the context of climate change. The

combination of high reproductive investment in females, energy demands to fight oxidative stress, and limited thermal safety margins raises concerns about this species' ability to withstand future climate extremes.

Keywords: Critical Thermal maximum, Routine Metabolic Rate, Palaemon varians, Phenotypic Plasticity, Aquaculture

OC A11 - COMPARISON OF SKIN TRANSCRIPTOMIC PROFILES IN THE CONTEXT OF TRANSPORT INDUCED ACUTE STRESS RESPONSE IN ATLANTIC SALMON, EUROPEAN SEABASS AND RAINBOW TROUT

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Fish transport is a widely used and unavoidable essential practice in aquaculture for purposes such as stocking, breeding, and growth. However, this process often exposes fish to acute stress, which can significantly affect their physiology and overall welfare. The severity of transport-induced stress can vary depending on the initial health status of the fish and transportation conditions, including duration, stocking density, temperature, water quality, and ammonia concentration. While transport rarely causes immediate mortality, secondary and tertiary stress-related responses can compromise both specific and nonspecific immunity, increasing susceptibility to disease and osmoregulatory imbalances, which may lead to mortality. Current methods for assessing immune and health status rely on invasive procedures (e.g., blood and tissue collection, biosensor surgical implantation). Established welfare indicators, which help assess how well fish needs are met, are often observed too late to enable timely intervention, and remain limited for many farmed species. Further research is needed to understand how common aquaculture practices, such as transport, impact farmed fish. To investigate the molecular signatures following acute stress, three key European aquaculture species – Atlantic salmon (*Salmo salar*), European seabass (*Dicentrarchus labrax*), and rainbow trout (*Oncorhynchus mykiss*) were submitted to transport induced stress. The transportation trials were designed to simulate current transport conditions specific to each species. Skin tissue samples were collected at three time points: before transport, immediately after stress exposure, and following a 24-hour recovery period. RNA sequencing (RNA-seq) followed by differential gene expression (DGE) analysis and functional enrichment were performed to examine transcriptomic responses. A comparative analysis across species revealed shared molecular pathways, as well as differentially expressed genes—most notably, the upregulation of 36 genes following stress exposure in all three species. Among the upregulated genes are several involved in key biological functions such as immunity, apoptosis, stress modulation and epithelial regeneration. These preliminary findings offer valuable insights into conserved stress response mechanisms. Future research will incorporate proteomic analysis of skin mucus and miRNA profiling from water samples to provide a more comprehensive understanding of the underlying biological processes and to support the identification of potential biomarkers for use in minimally invasive biosensors.

Keywords: Aquaculture, Transcriptomics, Mucus, Skin, Welfare indicators

OC A12 - SEA URCHIN IMMUNE RESPONSE TO SHORT AND LONG-TERM EXPOSURE TO CHALLENGING ENVIRONMENTAL CONDITIONS: BASIC RESEARCH TOWARDS SEA URCHIN WELFARE IN AQUACULTURE

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The sea urchin *Paracentrotus lividus* (Lamarck, 1816) is Southern Europe's most exploited and economically important species. Aquaculture has been identified as a sustainable way to meet the increasing market demand for sea urchin roe. A considerable amount of relevant information has been produced regarding rearing technology and nutritional requirements to produce sea urchins. However, the knowledge about sea urchin welfare in rearing facilities is still limited. In recent years, we have conducted a series of experiments to study the immune response of *P. lividus* to different rearing factors.
Experiment 1: Sea urchins were reared for 90 days on two diets: a vegetable-based diet and a seaweed diet. During that period, the sea urchins were exposed to 2 short-term environmental changes: (A) an acute increase in ammonia concentration and (B) an acute temperature rise of 5 ºC. After 24 hours of exposure, cellular and humoral immunity parameters were assessed. The results show that diet may affect the immune system's sensitivity to acute changes in the environmental conditions, and an increase in temperature affects coelomocyte densities and lysozyme concentration in the coelomic fluid.
Experiment 2: To investigate the effect of long-term exposure to high temperature in the immune response, sea urchins were exposed to an increase in temperature up to 24°C for 36 days. After this period, cellular and humoral immune parameters were assessed. As in the acute exposure to high temperature, both the number of coelomocytes and lysozyme concentration in coelomic fluid increased with increasing in temperature.
Experiment 3: The effect of diet in the immune response was evaluated after a 60-day feeding trial with three diets: a carotenoid-rich diet (HC), a carotenoid poor diet (LC) and a seaweed-based diet (KELP). At the end of the feeding trial, sea urchins were exposed to a pathogenic challenge by inoculation with *Vibrio parahaemolyticus* with a time-course measurement of cellular and humoral immunity parameters. The results show an increase in coelomocyte levels in sea urchins fed with HC and LC diets. When exposed to the pathogen, sea urchins on KELP diet exhibited a faster activation of their immune system, characterised by higher concentrations of lysozyme.
Our results indicate that diet provided has a meaningful impact on the sea urchin immune system and on how this responds to temperature changes. The knowledge acquired from the experiments conducted has the potential to provide indications about best practices to protect echinoderms' welfare in aquaculture and experimental facilities

Keywords: Paracentrotus lividus, rearing, temperature, ammonia, diet, pathogen challenge

OC A13 - MULTI-OMICS APPROACHES FOR MULTI-STRESSORS SCENARIOS: INSIGHTS FROM THE AQUA-CLIMADAPT PROJECT

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In the recent years, the use of “omic” tools, such as genomics, transcriptomics, metabolomics, metagenomics, has become increasingly popular in animal sciences and livestock biotechnology, as they allow to disclose complex biological pathways (e.g. growth, reproduction, immunity, metabolism) and to implement, in accordance, innovative approaches to optimize the performance, breeding, welfare and resilience to stressors of farmed animals. In alignment with this current trend, “omic” tools have also been recently introduced in aquaculture research, as the insights they provide allow to uncover cells and organisms adaptation mechanisms, functioning, growth and development under different rearing conditions and environmental stressors, ultimately improving production and management practices. So far, most studies on this direction have followed traditional “single-omics” approaches (which often fail to provide an overview of the full picture), rather than integrating multiple “omic” data to explore the complexity of biological systems as a whole and, on that basis, predict how its different components interact dynamically.

Within this context, project Aqua-CLIMADAPT aimed to explore farmed marine fish early responses under multi-stressors scenarios (including dietary shifts, disease outbreaks and climate variables/events) through an innovative and holistic “multi-omic” approach that

integrates conventional biochemical welfare biomarkers with genomics, transcriptomics, metabolomics and metagenomics.

S.3 EMERGING HAZARDS

OC A14 - FROM FEED TO FORK: ASSESSING MYCOTOXIN EXPOSURE IN AQUACULTURE AND HUMAN HEALTH RISK

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Aquaculture is an expanding sector essential to global food security. The shift towards plant-based proteins in aquafeed production, driven by economic and environmental concerns, has increased the risk of mycotoxin exposure. Mycotoxins are secondary fungal metabolites, commonly found in plant-derived feed ingredients, and are associated with adverse health effects in both animals and humans (1).
In this study, a multi-tiered approach was employed to evaluate the potential exposure of mycotoxins from fishfeed to both fish and humans. Firstly, fishfeeds (n=65) and plant-based ingredients (n=49) were analysed for 22 mycotoxins (including regulated and emerging ones). Based on these results, an *in vivo trial* was performed using specimens of *Sparus aurata,* exposed to fumonisin B1 (FB1) at 10 mg/kg and enniatin B (ENNB) at 5 mg/kg, both isolated and combined. To further evaluate potential human health risk, muscle tissues of *S. aurata* spiked with aflatoxin B1 (AFB1) and enniatin B1 (ENNB1), both selected due to their reported bioaccumulation in fish (2,3), underwent *in vitro* gastric and intestinal digestion followed by an intestinal transport simulation using a Caco-2/HT-29 MTX co-culture model (4, 5) to assess their human bioavailability.
Results indicate confirmed mycotoxin contamination in 12 fishfeeds and 8 ingredients, with aflatoxins (AFs), fumonisins (FMs), and enniatins (ENNs) being the most frequently detected. In fish plasma, only ENNB was detected, confirming its bioavailability in fish. In the human *in*

vitro model, bioaccessibility was confirmed, and absorption occurred across the intestinal barrier: AFB1 was transported both in mixture and alone, while ENNB1 was only transported after combined exposure.

These findings underscore the need to incorporate mycotoxins into routine quality control frameworks in aquaculture, considering their widespread occurrence and potential for cross-species exposure. While further *in vivo* studies involving chronic exposure are necessary to comprehensively assess bioaccumulation and long-term effects, the present data provides critical evidence of uptake and systemic bioavailability. Moreover, the application of *in vitro* digestion and intestinal transport models proved to be valuable for evaluating mycotoxin bioaccessibility and absorption under conditions that simulate human exposure. This study reinforces the relevance of a One Health approach in managing chemical risks within aquatic food systems.

References

1. doi: 10.3390/toxins13060403
2. doi: 10.1002/jsfa.11471
3. doi: 10.1021/jf5036838
4. doi: 10.1038/s41596-018-0119-1
5. doi: 10.3390/toxins16050205

Keywords: Mycotoxins, bioavailability, in vitro, in vivo, Caco-2, aquaculture

OC A15 - MYCOTOXINS EFFECTS ON FARMED FISH HEALTH AND NUTRITIONAL QUALITY: GILTHEAD SEABREAM AS CASE STUDY

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This work was funded by The Portuguese Foundation for Science and Technology (FCT I.P.), under the framework of the Project MycoFish - Occurrence, bioavailability and mitigation strategies for mycotoxins in farmed fish and associated feed ingredients: Gilthead seabream as case study (PTDC/CVT-CVT/2660/2021; https://doi.org/10.54499/PTDC/CVT-CVT/2660/2021).

Recent advances in aquafeeds promoted the use of plant-based ingredients with increased risks of mycotoxin contamination that can negatively affect the fish health and nutritional quality and pose potential risks to human consumers. However, the scientific knowledge on the adverse impacts of mycotoxins in these issues is still limited. In this context, this study aimed to evaluate the mycotoxins effects on the physiological and nutritional quality of gilthead seabream (*Sparus aurata*) when exposed to fumonisin B1 (FB1) and enniatin B (ENNB) in isolation or in mixture for 96h and 28 days (acute and chronic exposure, respectively). Fitness indexes, growth performance, plasma metabolites, biomarkers of oxidative stress, metabolism, cellular and neurotoxic damage were assessed in fish tissues (muscle, brain, liver and gills). Also, proximate chemical composition, fatty acid profile, essential macro and trace elements contents and chemical changes (protein and lipid oxidation) were analyzed in fish muscle.

ENNB revealed be the most harmful mycotoxin to juvenile *S. aurata* under both exposure assays. Chronic exposure (during 28 days; 150 µg/kg) to alone ENNB caused protein and lipid degradation in liver, and affect lipase and alkaline phosphatase activities in plasma. Additionally, the combined action of both mycotoxins (FB1+ENNB) can compromise the nutritional value of this species, where essential elements (K, Mg and Zn) proved to be the most affected nutritional compounds. On the other hand, acute exposure (during 96-h) to isolated ENNB (5 mg/kg) induced an increase in plasma cholesterol levels, while FB1 (10 mg/kg) promoted an increase in plasma glucose levels. Generally, lipid peroxidation occurred in brain for all contaminated-treatments, while in liver and gills occurred only to ENNB isolated or in combination with FB1. Additionally, the last two contaminated treatments caused an increase of acetylcholinesterase (AChE) activity. Therefore, the effects of both mycotoxins appear to be influenced by target tissues, dosage, and duration of exposure.

Overall, these studies contributes to highlight the toxicological attributes of the emerging mycotoxin (ENNB), thus reinforcing the need to regulate this mycotoxin in the future legislation regarding aquaculture feeds, prioritizing animal health and welfare. Furthermore, understanding mechanisms of action, metabolic pathways and potential effects in farmed fish species across different exposure scenarios it is of crucial importance. Finally, reinforces the importance to undertake consumer risk-benefit assessment to accurately ensure maximum protection for farmed fish consumers, and ultimately ensure and improve the sustainable development of marine resources and the aquaculture sector.

Keywords: Fumonisin B1, Enniatin B, Fish feed, Fish, Biomarkers

OC A16 - CLIMATE-DRIVEN DYNAMICS OF ANISAKIS SPP. ALLERGENS IN EUROPEAN HAKE: IMPLICATIONS FOR SEAFOOD QUALITY AND SAFETY

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Ocean warming associated with climate change is altering marine parasite distribution patterns, potentially increasing the prevalence of zoonotic parasites in commercially important fish species. This study investigates the relationship between seasonal variations and the prevalence of *Anisakis* allergens in European hake (*Merluccius merluccius*), while assessing impacts on nutritional quality parameters. Samples (n=240) were collected across four seasons from two fishing regions (Bay of Biscay and Southeast Ireland) during an annual cycle. Quantitative PCR assays (qPCR) targeting genes encoding four major allergens (Ani s 1, 4, 7, and 13) were employed for detection and quantification in fish dorsal muscle, while comprehensive nutritional profiling assessed proximate composition, texture, colour, pH, and antioxidant capacity. Our results revealed regional and seasonal variations in allergen prevalence, with detection rates of 56% (Ani s 1; 0.010-15.330 mg/kg), 53% (Ani s 4; 0.008-34.336 mg/kg), 42% (Ani s 7; 0.009-2.677 mg/kg), and 72% (Ani s 13; 0.007-21.802 mg/kg). Winter samples exhibited altered nutritional profiles with higher protein content (18.73±0.16% ww Southeast Ireland; 18.15±0.16% ww Bay of Biscay) and lower lipid levels (0.60±0.04% ww Southeast Ireland; 0.68±0.03% ww Bay of Biscay), along with decreased lightness (L*) and altered texture parameters (increased cohesiveness, decreased hardness and chewiness). While parasite loads primarily affected visual quality attributes without compromising nutritional value, the increasing ocean temperatures projected under climate change scenarios are expected to disrupt *Anisakis* spp. life cycles and expanding their geographical range into previously unaffected areas. These findings provide baseline data for developing climate-informed risk assessment models for *Anisakis* allergens in commercially important fish species. The established seasonal and geographical patterns enable the development of targeted monitoring strategies and improved processing interventions to enhance seafood safety in the context of ongoing climate change.

Keywords: Climate change, Anisakis allergens, qPCR, Food safety, Nutritional quality, European hake

OC A17 - ECOTOXICOLOGICAL AND SEAFOOD SAFETY IMPLICATIONS OF HARMFUL ALGAL BLOOMS DURING EXTREME WEATHER EVENTS: MYTILUS GALLOPROVENCIALIS EXPOSED TO PROROCENTRUM LIMA AS A CASE STUDY

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Marine heatwaves (MHWs) are becoming more frequent and intense around the globe, as a result of global warming. These extreme events often lead to widespread die-offs in bivalve populations and promote the growth and spread of toxin-producing microalgae, posing significant risks to seafood safety. However, the combined impact of MHWs and the uptake of marine biotoxins by marine organisms is a relatively new area of research, with limited understanding from both ecotoxicological and food safety perspectives. This study investigated how a MHW event influences the accumulation and elimination of diarrhetic shellfish toxins in *Mytilus galloprovincialis* exposed to *Prorocentrum lima*, as well as the physiological responses of mussels subjected to both stressors. Results revealed that a +4°C temperature increase significantly decreased toxin accumulation (by 49%) and elimination (by 77%) compared to normal conditions. Additionally, simultaneous exposure to MHW conditions and toxins altered antioxidant activity, caused lipid and protein damage, and affected metabolism in a tissue-specific manner. These findings suggest that *M. galloprovincialis* may become more susceptible to toxin exposure during marine heatwave events.

OC A18 - BIOREMEDIATION POTENTIAL OF MACROALGAE IN MUSSEL FARMING: RESPONSE TO COPPER AND OCTOCRYLENE EXPOSURE UNDER ENVIRONMENTAL CHALLENGES.

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Co-production of macroalgae with other species has gained representativeness among producers as it increases profitability while reduces nutrient loads. Additionally, macroalgae aquaculture is often performed in areas with high anthropogenic pressure (estuaries and coastal waters) and contaminants load that macroalgae can accumulate. Such accumulation can protect other species by reducing contaminant levels in the environment.
The objectives of the present work were to i) assess the bioremediation potential of two macroalgae species (*Laminaria ochroleuca* and *Ulva* sp.), when co-cultured with mussels (*Mytilus* sp.), to two contaminants commonly found near aquaculture sites, and ii) to evaluate the physiological responses of both macroalgae species exposed to contaminants and/or environmental stressors. The contaminants used were: a) copper-based compounds used as antifouling agents in paints to protect aquaculture infrastructures, and b) the UV-filter octocrylene, commonly used in sunscreens. Simulations were conducted to assess the effects

of a salinity decrease and UV radiation exposure combined with Cu (20 µg/L with *L. ochroleuca*) and octocrylene (10 µg/L L with *Ulva* sp.) contamination, respectively.
Preliminary results showed higher mortality of *Mytilus* sp. when exposed solely to Cu compared to co-cultures with macroalgae. Direct observation of morphological features and lipid peroxidation values suggested that this mortality is associated with the degeneration of *Mytilus* sp. gills. In *L. ochroleuca* treatments, Cu levels were reduced, and accumulated more in the macroalgae (≈100 µg/g) than in the mussels (≈50 µg/g). Copper-exposed macroalgae showed higher chlorophyll *a* degradation, leading to bleaching and death, as well as increased Cu chelating capacity.
In the octocrylene and UV exposure test, both stressors resulted in higher mortality of *Ulva* sp. and *Mytilus* sp. *Ulva* sp. exposed to UV radiation presented a slightly lower Fv/Fm, which is a proxy of photooxidative damage.
These findings underscore the macroalgae's potential to protect co-cultivated species in an integrated multitrophic aquaculture system.

Keywords: Laminaria ochroleuca, Ulva sp., Integrated Multi-Trophic aquaculture, Antifouling, Octocrylene, Physiological stress

POSTERS
S.1 - CLIMATE SMART STRATEGIES

PO1 - GROWTH PERFORMANCE, IMMUNOCOMPETENCE AND PHYSIOLOGICAL RESPONSES OF JUVENILE SPARUS AURATA SUPPLEMENTED WITH L. DIGITATA

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Introduction: Balancing economic sustainability and environmental responsibility is critical to the aquaculture industry. While technological advancements and innovation in feed formulations can drive economic gains, they must also align with environmental sustainability goals. The integration of functional feeds that reduce the need for antibiotics and chemicals in aquaculture production aligns with these dual objectives, promoting both economic viability and environmental stewardship. In this sense, seaweeds have emerged as a promising alternative aquafeed ingredient with several nutritional and functional attributes that can improve farmed marine species' performance and resilience. The bioactive compounds in their composition can enhance species growth, immune responses, and stress tolerance. In addition, the antioxidant properties of certain seaweed species help mitigate oxidative stress, enhancing the overall resilience of targeted farmed species. This study aimed to assess the effectiveness of aquafeeds biofortified with *Laminaria digitata* as an eco-innovative approach to improve growth performance, immunocompetence and physiological responses of juvenile *Sparus aurata* environmental stressors.

Material and Methods: Four experimental feeding treatments were performed: i) a commercial control diet without seaweed supplementation (CTR); ii) a diet supplemented with 0.3% *L. digitata* powder (P0.3%); iii) a diet supplemented with 1.5% *L. digitata* powder (P1.5%); and iv) a diet supplemented with 0.3% *L. digitata* extract (E0.3%). Animal condition and key physiological markers indicative of oxidative stress (catalase - CAT, glutathione S-transferase - GST, lipid peroxidation - LPO), protein degradation (ubiquitin content - UBI), heat shock response (HSP70/HSC70 content) and metabolism (lactate dehydrogenase - LDH) were determined after 30 days of biofortification..

Results and discussion

Fish biofortified with *L. digitata* demonstrated significantly higher Fulton's condition index (Kc) and Specific Growth Rate (SGR) values, as well as enhanced antioxidant defences, compared to the control treatment, particularly E0.3%
The reduction in lipid peroxidation (LPO) levels in the E0.3% treatment compared to other treatments, indicates that the extract form effectively provides bioactive compounds with antioxidant properties, which scavenge reactive oxygen species (ROS) and protect cellular components from oxidative damage. The extraction process concentrates these compounds, increasing their bioavailability and efficacy. Although P0.3% and P1.5% provided some animal conditioning benefits, they also increased oxidative stress, emphasizing the relevance of investigating optimal biofortification doses for *S. aurata*, which may vary according to seaweed species and available raw material.

Keywords: Aquaculture, climate change, functional aquafeeds, fish welfare, biomarkers

PO2 - TOWARDS CLIMATE-SMART HATCHERY PRACTICES: PH-DEPENDENT GROWTH PATTERNS IN VENERUPIS CORRUGATA UNDER SIMULATED OCEAN ACIDIFICATION

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Towards Climate-Smart Hatchery Practices: pH-Dependent Growth Patterns in *Venerupis corrugata* Under Simulated Ocean Acidification

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Climate change introduces significant challenges for sustainable aquaculture, particularly through ocean acidification. This phenomenon alters seawater pH, directly impacting calcifying organisms such as bivalves, whose shell formation and overall development are pH-sensitive processes. As part of a climate-smart approach to aquaculture, this study evaluated the effects of future ocean acidification scenarios on the early growth of *Venerupis corrugata*, a species of increasing relevance to Portuguese shellfish aquaculture.
A 64-day experiment simulated two pH conditions (8.2 and 7.8) in a controlled closed system, covering both the larval and post-larval phases. pH was adjusted using CO_2 injection, while temperature (17.6±0.3°C), oxygen (94.2±3.2%), salinity (33.2±0.6), and daily feeding consisted of a progressive mix of *Tisochrysis lutea* and *Chaetoceros calcitrans*. Growth was monitored through regular measurements of shell length and total area of the valve.
The results demonstrated that larvae reared at pH 7.8 outperformed those at pH 8.2, showing approximately 30 µm greater shell length, 8000 μm^2 larger shell area, and a 40% higher survival rate by day 12 after fertilization. However, this trend reversed in the post-larval stage, with juveniles at pH 8.2 developing shells nearly 200 µm longer and 0.21 mm^2 more area by the end of the trial.
These findings highlight a developmental trade-off in bivalve responses to ocean acidification, suggesting that optimal pH conditions shift between early and later stages. Lower pH conditions may benefit early larval growth and survival, while higher pH appears more favorable for post-larval development. To enhance resilience and productivity in bivalve hatcheries, dynamic pH management strategies (tailored to the developmental stage) could become a key component of climate-smart and safe aquaculture systems.

Keywords: Climate changes, Venerupis corrugata, Ocean acidification, Growth, Hatchery

S.2 NEW TOOLS FOR ANIMAL WELFARE

PO3 - STRATEGIC RELEVANCE OF CELLULAR AQUACULTURE FOR CLIMATE-SMART, SAFE, AND SUSTAINABLE SEAFOOD

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Cellular aquaculture represents a disruptive innovation addressing the intertwined challenges of global food security, climate resilience, and marine ecosystem preservation. With global food demand projected to increase by 50% by 2050 - and seafood already accounting for 17% of animal protein intake -conventional supply models are unsustainable. Overfishing has depleted marine biomass by approximately 60%, and over three million wild fish are harvested per minute, raising serious ecological and ethical concerns.
Cellular aquaculture consists of producing seafood through the cultivation of cells from marine organisms rather than from whole animals. This approach involves isolating cells from fish, mollusc or crustacean species and growing them in controlled conditions that provide the necessary nutrients and environmental signals for proliferation or tissue formation. By cultivating only the edible fractions of seafood, cellular aquaculture offers a resource-efficient, low-waste production model fully decoupled from ocean ecosystems and seasonal variability, and geographical constraints. Cellular Aquaculture is based on advanced biotechnology techniques, including the development of species-specific cell lines, serum-free and microalgae-based media, and scalable bioreactor platforms, to generate both structured and unstructured seafood prototypes - such as Cell4Food's proprietary bioink for 3D-printed octopus.
Cultivated seafood is produced in controlled, sterile environments, ensuring traceability, biosecurity, and consistently high food safety standards. It aligns with the growing consumer demand for clean-label, sustainable protein options, free from antibiotics and contaminants, such as microplastics or metals. This approach also has the potential to mitigate environmental impacts by reducing or potentially eliminating emissions associated with fishing fleets and long-distance cold-chain logistics. By removing the need for conventional feed inputs like fishmeal and soy, cellular aquaculture supports biodiversity conservation and helps to reduce deforestation. Moreover, it is expected to require substantially less land and freshwater than traditional aquaculture.
Although many technical and scientific challenges remain, cellular aquaculture holds promise as a complementary method to traditional seafood production. It has the potential to address issues such as overfishing, disease outbreaks, environmental impact, ethical concerns, and supply chain instability while contributing to a more resilient and sustainable food system.
Cell4Food is a first mover in the cultivated octopus' segment and a pioneer in cell-based mollusc and crustacean technologies. Operating with a B2B licensing model, the company is a deep tech biotechnology startup dedicated to the development of cellular agriculture solutions for seafood production. Its platform exemplifies alignment with ESG principles, particularly using a single, non-lethal biopsy to initiate cell cultures - minimizing harm to marine species.

Keywords: Cellular Aquaculture, Sustainability, Food Security, Climate Mitigation, Innovation

PO4 - NEW ANIMAL WELFARE TOOL TO ASSESS FISH IMMUNE CELLS: A PROTOCOL FOR ISOLATION, FIXATION AND CHARACTERIZATION OF JUVENILE GILTHEAD SEABREAM HEAD KIDNEY LEUKOCYTES BY FLOW CYTOMETRY

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Abstract

The immune system plays a vital role in maintaining fish homeostasis by protecting against pathogens and environmental stressors. The study of immune cells provides essential insights into host defense mechanisms, but the lack of standardized protocols for marine fish presents technical challenges that hinder accurate data interpretation and the understanding of species-specific immune responses.

In recent years, considerable progress has been made in the immunological characterization of various fish species, with a particular focus on leukocyte populations and their functional roles. Conventional hematological methods, such as manual counting using Bürker, Neubauer, or Thoma hemocytometers, along with stained blood smears using Wright, May-Grünwald-Giemsa, or Hemacolor dyes, are still widely used. However, these techniques are time-consuming, technically demanding, and prone to human error, requiring a high level of expertise to ensure reliable and reproducible results.

To overcome these limitations, we optimized a flow cytometry-based protocol to characterize and assess the viability of leukocytes isolated from the head kidney (the primary hematopoietic organ in teleost fish) of juvenile gilthead seabream (*Sparus aurata*). The procedure began with leukocyte isolation through a homogenization process using Hanks' balanced salt solution, followed by an optimized Percoll density gradient centrifugation method to ensure high recovery rates of leukocytes with minimal erythrocyte contamination. Additionally, a novel technique using a cell-reactive dye (LIVE/DEAD™ Fixable Dead Cell Stain Kit) was employed to distinguish viable from non-viable cells based on fluorescent staining patterns. Fixation was performed using 37% formaldehyde, preserving cell morphology, viability, and staining efficiency. Flow cytometry analysis successfully identified three predominant leukocyte populations — lymphocytes, monocytes, and granulocytes — while simultaneously allowing the assessment of viability within each leucocyte populations.

This protocol represents a step forward in fish immunology, offering a reliable, reproducible, and efficient method for immune cell analysis. In addition, by allowing cells to be fixed for later analysis, this protocol significantly reduces the time and effort required for immune assessments, making it a valuable animal welfare tool for practical applications in several research areas.

Keywords: flow cytometry, leukocytes, live-dead fixable dye, cell viability, immune responses, Sparus aurata

PO5 - OPTIMIZATION OF AN IN VITRO BIOACCESSIBILITY FISH MODEL: PROTEIN DIGESTIBILITY USING FISH ENZYMATIC EXTRACTS AND COMMERCIAL ENZYMES

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This work was funded by The Portuguese Foundation for Science and Technology (FCT I.P.), under the framework of the Project MycoFish - Occurrence, bioavailability and mitigation strategies for mycotoxins in farmed fish and associated feed ingredients: Gilthead seabream as case study (PTDC/CVT-CVT/2660/2021; https://doi.org/10.54499/PTDC/CVT-CVT/2660/2021).

Recent advances in aquaculture nutrition have raised the interest on studies regarding new feed products capable of effectively replacing fish meal and oil, which must be thoroughly analysed in terms of nutrients bioaccessibility to certify their efficacy. *In vitro* models could allow the assessment of nutrients bioaccessibility, particularly proteins, from new ingredients used to replace fish meal, in a faster, more ethical, and less expensive manner when taken into consideration the *in vivo* approach. To date, there are few studies focused on *in vitro* simulating the fish gastrointestinal tract, to quantify the feed digestibility, bioaccessibility and bioavailability of nutrients, since are commonly used in human studies, which motivates further efforts to develop and validate fish digestive *in vitro* models. This study aimed to validate an *in vitro* digestion model for marine fish capable of assessing the bioaccessibility of proteins, using a juvenile fish species (gilthead seabream, *Sparus aurata*) as biological model. Two different procedures were tested to conduct the bioaccessibility analyses of the samples: i) use of enzymatic extracts obtained from the fish digestive tract (stomach and pyloric ceca+intestine); and ii) use of commercial digestive enzymes. Additionally, the effects of temperature, digestion time and feed amount (enzyme-to-substrate [E:S] ratio) were evaluated in the *in vitro* protein digestibility, either individually or through their interactions.

Only commercial enzymes lead to similar protein bioaccessibilities to those obtained *in vivo* (90.8 ± 1.7%) under the following factorial design: i) 20 °C, 24h, 250 mg of feed (88.1 ± 2.6%); ii) 37 °C, 6h, 136.5, 250 and 500 mg (85.9 ± 2.5%, 90.1 ± 3.0% and 87.4 ± 1.0%, respectively); and iii) 37 °C, 24h, 500 and 1000 mg (86.0 ± 2.1%, 86.6 ± 5.2%, respectively). The distinct action mechanisms of non-fish commercial enzymes seem to significantly enhance protein bioaccessibility compared to fish digestive extracts. Moreover, an optimal balance between temperature and digestion time plays a crucial role in maximizing digestibility, supporting efficient nutrient breakdown and absorption.

The validated *in vitro* digestibility method using commercial enzymes for *S. aurata* provides a cost-effective, fast alternative and free of ethical constraints. Ultimately, this study will contribute to assess the efficacy of fish meal replacement ingredients and new aquafeeds throughout a validate model that in turn could contribute to the achievement of a truly

sustainable aquaculture development, while ensuring the production of safe and high quality farmed products for consumers.

Keywords: Fish feed, Gilthead seabream, Gastrointestinal fish model, Digestive extracts, Digestive enzymes

S.3 EMERGING HAZARDS

PO6 - MICROBIOME ANALYSIS OF WATER AND SEDIMENT NEAR SALMON CAGES: SEASONAL AND ENVIRONMENTAL IMPACTS

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Aquaculture is an important food production sector, whose rapid development and intensification have raised environmental concerns about marine microbial communities. Intensive aquaculture practices release large amounts of nutrients into the water column and benthic environments, modifying microbial diversity. This work aimed to evaluate bacterial community composition in water and sediment from a fish farm site during winter and summer in Norway (Storevika, GIFAS) compared to a control site.
Water samples were collected at 5 and 10 meters at a control site and in sea cages, and sediment samples were collected with a Van Veen dredger below sea cages and at the control site. Water samples were filtered using 0.22 µM filters, and total DNA was extracted using kits for water and sediment (Power water and Power soil kits, QIAGEN). Quality control was performed by PCR with amplification of the V3-V4 regions of the 16S rRNA. Then, samples were subjected to full-length 16S sequencing by PacBio Revio HIFI reads with 50k reads per sample. Data was filtered, and Amplicon Sequence Variants (ASVs) analysis was performed. ASVs determination was performed using the Qiime2 platform with the DADA2 package. Statistical analysis of the pathogenic groups among samples was done with SPSS v29 (IBM, USA).
The sequencing produced high-quality datasets. Alpha diversity indexes revealed that sediment samples, especially from control sites in summer, presented high richness and evenness. On the contrary, the reduced diversity and higher dominance observed for water samples suggested a microbial community dominated by fewer taxa. Statistical analysis of alpha diversity indices revealed significant differences among water samples, underlying how diversity of microbial communities is susceptible to seasonality, depth, and place of collection. Moreover, beta diversity showed greater separation among sediment samples and a clear clustering among seasons within water groups. Five genera of fish pathogens were found in sediment and water: *Mycobacterium*, *Tenacibaculum*, *Pseudomonas*, *Photobacterium*, and *Vibrio*. Depending on the season, sea cage water samples showed a significant increase in bacteria, particularly *Tenacibaculum* spp. and *Moritella* spp., compared to controls. An increased count of *Tenacibaculum* spp. and *Moritella* spp. in sea cage samples indicates potential for outbreaks, as both genera are associated with severe fish diseases - tenacibaculosis and winter ulcer disease.
These results support the need for continuous improvements regarding management practices in the aquaculture industry, especially under changing environmental conditions, as well as for the monitoring of microbial communities around aquaculture sites.

Keywords: Microbiome, Seasonal variation, Aquaculture impact, Sequencing, Fish pathogens