ENAQUA 25

National Aquaculture Meeting | CIMAR-LA

27–28 November 2025
1st edition

BOOK OF ABSTRACTS

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Associate Laboratory

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ENAQUA 25
National Aquaculture Meeting | CIMAR-LA

Book of Abstracts ENAQUA 25

Editors: Sofia Engrola, Rita Teodósio and Cláudia Aragão

Edition: Centro de Ciências do Mar do Algarve (CCMAR)

Publication date: November 2025

ISBN: 978-989-33-8817-4

National Aquaculture Meeting CIMAR LA – ENAQUA 25

27-28 November 2025

Faro, Portugal

Organisation:

CIMAR
Associate Laboratory

CCMAR
Centro de Ciências do Mar
Algarve

ciimar

Funding:

This event received Portuguese national funds from FCT - Foundation for Science and Technology, and by the European Commission's Recovery and Resilience Facility, within the scope of UID/04326/2025, UID/04423/2025 (https://doi.org/10.54499/UID/04423/2025), UID/PRR/04326/2025, UID/PRR/04423/2025 (https://doi.org/10.54499/UID/PRR/04423/2025), and LA/P/0101/2020 (https://doi.org/10.54499/LA/P/0101/2020).

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Welcome to the first edition of the National Aquaculture Meeting CIMAR-LA (ENAQUA25)

It is a great pleasure to host you at the University of Algarve for this inaugural event, jointly organised by CCMAR and CIIMAR, the two research institutes that together form CIMAR-LA, Portugal's largest marine and environmental research laboratory. This meeting marks an important milestone for our community, and our ambition is to establish ENAQUA as a biennial gathering that strengthens collaboration, promotes scientific excellence, and advances innovation in aquaculture.

We are pleased to welcome participants from 15 institutions, including researchers, students, industry representatives, and stakeholders from across the sector. Your presence reflects the diversity, vitality, and growing strategic importance of aquaculture in Portugal and Europe.

This year, ENAQUA25 features a dedicated Industry Session focusing on Bivalves, a major pillar of national and international aquaculture. This session underscores our commitment to engaging with industry partners, understanding their challenges, and contributing meaningful scientific solutions that support sustainable growth, food sovereignty, and the societal relevance of aquaculture.

We thank all contributors for their abstracts, presentations, and posters, which form the core of this scientific programme. We also extend our gratitude to our keynote speakers, supporters, sponsors as well as organising and scientific committees for their dedication and essential support in making this event possible.

We wish you an inspiring and productive meeting, full of opportunities for learning, discussion, and the strengthening of future collaborations.

On behalf of the Organising Committee CIMAR LA,
Sofia Engrola (CCMAR) and Benjamin Costas (CIIMAR)

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ENAQUA 25 Programme

Thursday, 27th of November

09h00	**Opening Ceremony**
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09h45	*In vitro* functional screening of circular protein hydrolysates for aquafeeds: **L. Rodrigues-dos-Santos**, M. Monteiro, A. Filipa-Silva, D.A. Marques, M. Pintado, A. Almeida, L.M.P. Valente
10h00	Nutritional modulation of stress responses in European seabass under low-fishmeal diets: **M. Cabano**, B. Costas, C. Aragão
10h15	Low-trophic organisms as functional ingredients in high vegetable-protein diets for *Sparus aurata*: **D. Filipe**, S. Moutinho, P. Canada, A. Monteiro, R. Calado, L. Valente
10h30	Flash-Talks (Daniela Castro – CCMAR; Ana Claro – CIIMAR)
10h40	**COFFEE-BREAK**
11h15	*Gammarus* and *Hediste* meals improve growth and fillet lipid stability in gilthead seabream: **P. Canada**, F. Rey, S. Moutinho, T. Sousa, A. Marques, A. Monteiro, D. Moreira, R. Domingues, R. Calado, L.M.P. Valente
11h30	Tailoring peptide size to enhance growth and physiological performance in sole larvae: **A. Sharif**, R. Barruncho, R. Teodósio, R. Colen, W. Pinto, A. Santos, C. Castro, R. Serradeiro, L.E.C. Conceição, S. Engrola
11h45	Putative biomarkers for the assay of nutritional approaches to mitigate oxidative stress and enhance reproduction in flatfish: **C. Marrero-Alemán**, E. Fatsini, C. Oliveira, C. Magalhães-Raposo, H. Pereira, E. Cabrita
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12h15	Transcriptomic profiling of muscle in fast- and slow-growing *Sparus aurata*: preliminary results: **M. Ângelo**, M. Barata, M. Rocha, P. Pousão-Ferreira, I.A.L. Silva, C. Lourenço-Marques
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11h45	Methionine supplementation enhances viral haemorrhagic septicemia virus resistance in rainbow trout (*Oncorhynchus mykiss*): insights into the role of viperin: **M. Vaz**, G.E. Themudo, A. Florea, I. Carvalho, F.B. Schöninger, P. Díaz-Rosales, C. Tafalla, T. Bean, D. Robledo, B. Costas, M. Machado
12h00	**Closing ceremony**

KEYNOTE PRESENTATIONS

Closing the loop: protein and oil sources in the circular economy

Luísa M.P. Valente

Interdisciplinary Centre of Marine and Environmental Research (CIIMAR) and School of Medicine and Biomedical Sciences (ICBAS), University of Porto, Portugal; valente@icbas.up.pt

The recovery of protein and oil sources within a circular economy framework offers a viable route toward sustainable resource management and improved food security. Conventional linear production systems drive resource depletion, environmental degradation, and food loss, whereas circular approaches focus on reuse, recycling, and regeneration to minimise waste and optimise value. This paradigm is particularly relevant to the aquafeed sector, where the rising global demand for protein and oil necessitates alternative, sustainable inputs. Large volumes of agricultural and food-processing biomass—both marine and terrestrial—remain underutilised yet can be valorised into high-value ingredients. Emerging technologies, including insect bioconversion, microalgae cultivation, and precision fermentation, enable the transformation of organic residues into nutrient-rich protein and lipid products. Embedding these innovations into closed-loop production systems enhances resource efficiency while reducing environmental burdens. Progress in technology readiness levels is accelerating the availability of novel, nutritionally balanced feed ingredients at scale. Nevertheless, issues related to consumer perception, regulatory frameworks, market integration, and the energy demands of production processes must be carefully addressed to fully realise the potential of circular protein and oil production.

Keywords: Sustainable aquaculture, by-products valorisation, processed-animal-protein (PAP), insect meal and oil, single-cell-protein (SCP), environmental impact.

Acknowledgements: This work has been supported by the Blue Bioeconomy Innovation Pact (C644915664-00000026) and InsectERA Mobilising Agenda (PRR - InsectERA - The ERA of the insect industry), funded by Portugal and European Union - NextGenerationEU, with the identifier C644917393-00000032.

Fish welfare in aquaculture: collective goals, challenges and solutions

João L. Saraiva

Centro de Ciências do Mar do Algarve (CCMAR / CIMAR LA), Faro, Portugal;
FishEthoGroup, Faro, Portugal; jsaraiva@ualg.pt

The welfare of farmed fishes constitutes a collective goal that should empower all stakeholders in the aquaculture sector to work together in pursuit of better lives for the animals we rely on. The challenges ahead are far from easy, starting from the multitude of species currently being farmed, the lack of knowledge regarding many of them even in crucial aspects of their biology, and the limited dialogue between parties. An essential aspect is the definition of the term “welfare” and how it can be addressed scientifically in fish. While animal welfare science is well consolidated in land animals, the field is still very young in fish. However, it is picking up pace, especially in the last 20 years. In this talk I will address recent relevant discoveries in fundamental fish science and why they are highly relevant for our understanding of the prevailing welfare bottlenecks. Challenges include 1) the establishment of species-specific welfare indicators that can be used on farms and allow real-time monitoring of the animals as they cope with their rearing environment; 2) the implementation of protocols and techniques that are directed to meet the biological needs of the animals, namely through environmental enrichment; and 3) the use of effective humane stunning and slaughter methods. I will also provide examples of why and how collaboration is essential to address these challenges and propose a framework to tackle fish welfare issues systematically.

Keywords: farmed fish welfare, welfare indicators, environmental enrichment, humane stunning and slaughter

Acknowledgements: This study received Portuguese national funds from FCT - Foundation for Science and Technology through contracts UID/04326/2025, UID/PRR/04326/2025 and LA/P/0101/2020 (DOI:10.54499/LA/P/0101/2020).

ORAL PRESENTATIONS

In vitro functional screening of circular protein hydrolysates for aquafeeds

Luciano Rodrigues-dos-Santos[1-5,*], Marta Monteiro[1,2], Andreia Filipa-Silva[1], Diana A. Marques[1],
Manuela Pintado[3], André Almeida[4,5], Luisa M. P. Valente[1,2]

[1]CIIMAR/CIMAR LA, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Matosinhos, Portugal; [2]Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal; [3]Laboratório Associado, Escola Superior de Biotecnologia, Centro de Biotecnologia e Química Fina, Universidade Católica Portuguesa, Porto, Portugal; [4]ETSA, I.T.S., Indústria Transformadora de Subprodutos, Loures, Portugal; [5]SEBOL, Comércio e Indústria do Sebo, SA (ETSA Group), Loures, Portugal; *Presenting author.

The transition toward sustainable food systems demands efficient resource utilisation and adherence to circular economy principles. The valorisation of animal by-products into protein hydrolysates (PHs) offers an innovative pathway to produce high-quality, functional ingredients for aquafeeds. PHs derived from swine trimmings, insect biomass, and fish by-products represent a promising approach to reduce waste while offering bioactive peptides with antioxidant and antimicrobial potential. This study aimed to compare the *in vitro* nutritional and bioactive properties of five PHs derived from circular animal by-products—specifically from blue shark (*Prionace glauca*), fish, swine, and whole insect larvae (*Hermetia illucens*)—using CPSP90 as a commercial hydrolysate reference. Hydrolysates were obtained by enzymatic or thermal hydrolysis and analysed for proximate composition, amino acid (AA) and peptide profiles, solubility, antioxidant capacity, and antibacterial activity against relevant fish pathogens. Results revealed distinct profiles. INSECT and FISH hydrolysates showed the strongest antioxidant activity in ABTS and ORAC assays, associated with aromatic AAs or phenolic compounds, while SHARK hydrolysate, rich in collagenic AAs, showed low solubility and antioxidant capacity. SWINE hydrolysate exhibited consistent antibacterial activity against *Vibrio* spp. *and Aeromonas salmonicida*, whereas FISH and SHARK hydrolysates tended to promote bacterial growth. INSECT hydrolysate showed a dual effect—supporting or inhibiting bacterial growth depending on the strain. Overall, PH functionality depended on the raw material and hydrolysis method. The functional and nutritional properties of the PHs tested underscore their potential for by-product valorisation and reuse in aquaculture and biotechnological systems, supporting the principles of circular bioeconomy.

Acknowledgements: This work was supported by the Blue Bioeconomy Pact (C644915664-00000026) within the scope of the Recovery and Resilience Plan (PRR). Luciano Rodrigues-dos-Santos acknowledges FCT for the PhD grant (2023.00947.BDANA) – http://doi.org/10.54499/2023.00947.BDANA.

Nutritional modulation of stress responses in European seabass under low-fishmeal diets

Miguel Cabano[1,2,3,*], Benjamín Costas[3,4], Cláudia Aragão[1]

[1]Centro de Ciências do Mar do Algarve (CCMAR/CIMAR LA), Faro, Portugal; [2]Universidade do Algarve, Faro, Portugal; [3]Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR LA), Matosinhos, Portugal; [4]Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, Porto, Portugal;
*mcabano@ualg.pt

Introduction

Finding sustainable alternatives for finite marine-derived proteins is crucial for the growth of aquaculture. However, low-fishmeal diets often lack taurine, a key amino acid in several metabolic processes, which may reduce fish resilience. This study aimed to evaluate whether taurine supplementation could mitigate stress effects in European seabass fed low-fishmeal diets.

Material and methods

Three isonitrogenous, isolipidic diets were tested: a marine protein-based (MARINE), a low-fishmeal diet using animal by-products (PAP), and a similar PAP formulation supplemented with 1% taurine (TAU). Triplicate groups of European seabass (34.5 ± 0.7 g) were fed for 15 days and then exposed to a stress event (crowding and chasing followed by 1 min of air exposure). Samples were collected at 0, 1, 4, and 24 h post-stress for evaluation of stress and antioxidant response parameters.

Results

Taurine supplementation significantly reduced cortisol release at 1 h compared to the other diets. Fish fed the MARINE diet showed higher catalase activity in the intestine and lower lipid peroxidation in the liver and intestine compared with fish from the PAP treatment. Taurine supplementation partially restored redox balance, with TAU-fed fish showing intermediate values between MARINE and PAP groups.

Discussion and Conclusion

Taurine supplementation improved stress resilience, limiting cortisol release and reducing oxidative damage, partially restoring the antioxidant capacity of fish fed low-fishmeal diets. These results reinforce the value of taurine as a functional additive to enhance the physiological robustness of European seabass.

Acknowledgements

This study received Portuguese national funds from the Foundation for Science and Technology through contracts UID/04326/2025, UID/PRR/04326/2025, LA/P/0101/2020 and 2021.06497.BD (DOI:10.54499/LA/P/0101/2020).

Low-trophic organisms as functional ingredients in high vegetable-protein diets for *Sparus aurata*

Diogo Filipe[1,*], Sara Moutinho[1], Paula Canada[1], André Monteiro[1,2], Ricardo Calado[3], Luísa Valente[1,2]

[1]Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR LA), University of Porto, Matosinhos, Portugal; [2]ICBAS–School of Medicine and Biomedical Sciences, University of Porto, Porto, Portugal; [3]ECOMARE, CESAM – Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, Aveiro, Portugal; *Presenting author.

Replacing fishmeal (FM) with plant proteins can affect oxidative balance and stress in fish through changes in antioxidant enzymes and endocrine regulation. Functional ingredients can counter these effects. Low-trophic organisms remain underexplored, despite their culture potential and bioactive antioxidant and antimicrobial compounds. This study evaluated whether supplementation with *Gammarus locusta* (GAM) and *Hediste diversicolor* (HED) could mitigate the drawbacks of high vegetable protein inclusion in *Sparus aurata*. Four diets were tested: a positive control (PC, 20% FM), a negative control (NC, 7% FM), and NC supplemented with 2.5% GAM or HED (both 7% FM). The antioxidant capacity of diets differed: NC and HED had the highest total antioxidants (DPPH, ABTS), while GAM had the highest phenolic content. An 8-week growth trial was conducted with *S. aurata* juveniles (~16 g) distributed into 12 tanks and hand-fed to satiation three times daily at 23 °C. At the end of the trial, fish were subjected to combined cold (−6 °C overnight) and handling (1 min air exposure) stress. Hepatic lipid peroxidation (LPO) was reduced in the HED group under both conditions and muscle (LPO) was reduced in basal conditions. Under basal conditions, PC increased plasma triglycerides and phospholipids, GAM increased glucose, and NC increased peroxidase activity. Hepatic SOD activity was lowest in PC fish, CAT was higher in GAM and HED, and GR was highest in NC. Under stress, NC decreased plasma glucose, triglycerides, and cholesterol, while PC fish showed higher lactate levels. Hepatic SOD was lower in GAM and HED, CAT was reduced in PC, and GPX increased in GAM, possibly due to supplemented antioxidants. Redox defences appeared upregulated in fish fed the supplemented diets: hepatic CAT and SOD increased in basal conditions, while SOD activation during stress was reduced. In conclusion, supplementation of low-trophic organisms is effective in improving oxidative balance of *S. aurata*, ameliorating the effects of high vegetable protein diets.

Acknowledgements

This work was funded by: Innovation Pact (Project No. C644915664-00000026/Notice No. 02/C05-i01/2022, “Blue Bioeconomy Pact”, funded by the European Union through the Recovery and Resilience Plan - PRR) and by project PUFAPODS (2022.01620.PTDC), funded by FEDER, through PT2020 Partnership Agreement and Compete 2020 and by national funds (OE), through FCT/MCTES.

Gammarus and *Hediste* meals improve growth and fillet lipid stability in gilthead seabream

Paula Canada[1,*], Felisa Rey[2], Sara Moutinho[1], Tiago Sousa[2], Alexandra Marques[1], André Monteiro[1,4], Diogo Moreira[1], Rosário Domingues[2], Ricardo Calado[3], Luísa M.P. Valente[1,4]

[1]Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR LA), University of Porto, Matosinhos, Portugal; [2]CESAM, Department of Chemistry, University of Aveiro, Portugal; [3]ECOMARE, CESAM – Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, Aveiro, Portugal; [4] ICBAS–School of Medicine and Biomedical Sciences, University of Porto, Porto, Portugal; *Presenting author.

The dietary inclusion of plant-ingredients has been a strategy to reduce the reliance of aquafeed industry on fishmeal (FM), but often impairs palatability, amino acid balance, and fillet lipid quality due to changes in fatty acid profile and oxidative stability. To face such limitations, there has been an effort to identify novel ingredients that can mitigate the reduction of FM in aquafeeds. In this study *Hediste diversicolor* and *Gammarus locusta* were evaluated as dietary supplements, through the effects on feed efficiency, growth, nutrient utilisation, and fillet lipid quality. A growth trial was performed with gilthead seabream (~16 g) at 23 °C using four isoproteic, isolipidic, and isoenergetic diets: a positive control (PC, 20% FM), a negative control (NC, 7% FM), and NC + 2.5% *H. diversicolor* or *G. locusta* (HED and GAM, 7%FM). Fish were hand-fed to apparent satiation for 8 weeks. All diets were well accepted, but fish fed the GAM and HED diets displayed higher feed intake than those of the NC group, but comparable to PC. The final body weight of fish fed the GAM diet was close to those on the PC diet, but fish feeding on HED diet displayed lower weight, similar to fish feeding on NC. There were no major changes in nutrient gain or retention, though a significant increase in the hepatosomatic index of fish feeding on PC when compared to others, point to possible metabolic changes. The induced changes in muscle fatty-acids (FA) profile were also found between PC fish *vs.* all others: PC led to increased SFA content and SFA/PUFA ratio, and a decrease in omega−6 FAs. However, those changes seemed not to influence lipid peroxidation (LPO). Only HED diet significantly reduced muscle LPO, when compared to PC and GAM. This effect appears unrelated to SFA/PUFA or omega−6 FA content but rather to the intrinsic antioxidant capacity of the ragworm meal, which showed strong radical scavenging activity. These properties likely enhanced oxidative stability and lipid quality in the muscle of fish feeding on the HED diet. Our results highlight the potential of low-trophic species as dietary supplements in aquafeeds for gilthead seabream. Supplementation with *G. locusta* improved growth performance, while *H. diversicolor* enhanced muscle lipid stability, potentially extending product shelf life. **Acknowledgements:** This work was funded by Innovation Pact (Project No. C644915664-00000026/Notice No. 02/C05-i01/2022, "Blue Bioeconomy Pact", funded by the European Union through the Recovery and Resilience Plan - PRR).

Tailoring peptide size to enhance growth and physiological performance in sole larvae

Aqsa Sharif[1,2,*], Rafael Barruncho[1], Rita Teodósio[1], Rita Colen[1], Wilson Pinto[3], André Santos[3], Carolina Castro[4], Renata Serradeiro[4], Luis E.C. Conceição[3], Sofia Engrola[1]

[1]Centro de Ciências do Mar do Algarve (CCMAR/CIMAR-LA), Faro, Portugal; [2]University of Cadiz, Spain; [3]SPAROS Lda., Portugal; [4]FLATLANTIC - Actividades piscícolas, S.A., Praia de Mira, Portugal; *Presenting author.

Marine fish larvae have limited digestive capacity and high metabolic demands due to rapid growth, requiring tailored microdiets that optimise nutrient assimilation and energy utilisation. This study evaluated how the molecular weight of fish protein hydrolysates influences growth and metabolic responses of Senegalese sole (*Solea senegalensis*) during early and later developmental stages. Larvae were fed from first feeding (3 days after hatching, DAH) to 16 DAH under a co-feeding regime with microdiets containing low (LPH), mixed low and high, commercial-like (MID), or high (HPH) molecular weight protein hydrolysates. Long-term effects were assessed during the benthic phase (18–38 DAH), when all groups received a common commercial-like diet (COM). Fish were reared under standardised conditions in a recirculating aquaculture system (RAS), and at 16 DAH subjected to a 24 h thermal challenge (+5°C). Sampling occurred at 12, 16, 17, and 38 DAH for growth, and at 16, 17, and 38 DAH for metabolic and molecular analyses. At 12 DAH, MID and HPH larvae were heavier than LPH. By 16 DAH, this trend reversed, with LPH surpassing HPH and matching MID. At this stage, LPH larvae upregulated genes related to nutrient transport, digestion, gut integrity, and stress regulation (*lat1, ampn, lap3, tjp1, sirt3*), while changes were less evident in HPH. After the thermal challenge (17 DAH), LPH larvae showed lower lipid peroxidation (LPO) than MID, with increased expression of antioxidant (*cat, nrf2*), mitochondrial (*sirt3*), and stress-response (*hsp60*) genes. By 38 DAH, LPH/COM had higher LPO than other groups, along with upregulation of *gpx4* versus HPH/COM. Low molecular weight peptides enhanced stress resilience, as reflected by antioxidant, mitochondrial, and stress-response gene activation and reduced LPO. Dietary peptide size effects were stage-dependent: high-MW peptides supported early growth, whereas low-MW peptides improved performance and stress resilience during metamorphosis. These findings highlight early nutrition as a key determinant of larval physiological readiness and suggest that tailoring peptide profiles to developmental stages can optimise growth, metabolic performance, and long-term juvenile robustness in sole aquaculture.

Acknowledgements: This work is part of project E!4876 FlatFIRST_1171, supported by EUROSTARS-3 program, and by Portugal and the European Union through ERDF, Algarve 2030, and COMPETE 2030, in the framework of Portugal 2030 and Portuguese national funds from FCT - Foundation for Science and Technology through contracts UID/04326/2025, UID/PRR/04326/2025 and LA/P/0101/2020 (DOI:10.54499/LA/P/0101/2020), and from the operational programmes CRESC Algarve 2020 and COMPETE 2020 through contract EMBRC.PT ALG-01-0145-FEDER-022121 to CCMAR.

Putative biomarkers for the assay of nutritional approaches to mitigate oxidative stress and enhance reproduction in flatfish

C. Marrero-Alemán[1,2,*], E. Fatsini[1], C. Oliveira[1], C. Magalhães-Raposo[1], H. Pereira[2], E. Cabrita[1]

[1]Centro de Ciências do Mar do Algarve (CCMAR/CIMAR-LA), University of Algarve, Campus de Gambelas, Faro, Portugal; [2]GreenCoLab, University of Algarve, Campus de Gambelas, Faro, Portugal; *Presenting author.

Reproductive dysfunction in some flatfish aquaculture remains a significant limitation, necessitating the development of novel biomarkers to assess nutritional strategies. Extracellular Vesicles (EVs) offer a promising tool to assess gamete quality. Given the impact of oxidative stress on sperm function, particularly in marine spermatozoa, antioxidant supplementation in breeders feeds is essential. Microalgae, rich in bioactive antioxidants, may mitigate oxidative damage and enhance male reproductive performance. The primary objective was to contribute to the increase in vitamin E content in *Phaeodactylum tricornutum*, thereby facilitating the development of a functional feed for flatfish breeders. EVs were isolated from the seminal plasma (SP-EVs) of different fish species, turbot (*Scophthalmus maximus*), Atlantic halibut (*Hippoglossus hippoglossus*), and Senegalese sole (*Solea senegalensis*) using SEC, quantified by Tunable Resistive Pulse Sensing (TRPS), and their protein cargo analysed by LC-MS/MS. In parallel, the diatom *P. tricornutum* was cultured with α-tocopherol-acetate (2–10 $mg\cdot L^{-1}$) to enhance vitamin E content, with growth, productivity, and antioxidant enrichment evaluated under controlled conditions. Using the TRPS, the SP-EVs determination ranged from 118–144 nm, with concentrations up to 2.1×10^{10} EVs/mL. The proteomic analysis revealed over 1,500 proteins, 209 in common. Microalgal cultures supplemented with 6 $mg\cdot L^{-1}$ α-tocopherol achieved a twentyfold increase in vitamin E without affecting growth. Our findings support SP-EVs as potential non-invasive biomarkers of sperm quality since some proteins key regulators of sperm motility and maturation were detected. Vitamin E-enriched *P. tricornutum* may improve gamete protection and fertilisation success, offering a sustainable alternative in feed to synthetic vitamin sources. Integrating EV-based biomarkers with antioxidant-rich microalgae offers an eco-innovative strategy to enhance broodstock reproduction in aquaculture.

Acknowledgements: This study received funds from CCMAR strategic program (UIDB/04326/2020, UIDP/04326/2020, LA/P/0101/2020 https://doi.org/10.54499/LA/P/0101/2020) and from FCT to C. Marrero Alemán (2023.00304.BDANA) https://doi.org/10.54499/2023.00304.BDANA. I am grateful to the team from GreenCoLab for their support during this work, with special thanks to Dr Sara Ferreira, Dr Vítor Spínola, Gabriel Bombo and María Lourenço.

Dietary effect of *Tenebrio molitor* on brown trout physiological conditions and behavioural responses

Filipe Chalangala[1,2,3,*], Andreia Domingues[1], Rafael Pechorro[1], Rita Colen[2], Rita Teodósio[2], Pedro R. Almeida[1,4], Sofia Engrola[2], Carlos M. Alexandre[1]

[1]Marine and Environmental Science Centre, University of Évora, Portugal (MARE). [2]Centro de Ciências do Mar do Algarve (CCMAR/CIMAR LA), Faro, Portugal. [3]University of Namibe (UNIMBE/FCN), Faculty of Natural Sciences, Angola. [4]Biology Department, School of Science and Technology, University of Évora, Évora, Portugal; *filipechalangala@gmail.com

Freshwater habitat degradation and fragmentation, exacerbated by climate change, have contributed to the decline of salmonid populations in Europe. Despite several restocking programmes, high post-release mortality of brown trout, *Salmo trutta,* limits their effectiveness. Early feeding with live organisms improves growth, prey capture, immunity and behaviour, increasing post release survival. Insects such as *Tenebrio molitor* emerge as sustainable and nutritious alternatives to increase the success of salmonid restocking. This study evaluated how a feeding regime could enhance the physiological condition of brown trout prior to restocking. Growth, survival, oxidative stress and predatory behaviour were evaluated in trout to assess the impact of feeding regime modulation. Three diets were evaluated, including a commercial diet used as a treatment control (T0) and two experimental formulations supplemented with *T. molitor* larvae (T1 and T2). In T1, the fry was fed on a co-feeding regime throughout the experimental period, while in T2, supplementation with live food was provided only in the sixth week of rearing. *S. trutta* fry 90 (dph) were reared for 10 weeks in a recirculating aquaculture system under optimised environmental conditions. At the end of the experience, a thermal stress test was performed to assess the effect of diets on physiological responses, and a behavioural test was conducted to determine feeding efficiency. Physiological and molecular markers were analysed. The inclusion of *T. molitor* did not affect growth/survival but increased the condition index in trout from T1 and T2, elevated total glutathione levels after thermal stress in T1 trout, and significantly improved capture efficiency and predatory capacity in trout from T1 and T2. Overall, dietary supplementation with *T. molitor* modulated oxidative status and stress response after temperature exposure, improved physical condition and capture efficiency.

Acknowledgements: The authors acknowledge FCT - Foundation for Science and Technology research grant to F. Chalangala (PRT/BD/154729/2022) and A. Domingues (2021. 05644.BD). FCT also supports this study through contracts UIDB/04292/2020 and LA/P/0069/2020, granted to MARE and ARNET, as well as UID/04326/2025, UID/PRR/04326/2025, and LA/P/0101/2020 (DOI:10.54499/LA/P/0101/2020) to CCMAR.

Transcriptomic profiling of muscle in fast- and slow-growing *Sparus aurata*: preliminary results

M. Angelo[1,*], M. Barata[2], M. Rocha[1], P. Pousão-Ferreira[1,2], I.A.L. Silva[1,#], C. Lourenço-Marques[1,2,#]

[1]S2AQUA, Olhão, Portugal; [2]EPPO/IPMA, Olhão, Portugal; [#]These authors contributed equally; *Presenting author.

Introduction

Growth variability in gilthead seabream (*Sparus aurata*) represents a major challenge for Mediterranean aquaculture, as individuals raised under identical conditions often show large differences in growth rate. The underlying molecular causes of this variability remain poorly understood because conventional analyses target only a limited number of genes.

Materials and Methods

A trial using gilthead seabream was conducted at EPPO/IPMA, where growth differences were identified at initial sampling. After sampling, fish were sorted into slow- and fast-growing based on the average batch weight, with density maintained across all tanks. Muscle samples from six fish per condition were collected for differential gene expression (DGE) analysis.

Results

Results show that fast-growing fish outperformed slow-growing ones, demonstrating better growth potential and physiological performance. There was a total of 1190 differentially expressed genes between fast and slow-growing fish, of which 524 were up-regulated and 666 were down-regulated. The most five up-regulated genes were *tmprss9, ctxn3, nlrp12, dpy30* and *znf608*, supporting muscle growth involvement, cytoskeletal organisation, and transcriptional regulation. The top five down-regulated genes were *mfap4, dhys, mdn1, lgals3* and *gpr12,* that are primarily involved in immune response, extracellular matrix remodelling, and energy-intensive processes.

Discussion and Conclusion

This study provides new insights into the molecular mechanisms underlying growth variability in gilthead seabream. The RNA-seq analysis of muscle tissue proved highly effective for identifying genes and regulatory networks directly associated with growth performance. Altogether, these findings highlight a set of candidate genes that can serve as potential biomarkers for growth capacity, offering valuable tools for growth prediction, selective breeding, and the optimisation of aquaculture production strategies.

Acknowledgements

This study was funded by the project NanoPEIXE (ALG-01-0247-FEDER-070032), INOVAQUA (MAR-021.1.3-FEAMPA-00004) and Interface Mission cofinanced by PRR - Plano de Recuperação e Resiliência by the European Union (operation code 01/C05-i02/2022.P148).

Cultivate change for marine forests restoration

Tomás F. Pinheiro*, Tânia R. Pereira, Francisca Sá, Catarina Coutinho, Leonor Castro, Isabel Sousa-Pinto, Isabel Costa, Silvia Chemello

Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR LA), University of Porto, Matosinhos, Portugal; *Presenting author.

Introduction

Marine forests are highly productive ecosystems that provide key ecological services but are increasingly threatened by climate change and coastal pressures such as eutrophication, habitat degradation, and overgrazing. While restoration has traditionally relied on natural recruitment, aquaculture-based methods now offer active and scalable solutions to accelerate recovery. This study explore how aquaculture can optimise settlement, enhance survival, and supply material for large-scale out-planting, by producing propagules and juvenile sporophytes under controlled conditions.

Materials and Methods

A series of laboratory experiments were conducted to evaluate the performance of different cultivation techniques using three kelp species of ecological and commercial importance. For *Laminaria ochroleuca*, the green gravel method was applied under varying cultivation conditions (substrate type, light intensity, and seeding density). Seeding density effects were tested for *Laminaria hyperborea* using synthetic fibres, while *Saccharina latissima* was cultivated on natural fibre meshes (sisal and cotton) to compare their suitability for juvenile development.

Results

For *L. ochroleuca*, juvenile growth was influenced by substrate type, light conditions, and seeding density. *L. hyperborea* exhibited greater juvenile length under low seeding density, although overall surface coverage was reduced. In the *S. latissima* experiment, performance differed between the two natural fibre substrates, with sisal supporting higher juvenile length and larger coverage area compared to cotton.

Discussion and Conclusion

These findings show that aquaculture techniques can greatly enhance the success and scalability of kelp forest restoration. Refining cultivation parameters, such as substrate, light, and seeding density, can provide a reliable source of recruits for out-planting. Further research should focus on protocol optimisation and field validation to assess long-term performance and ecosystem recovery.

Acknowledgements

The authors gratefully acknowledge the support of CIIMAR/CIMAR LA and the Laboratory of Coastal Biodiversity (LBC) for providing research facilities and technical assistance.

Microbiome analysis of water and sediment near salmon cages: seasonal and environmental impacts

I.A. Ferreira[1,*], G. Micallef[2], B. Costas[1], S. Fernández-Boo[1]

[1]Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Portugal;
[2]Gildeskål Forskningsstasjon AS (GIFAS). Inndyr. Norway; *Presenting author.

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 evaluated 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 the 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 (PowerWater and PowerSoil kits, QIAGEN). Quality control was performed by PCR with amplification of the V3–V4 regions of 16S rRNA. Samples were subjected to full-length 16S sequencing by PacBio Revio HiFi reads with 50k reads per sample. Data were filtered, and Amplicon Sequence Variant (ASV) analysis was performed using Qiime2 with the DADA2 package. Statistical analysis of 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. Conversely, reduced diversity and higher dominance in water samples suggested a microbial community dominated by fewer taxa. Statistical analysis of alpha diversity indices revealed significant differences among water samples, highlighting how microbial diversity is influenced by seasonality, depth, and location. Moreover, beta diversity showed greater separation among sediment samples and 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. The increased abundance 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 improvement in aquaculture management practices, especially under changing environmental conditions. This work was funded by the European Union’s Horizon Europe research and innovation program (GA No. 101084651 - project IGNITION).

Challenges in tuning cation-exchange resins as adsorbents of marine paralytic shellfish toxins

Joana F. Leal[1,*], Maria L. S. Cristiano[1,2]

[1]Centro de Ciências do Mar do Algarve (CCMAR/CIMAR LA), University of Algarve, Faro, Portugal;
[2]Departamento de Química e Farmácia, Faculdade de Ciências e Tecnologia, University of Algarve, Faro, Portugal; *Presenting author.

Aquaculture has grown in response to the growing demand for animal protein, and has already surpassed fishing, presenting numerous benefits and a reduced carbon footprint compared to other animal protein production activities. However, this activity is often threatened by external factors, such as infections by protozoan parasites such as *Perkinsus olseni*, in bivalves, and *Amyloodinium ocellatum*, in fish, or contaminations by marine biotoxins produced in harmful algae blooms (HAB). These topics have been tackled in recent years by the OrgMedChem group, at CCMAR.

This contribution will focus primarily on the issue of marine paralytic shellfish toxins (PST), which cause paralytic shellfish poisoning (PSP). Our main goal is the development of chemical methodologies to remove these toxins from marine aquatic systems. For that, cation-exchange resins were prepared and tested for their ability to adsorb biotoxins, over different times. Experiments were conducted using PST-producing microalgae, and mussels *Mytilus edulis*. Toxins were quantified by high-performance liquid chromatography with fluorescence detector (HPLC-FLD). Studies using microalgae cultures revealed a decrease of around 80% in overall toxicity after 48h, and a differentiated adsorption by the resin, depending on the molecular structure of the toxins. This preferential adsorption was also observed in live mussels, although the reduction in overall toxicity was not as great as in cultures. This may be explained by different types of interactions between toxins and resins with the natural constituents of water. Our study highlights the importance of testing methodologies *in vivo* and reveals some aspects that may affect experimental results, contributing to the optimisation of solutions that ensure food safety.

Acknowledgements: This work was funded by national funds through FCT — Fundação para a Ciência e a Tecnologia, I.P. (Portugal), under the Scientific Employment Stimulus — Individual Call (J. F. Leal) — 2022.08181.CEECIND/CP1729/CT0002 (DOI: 10. 54499/2022.08181.CEECIND/CP1729/CT0002), and through contracts UID/04326/2025, UID/PRR/04326/2025 and LA/P/0101/2020 (DOI:10.54499/LA/P/0101/2020) (CCMAR/CIMAR LA). Also, it was funded from the European Commission through the Interreg Atlantic Area under EAPA_0032/2022 (BEAP-MAR). The authors thank G. Bombo and H. Pereira from GreenCoLab (Faro) for their support in the experiments, cultivation and supply of microalgae.

Physiological and genomic insights of the Grooved Carpet Shell clam *Ruditapes decussatus* to temperature and salinity stress events

Simão Correia[1,2], Andreia Cruz[3], Eric Guévélou[3], Helena Barbosa[1], Inês Almeida Ferreira[1], Domitília Matias[1,4], Sergio Fernández-Boo[1,*]

[1]Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR LA), University of Porto, Matosinhos, Portugal. [2]IHPE, CNRS, Ifremer, Univ. Montpellier, Univ. Perpignan, France. [3]Oceano Fresco S.A, Nazaré, Portugal. [4]Department of Sea and Marine Resources, Portuguese Institute for Sea and Atmosphere (IPMA, I.P.), Olhão, Portugal. *Presenting author.

Introduction

The grooved carpet shell clam, *Ruditapes decussatus*, is a bivalve species of significant economic and ecological importance. Native to Europe and North Africa, this species is intensively harvested in Portugal, being the major producer by far. However, coastal communities are increasingly affected by climate change, with rising temperatures and significant shifts in salinity. In this study, the physiological and molecular responses of the European clam were examined when first exposed to high temperature, followed by low salinity while remaining under elevated temperature conditions.

The main objective of the work it was the identification of single nucleotide polymorphisms (SNPs) associated with the tolerance to low salinities to provide new data for a selective breeding program.

Material and methods

Clams (10-12 mm length) were exposed to a high temperature event (>30ºC) for 30 days. After that, no mortality was observed, showing a high resistance increased temperatures and marine heatwaves events. Subsequently, clams were exposed to low salinity (10ppm) at 22 ºC, which led to a progressive increase in mortality, starting on day 5, with 50% mortality observed by day 11. Surviving clams were then returned to normal salinity (33ppm) to assess their capacity for recovery. All clams were genotyped using a novel 15k SNP-Chip array developed for the species. After this, a GWAS (Genome Wide Association Study) was conducted to identified SNPs related with tolerance to salinity.

Results and conclusions

Nine SNPs were significantly different on clams resilient to low salinity stress. After mapping the SNPs in the genome, four of them were present in exons related with homeostasis, cytoskeleton regulation and stress response. Using Colony software, the heritability of the trait was calculated with a value of 0.46, indicating that a selective breeding program for low salinity strain is possible.

Acknowledgements: This work was financed by the project Shellfishboost (Boosting the resilience of European shellfish production against climate change-related challenges through genetic selection) with reference SBEP/009/2023 under the Sustainable Blue Economy Partnership and FCT.

Translating composite fish skin biomarker signatures into actionable metrics of fish health and biosensing technologies

Cláudia Raposo de Magalhães[1,2,*], Bruno Reis[3], Tonka Buha[1,3], Paula Simó-Mirabet[4], André Barreto[2], Paulo Rema[5], Benjamin Costas[3], Ana Teresa Gonçalves[1,2]

[1]SPAROS, Lda, Olhão, Portugal; [2]RIASEARCH, Lda, Murtosa, Portugal; [3]CIIMAR, Universidade do Porto, Portugal; [4]INMAR, Universidad de Cádiz, Cádiz, Spain; [5]Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal; *Presenting author.

The combined pressures of climate change and rising global demand for high-quality animal protein are amplifying the challenges faced by aquaculture, underscoring the need for sustainable practices that safeguard animal welfare. The IGNITION project aims to develop innovative strategies to enhance fish resilience in key aquaculture species, including European seabass (*Dicentrarchus labrax*), Atlantic salmon (*Salmo salar*), and rainbow trout (*Oncorhynchus mykiss*). A main objective is the identification of composite biomarker signatures in the skin interface, capable of accurately reflecting the stress and health status. Through advanced multiomics and machine learning, we are uncovering minimally invasive biomarkers to enable precise fish health assessments and their integration into multiplex biosensors. Messenger and small RNA sequencing, and mass spectrometric analyses of the proteins have been conducted on the skin and skin mucus of the three species, respectively, subjected to different acute and chronic challenges, followed by bioinformatics analyses. Multiomic and physiological data were jointly analysed using machine learning to pinpoint biomarkers for specific surrogate endpoints and derive a composite stress scoring system. Multiomics analyses showed genes, miRNAs and proteins commonly regulated in the skin and skin mucus of all three species in response to both temperature and transport challenges, primarily involved in transcription regulation, response to stress, and immune related pathways. The composite stress score based on the selected biomarker panel accurately distinguished challenged from non-challenged groups. Future work will embed these biomarker signatures into advanced biosensors for continuous, non-invasive monitoring, enabling real-time health surveillance and early intervention in aquaculture systems.

Acknowledgements

Work Co funded by UKRI and by the European Union's Horizon Europe research and innovation programme (GA No 101084651 project IGNITION). T.B. received funds from FCT - Fundação para a Ciência e a Tecnologia through grant 2023.04651.BDANA.

Integrating nutrition and vaccination: exploring the immunomodulatory potential of methionine supplementation in vaccinated rainbow trout

I. Carvalho[1,2,*], F. Bolgenhagen Schoninger[1,3], A. Cunha[1,2,4], D. Peixoto[1,2], F. Brito[1], L. Simões[1], M. Vaz[1,5], A. Stensballe[6], I. Ferreira[1,2], P. Santos[1,2], C. Tafalla[7], M. Machado[1], B. Costas[1,2]

[1]Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Matosinhos, Portugal; [2]Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Porto, Portugal; [3]Faculty of Sciences (FCUP), University of Porto, Porto, Portugal; [4]GreenCoLab, Associação Oceano Verde, University of Algarve, Faro, Portugal; [5]Faculty of Mathematics and Natural Sciences, University of Bergen, Bergen, Norway; [6]Aalborg University, Aalborg, Denmark; [7]Fish Immunology and Pathology Group, National Institute for Agricultural and Food Research and Technology (INIA), Spanish National Research Council (CSIC), Madrid, Spain; *Presenting author.

Enhancing vaccine efficacy remains a central goal in aquaculture health management. Given its recognised immunomodulatory role, dietary methionine supplementation may offer a promising approach. This study assessed the combined effects of methionine supplementation and vaccination on the immune response and resistance of rainbow trout (*Oncorhynchus mykiss*) to *Yersinia ruckeri*. Juvenile trout were fed either a control diet (CTRL, 8.0 mg/g DW) or a methionine-supplemented diet (MET, 17.8 mg/g DW) for 4 weeks. Subsequently, half of the fish per dietary group were dip-vaccinated against *Y. ruckeri*, and after 21 days were intraperitoneally challenged with the same pathogen (5×10^5 CFU/ml) or injected with HBSS. Tissues were sampled at early timepoints post-infection and mortality was monitored for 2 weeks. In non-immunised fish, methionine supplementation appeared to impair antioxidant defences and prolong immune activation, contributing to higher bacterial prevalence and lower survival. Both vaccinated groups survived the challenge and dietary effects were minor. Still, at 24 hpi, MET-fed vaccinated fish showed higher plasma abundance of haemostasis-related proteins, whereas CTRL-fed fish displayed proteomic profiles closer to pre-infection groups. These patterns coincided with a slightly faster bacterial clearance in vaccinated MET-fed fish. Overall, the methionine level tested may have surpassed the optimal range for non-vaccinated fish, while vaccination mitigated these effects. The faster bacterial clearance observed in vaccinated MET-fed fish warrants further investigation under more severe infection conditions to confirm its biological relevance. This work was funded by the European Union's Horizon Europe research and innovation programme (GA No. 101079467, GRINNAQUA) and Fundação para a Ciência e a Tecnologia (FCT), Portugal, through the project IMMUNAA (PTDC/CVT-CVT/7741/2020). IC was supported by FCT (2021.04867.BD).

Sublethal *Photobacterium damselae* subsp. *piscicida* challenge reveals dietary animal protein hydrolysates modulate early immune, oxidative and barrier responses in European Seabass juveniles

Mafalda M. Miranda[1,4,*], Luciano Rodrigues-dos-Santos[1,2,5,6], Micheli Zaminhan-Hassemer[3], Paulo Santos[1], Benjamín Costas[1,2], André Almeida[5,6], Luísa M. P. Valente[1,2], Marta Monteiro[1,2]

[1]CIIMAR/CIMAR-LA, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Matosinhos, Portugal; [2]ICBAS, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Porto, Portugal; [3]Universidade de Moji das Cruzes, São Paulo, Brasil; [4]B2E, Laboratório Colaborativo para a Bioeconomia Azul, Porto, UPTEC Mar – Sala B5 Avenida da Liberdade, s/n 4450-718 Leça da Palmeira, Portugal; [5]ETSA, I.T.S., Indústria Transformadora de Subprodutos, S.A. (ETSA Group); [6]SEBOL, Comércio e Indústria do Sebo, S.A. (ETSA Group); *Presenting author.

Reducing dependence on marine-derived ingredients like fishmeal, while maintaining fish health and performance, is a priority for aquaculture sustainable development. In carnivorous species such as European sea bass (*Dicentrarchus labrax; ESB*), pasteurellosis, caused by *Photobacterium damselae* subsp. *piscicida* (Phdp) is responsible for huge economic losses. Functional feed ingredients like protein hydrolysates are of particular interest, as they provide bioactive peptides with immunostimulant and gut-protective properties, improving disease resilience. This study evaluated the response of ESB to a Phdp challenge after feeding different animal by-product protein hydrolysates incorporated into plant-based diets. Fish were fed with four balanced experimental diets for 89 days: a plant-based control (CTRL) and three diets including three protein hydrolysates (SHARK, FISH and SWINE) at the expense of fishmeal protein. After the feeding trial, fish were intraperitoneally injected with Phdp and sampled at 0, 4, and 24 hours post-infection (hpi) for hepatic oxidative stress, plasma biochemical profile and gene expression analyses related to intestinal integrity and local and systemic immune responses. All diets were well accepted and supported similar cumulative survival rates (80% at 8 days post-infection). The SHARK diet enhanced antioxidant activity, decreasing hepatic lipid peroxidation (LPO) 24 hpi compared to CTRL. Conversely, the SWINE diet had higher GR activity compared to CTRL, without improvement of the oxidative status. SHARK-fed fish maintained stable glycemia compared to other groups but downregulated intestinal integrity markers (e.g. cldn12), suggesting fish are more susceptible to secondary infection. The FISH group had the lowest plasma total protein and globulin levels, and higher hepatic LPO than SWINE and SHARK, indicating a weaker oxidative and metabolic response to infection. Overall, SWINE improved oxidative stress responses in infected fish, without hindering local immune and intestinal barrier highlighting its potential as a functional, sustainable alternative protein source that supports the circular bioeconomy.

Acknowledgements: This work was partially funded by the Blue Bioeconomy Innovation Pact (C644915664-00000026) and supported by the Portuguese Foundation for Science and Technology (FCT) through a grant to Luciano Rodrigues-dos-Santos (2023.00947.BDANA).

A bioengineered outer membrane vesicles platform for multivalent fish vaccines

Inês Loureiro*, Nuno M. S. dos Santos, Ana do Vale

Fish Immunology and Vaccinology Group. i3S- Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal; *Presenting author.

Vaccination is crucial for controlling infectious outbreaks and reducing antibiotic use in aquaculture. Existing vaccines often provide incomplete protection. As a result, infectious diseases continue to cause major economic losses, highlighting the need for innovative vaccination strategies. Outer membrane vesicles (OMVs) are nanosized particles released by Gram (-) bacteria. They are promising vaccine candidates due to their immunostimulatory content, stability, and intrinsic adjuvanticity. OMVs can induce immune responses and can be bioengineered to remove toxic elements and incorporate antigens from multiple pathogens, enabling multivalent vaccine design. Although OMVs are already used in licensed human vaccines, their application in fish vaccination remains largely unexplored. Extending OMV-based vaccine technology to aquaculture, particularly for oral delivery, would represent a major advance. Recently, we showed that *Photobacterium damselae* subsp. *piscicida* MT1451 pathogen releases high numbers of OMVs. Their analysis revealed the presence of several virulence factors, including the plasmid-encoded AIP56 toxin, making them lethal to European sea bass, and unsuitable for vaccination. However, we generated a plasmid-cured strain and showed that the AIP56-negative OMVs produced by this strain, administered i.p. with no adjuvant to sea bass, provided significant protection (35–38% RPS) against experimental *Phdp* infection. In this work, we further enhanced the protective efficacy of these OMVs by deleting an additional toxin targeting macrophages and developed methodologies for incorporating heterologous antigens into the detoxified OMVs. For efficient incorporation, mature antigens were expressed in the detoxified *Phdp* strain fused to a Sec signal peptide for periplasmic targeting, under control of a strong promoter to maximise expression. Using this approach, we successfully incorporated antigens from a hyper-virulent *Phdp* strain, *Tenacibaculum maritimum*, Nervous Necrosis Virus, and *Vibrio harveyi*. I.p. immunisation of sea bass with OMVs loaded with antigens from the hyper-virulent *Phdp* strain elicited antibody responses against the incorporated antigens, demonstrating the feasibility of using these OMVs as a platform for developing multivalent fish vaccines. Future studies should investigate their potential for oral immunisation. Creating effective, orally administered, multivalent fish vaccines would enable large-scale low-stress vaccination, improving fish health and promoting more sustainable aquaculture practices.

Acknowledgements: This work received funding from the EU Horizon Europe research and innovation program (GA No. 101084651 - project IGNITION) and ESCMID.

Assessment of *Salicornia ramosissima* bioactives for controlling *bacterial* infections in European seabass through *in vitro* and *in vivo* approaches

Aminur Rashid[1,2,3,*], Marina Machado[1], Sergio Fernández-Boo[1], Mette Hedegaard Thomsen[4], Henrik Tribler[5], Malthe Fredsgaard[5], Benjamín Costas[1]

[1]Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR LA), Matosinhos, Portugal. [2]Faculty of Sciences, University of Porto, Porto, Portugal. [3]Department of Aquaculture, Sylhet Agricultural University, Sylhet, Bangladesh. [4]AAU Energy, Aalborg University, Esbjerg, Denmark. [5]Halorefine, Esbjerg, Denmark. *Presenting author.

The halophyte *Salicornia ramosissima* has been recognised as a potential source of phytobiotics. Recent studies revealed that dietary inclusion of non-edible residual fraction could induce transient anti-inflammatory and antioxidant responses in fish and shrimp. However, information on the antibacterial activity of these plant's phytobiotics is still lacking. This study aimed to screen *S. ramosissima* extracts for bactericidal and bacteriostatic activities against several fish pathogens and thereafter evaluate their potential for inclusion in European seabass (*Dicentrarchus labrax*) functional diets. The bactericidal and bacteriostatic activities of seven fibre extracts (Ext1, Ext2, Ext3, XAD, XAD3, FERM, FRAC3) were tested at 0.125, 0.25, and 0.5 mg/mL against *Aeromonas hydrophila, Edwardsiella piscicida, Photobacterium damselae piscicida, Tenacibaculum maritimum, Vibrio anguillarum, Vibrio parahaemolyticus*, and *Yersinia ruckeri.* Best promising extracts were selected according to data from *in vitro* trials, and experimental diets were designed and tested for its prophylactic potential in juvenile European seabass (23.56 ± 0.65 g). Diets were designed to incorporate Ext3 and XAD extracts at two levels (0.1 and 0.5% of feed; EXT3_0.5, EXT3_0.1, XAD0.1, XAD0.5). A 4-week feeding trial was performed in triplicates and followed by a 10-day challenge against *P. damselae piscicida*. Screening revealed that XAD3 and FERM showed significant bactericidal activities (17.63-18.65% inhibition, $p<0.05$) against *T. maritimum* while Ext1, 2 and 3 showed significant bactericidal activities (33.96–40.55% inhibition, $p<0.05$) at 0.5 mg/mL against *Y. ruckeri*. Ext1 demonstrated significant bacteriostatic effect against *P. damselae piscicida* (28.08% growth inhibition at 0.5 mg/mL) while XAD3 showed significant bacteriostatic effect (35.77%) against *V. anguillarum*. At the end of the feeding trial, no significant differences were observed in weight gain, FCR, survival and haematological profile of fish after a 4 weeks feeding period with the experimental diets. However, following bacterial challenge, EXT3_0.1 and XAD_0.1 showed significant difference in survival, 33.33% and 36.11% respectively, compared to CTRL 16.67% ($p<0.05$). In conclusion**,** *S. ramosissima* extracts could be a sustainable source of antibacterial compounds and its inclusion in fish feeds could improve the disease resistance. **Acknowledgements:** This work received funding from the European Union's Horizon Europe research and innovation program (GA No. 101084651 - project IGNITION) and UKRI.

Characterisation of *Tenacibaculum maritimum* infection on juvenile seabream

R. Carrilho[1,2,*], M. Moreira[3], M. Livramento[4], F. Soares[4], P. Rodrigues[1,2], M. Cerqueira[1]

[1]Centro de Ciências do Mar (CCMAR/CIMAR LA), Campus de Gambelas, Universidade do Algarve, Faro, Portugal; [2]Universidade do Algarve, Campus de Gambelas, Faro, Portugal; [3]Bio1OneHealth, UALG Tec Start Bloco B1, Campus de Gambelas, Faro, Portugal; [4]IPMA – EPPO, Olhão, Portugal ; *rvcarrilho@ualg.pt

Introduction: Tenacibaculosis, caused by *Tenacibaculum maritimum*, is a widespread bacterial disease affecting numerous marine species, however, its specific effects on certain hosts remain poorly understood. Consequently, the present study investigates the impact of *T. maritimum* infection on juvenile gilthead seabream (*Sparus aurata*), a key species in Mediterranean aquaculture, where outbreaks of tenacibaculosis are becoming increasingly frequent.

Materials and Methods: Twelve gilthead seabream juveniles (21.17 ± 4.57 g) were allocated per tank (n=3 tanks, 36 fish total) and bath-challenged with *T. maritimum* (CECT4276) at a concentration of 1 x 10^5 CFU/ml, or PBS, in 40 L rectangular tanks, in triplicate, for 28 h. Subsequently, the tank water was completely replaced three times. At five sampling times (0, 18h, 24h, 48h, 72h), two fish per tank (n = 6 per treatment) were sampled for mucus, blood, plasma, liver and spleen collection for infection characterisation and proteomic analysis.

Results: Proteomic analysis showed that the infected group exhibited the highest number of differentially abundant proteins (DAPs) across sampling times, with 133 proteins compared to 27 in controls. Furthermore, seven proteins changed over time and between controls and infected groups. Lactate content in infected fish showed an increasing tendency through time and was significantly higher at 72h post-infection compared to controls.

Discussion and Conclusion: The higher number of DAPs detected in infected fish suggests substantial metabolic and immune reprogramming in response to infection. The seven proteins that varied both over time and differed between treatments likely play key roles in host defence mechanisms and warrant further investigation. Additionally, elevated lactate levels indicate metabolic stress and a shift toward anaerobic metabolism. Overall, *T. maritimum* infection induces significant molecular and metabolic alterations associated with the host's immune defence response.

Acknowledgements: This work was financed by project BIOIMMUNE (MAR-02.05.01-FEAMP-0052) and SAUDE&AQUA (MAR-02.05.01-FEAMP-0009) and received Portuguese national funds from FCT through contracts UID/04326/2025, UID/PRR/04326/2025 and LA/P/0101/2020 (DOI: 10.54499/LA/P/0101/2020). Raquel Carrilho acknowledges a FCT PhD scholarship, Refª 2021.06786.BD.

Methionine supplementation enhances viral haemorrhagic septicemia virus resistance in rainbow trout (*Oncorhynchus mykiss*): insights into the role of viperin

Mariana Vaz[1,2,*], Gonçalo Espregueira Themudo[1], Alexandra Florea[3], Inês Carvalho[1], Felipe Bolgenhagen Schöninger[1], Patricia Díaz-Rosales[4], Carolina Tafalla[4], Tim Bean[3], Diego Robledo[3], Benjamín Costas[1,5], Marina Machado[1]

[1]Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR-LA), University of Porto, Portugal; [2]Faculty of Mathematics and Natural Sciences, University of Bergen, Norway; [3]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, UK; [4]Fish Immunology and Pathology Group, Biotechnology Department, National Institute for Agricultural and Food Research and Technology (INIA), Spanish National Research Council (CSIC), Madrid, Spain; [5]Instituto de Ciências Biomédicas Abel Salazar (ICBAS-UP), University of Porto, Portugal. *Presenting author.

European aquaculture of rainbow trout faces substantial losses due to viral haemorrhagic septicaemia virus (VHSV). While methionine supplementation enhances fish antibacterial resistance, its influence on antiviral immunity remains poorly understood. This study investigated the effects of dietary methionine supplementation on the immune response of rainbow trout (*Oncorhynchus mykiss*), under viral challenge. To do so, juvenile rainbow trout (5.9 ± 0.9 g) were fed either a control diet (CTRL) or a methionine-supplemented diet (MET; twice the species' requirement) for four weeks. Fish were then sampled, and remaining individuals were bath-challenged with VHSV (10^5 $TCID_{50}$ mL^{-1}) and sampled over a time course. Viral load quantification across multiple tissues revealed a peak of infection at 72 h post-challenge, with gills and skin acting as primary entry points where the virus was first detected as early as 24 h. Transcriptomic analysis (RNA-seq) of these tissues showed that MET-fed fish exhibited significant upregulation of immune-related pathways, particularly in the gills. In contrast, the skin displayed a more antiviral-specific transcriptional profile, characterised by enrichment of the type I interferon signalling pathway—a central component of innate antiviral defence. Among the most significantly differentially expressed genes (DEGs) were several associated with antiviral responses. Notably, viperin (coded as vig1/RASD2), a key antiviral effector, was significantly upregulated in the gills of MET-fed fish, as confirmed by quantitative PCR, supporting a modulatory role of methionine in enhancing host antiviral defence. To further elucidate viperin's role in the trout antiviral response, an in vitro trial was conducted using the rainbow trout spleen cell line (RTS11). The viperin gene was knocked out via CRISPR–Cas9, and cells were subsequently stimulated with inactivated VHSV to assess transcriptional responses. RNA sequencing data from two CRISPR guides (achieving ~50% knockout efficiency) are currently under analysis. This work was funded by the European Union's Horizon Europe research and innovation programme (Grant Agreement No. 101079467, project GRINNAQUA).

POSTERS PRESENTATIONS

Dietary inclusion of black soldier fly larvae oil reduces the omega-6/omega-3 ratio in the flesh of European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) juveniles

Daniel Acebes[1,2,*], Paula Canada[1], Tiago Sá[1], Andreia F. Silva[1], Sara Magalhães[3], Daniel Murta[4], Luisa M.P. Valente[1,2], Ana Basto[1]

[1]CIIMAR/CIMAR LA – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, 4450-208 Matosinhos, Portugal; [2]Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Porto, Portugal; [3]Sorgal – Sociedade de Óleos e Rações S.A., S. João de Ovar, Portugal; [4]EntoGreen®, Ingredient Odyssey S.A., Santarém, Portugal; *Presenting author.

The inclusion of terrestrial fats (vegetable and animal) in aquafeeds has increased the omega-6/omega-3 ratio in fish flesh, thereby reducing its nutritional value for human consumption. In this context, this study aimed to evaluate the potential of black soldier fly larvae oil (BSFLO), which contains markedly less omega-6 than conventional terrestrial lipid sources, in diets for European seabass (ESB) and gilthead seabream (GSB). Two parallel feeding trials were conducted with juvenile fish (initial body weight: 11 g for ESB; 18 g for GSB) maintained at 23°C. Four isoenergetic diets (23 kJ g/DM) with identical protein basis and 6% fish oil were formulated. The control diet (CTRL) contained 5% poultry oil (PO), while three experimental diets included 1.7%, 3.4%, and 5.2% BSFLO replacing PO (BSFLO1.7, BSFLO 3.4 and BSFLO5.2). Fish were hand-fed to apparent satiety three times daily until their body weight had quadrupled. All diets were tested in triplicate. In ESB, voluntary feed intake was not affected by dietary inclusion of BSFLO, but feed conversion ratio was lower in fish fed BSFLO5.2 that displayed the highest final body weight at the end of trial. A similar trend occurred in GSB, but without statistical significance. Somatic indexes were unchanged in ESB fed BSFLO diets, whereas both hepatosomatic and viscerosomatic indexes decreased in GSB fed BSFLO5.2. This alterations in somatic indexes of GSB suggest metabolic alterations, but further clarification warrant additional investigation. In both species, muscle omega-6/omega-3 ratio was lower in fish fed the BSFLO5.2 diet. This diet has also led to decreased lipid peroxidation and enhanced antioxidant capacity in the muscle of both species. Overall, the inclusion of 5.2% BSFLO appears to be a promising alternative to PO in diets for ESB and GSB, improving growth and feed efficiency, but also muscle antioxidant capacity and nutritional value.

Acknowledgements: This work has been supported by InsectERA Mobilising Agenda (PRR - InsectERA - The ERA of the insect industry), funded by Portugal and European Union - NextGenerationEU, with the identifier C644917393-00000032 and Daniel Acebes is supported by Fundação para a Ciência e Tecnologia (FCT), I.P., through a PhD. grant (2024.02126.BDANA). Strategic Projects UIDB/04423/2020 and UIDP/04423/

Advancing macroalgae biobanking through cryopreservation and molecular identification

Morgana Angelo[1,*], Iris A.L. Silva[1], Inês Freitas[1], Raquel Quintã[1], Pedro Pousão-Ferreira[1,2], Cátia Lourenço-Marques[1,2]

[1] S2AQUAcoLAB, Olhão, Portugal; [2] EPPO/IPMA, Olhão, Portugal; *Presenting author.

Introduction

Biobanking is a key tool for the long-term conservation of macroalgae, enabling the maintenance of genetic resources for biotechnology, aquaculture, and ecological studies. However, standardised protocols for green macroalgae such as *Ulva* are still scarce.

Methodology

Fresh *Ulva* thalli were cut into discs and placed in cryotubes with sterile seawater. Three concentrations of glycerol were tested (10%, 20%, and 30%) and a seawater-only treatment served as cryo-control, and a non-cryopreserved growth control was maintained with f/2 medium. Samples were frozen in a Mr. Frosty container and stored at -80°C. After one month, cryotubes were thawed, and *Ulva* discs were rinsed in cold HEPES-buffered seawater to remove cryoprotectants. Cultures were maintained at 15°C under 80 µmol photons m^{-2} s^{-1} and a 12:12 h light:dark cycle, with f/2 medium renewed every 3 days.

Results

At day 0, all treatments retained pigmentation; by day 7, the cryo-control and 10% glycerol treatments lost all colour, while higher glycerol concentrations showed partial loss. By day 20, regrowth appeared as disc-like and filamentous structures. Molecular identification confirmed that the control culture and the disc-like regrowth from the 30% glycerol treatment corresponded to *Ulva ohnoi* (100% identity), while the filamentous regrowth was identified as *Ulva flexuosa* (100% identity).

Discussion and Conclusion

The cryopreservation results demonstrate that *Ulva* can regenerate following cryopreservation with glycerol. Molecular identification confirmed that viable tissues corresponded to *Ulva ohnoi*, consistent with the control, while filamentous regrowth identified as *Ulva flexuosa* suggests possible contamination from mixed cultures prior to preservation. These findings emphasise the feasibility of glycerol-based cryopreservation for *Ulva*, highlight the need for thorough culture purification and the importance of molecular tools in assessing post-thaw recovery for species identification.

Acknowledgements: This study had the support of the projects WP9- Portuguese Blue Biobank under the Blue Economy Pact - Project Nº. C644915664-00000026, Interface Mission cofinanced by PRR - Plano de Recuperação e Resiliência by the European Union (operation code 01/C05-i02/2022.P148) and SAUDE&AQUA II (MAR-021.1.3-FEAMPA-00018).

Nutritional interactions between protein source and lipid level in Senegalese sole

Cláudia Aragão[1,*], Rita Colen[1], Rita Teodósio[1], Jorge Dias[2], Luís E.C. Conceição[2], Laura Ribeiro[3]

[1]Centro de Ciências do Mar do Algarve (CCMAR/CIMAR LA), 8005-139 Faro; [2]SPAROS Lda., 8700-221 Olhão;
[3]Faculdade de Ciências e Tecnologia, Universidade do Algarve, 8005-139 Faro; *caragao@ualg.pt

Introduction

Studies on Senegalese sole (*Solea senegalensis*) have shown that this species tolerates plant-based diets relatively well and prefers low dietary lipid levels. This study aimed to evaluate the impact of dietary protein sources and lipid inclusion levels on Senegalese sole performance, and to assess whether taurine supplementation to plant-based diets may enhance lipid utilisation.

Materials and Methods

Four isoproteic (55%) diets were tested in Senegalese sole juveniles (13.6 g). The basal formulations included an FM diet, containing a high proportion of marine ingredients, and a PP diet, where plant protein sources replaced 85% of marine ingredients. The latest was supplemented with taurine. Each basal diet was formulated with two lipid inclusion levels: 8.6% (LL) or 16.8% (HF). All diets (FMLF, FMHF, PPLF, PPHF) were tested in triplicate over six weeks.

Results

A 2-way ANOVA showed that fish growth was negatively affected by HF diets, whereas survival decreased with PP diets. A significant interaction between protein source and lipid level was found for feed intake, which was significantly reduced in fish fed the PPHF diet, increasing the FCR. Bile acid concentration responded to taurine supplementation but declined with HF diets. Lipid catabolism decreased in fish receiving HF diets, while hepatosomatic index (HSI) increased with both PP and HF diets.

Discussion and Conclusion

Fish fed the PPHF diet produced the lowest biomass due to reduced growth and survival, likely linked to lower feed intake. Bile acid concentration appeared to influence lipid catabolism, and the reduced lipid catabolism associated with high inclusion of plant proteins may explain the higher HSI. Therefore, high dietary lipid levels negatively affect Senegalese sole performance, especially when using plant-based diets, even with taurine supplementation.

Acknowledgements

This study received Portuguese national funds from FCT through contracts UID/04326/2025, UID/PRR/04326/2025 and LA/P/0101/2020 (DOI:10.54499/LA/P/0101/2020).

Management of the reproductive cycle and body growth of purple sea urchins (*Paracentrotus lividus*) through nutrition and environmental conditions manipulation

João Araújo[1], Sandra Ramos-Júdez[2], Ana Gamboa[1], Ana Catarina Carvalho[1,*], Ana Candeias-Mendes[1],
Florbela Soares[1,2], Pedro Pousão-Ferreira[1,2]

[1]Instituto Português do Mar e da Atmosfera, Olhão, Portugal; [2]S2AQUAcoLAB, Olhão Portugal;
[*]Presenting author.

Introduction

The success of *Paracentrotus lividus* aquaculture depends on effective control of growth and reproduction. This study evaluated the effects of temperature, photoperiod, and a glycerol-enriched diet on somatic and gonadal development.

Materials and Methods

Tanks (110 L) were assigned to three environmental regimes (in triplicate): natural conditions (initial/final photoperiod: ~9h / ~13h), heated seawater (20.9 ± 0.2 °C) with advanced photoperiod (~12h → ~15h), and ambient temperature with the same advanced photoperiod. Within each regime, urchins received either a control diet or a glycerol-enriched diet (GLY, 7.5%). Test diameter was measured at the start, day 47, and day 76. Gonads were sampled at start and end to determine gonadosomatic index (GSI) and histological maturation stage.

Results

Specific growth rate (SGR % day^{-1}) did not differ significantly among treatments, despite higher mean values under heated seawater. However, elevated temperature and advanced photoperiod promoted faster gonad maturation. GSI was significantly higher in animals under natural conditions. The GLY diet showed moderate enhancement of gonad development, especially in warm conditions.

Discussion and Conclusion

Environmental conditions had a stronger influence on gonadal development than diet. The glycerol-enriched diet may have contributed energy preferentially to reproduction over somatic growth. These results highlight the potential of combining environmental and nutritional manipulation to optimise reproductive control in *P. lividus* aquaculture.

Acknowledgements

INOVAQUA (MAR-021.1.3-FEAMPA-00004) and AQUAFISH 0.0 (Interreg Atlantic Area Programme).

Sustainability tools for Mediterranean aquaculture

Ravi Araújo[1,*], Ana Catarina Matias[1], Laura Ribeiro[2], Jorge Dias[3], Tatiana Poletto[3], Juan Martos-Sitcha[4], Anyell Caderno[4], Amparo Gonçalves[5], Pedro Pousão-Ferreira[1]

[1]Estação Piloto de Piscicultura de Olhão (EPPO), Portuguese Institute of the Sea and Atmosphere (IPMA), Av. do Parque Natural da Ria Formosa s/n, 8700-194 Olhão, Portugal; [2]Faculty of Sciences and Technology, University of Algarve, Campus de Gambelas, 8005-139, Faro, Portugal; [3]Sparos Lda, Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal; [4]Department of Biology, Faculty of Marine and Environmental Sciences, Instituto Universitario de Investigación Marina (INMAR), Campus de Excelencia Internacional del Mar (CEI_MAR), University of Cádiz, 11510 Puerto Real, Cádiz, Spain; [5]Division of Aquaculture, Valorization and Bioprospecting, Department for the Sea and Marine Resources, Portuguese Institute for the Sea and Atmosphere (IPMA, I.P.), Av. Dr. Alfredo Magalhães Ramalho, 6, 1495-165 Lisboa, Portugal; *Presenting author.

Introduction: By 2030, aquaculture is expected to produce around 106 million tons of seafood, nearly 60% of global supply. The FAO's *Blue Transformation* promotes renewable energy, stakeholder collaboration and alignment with the United Nations Sustainable Development Goals (UN SDGs) to ensure sustainable growth. Innovative systems like Integrated Multi-Trophic Aquaculture (IMTA) and Recirculating Aquaculture Systems (RAS) are making aquaculture sector more efficient, circular, and environmentally responsible.

Material and Methods: Three experimental trials were carried out to develop and evaluate sustainability tools suited to Mediterranean aquaculture. The studies focused on key factors affecting sustainability, including environmental conditions, production systems, and nutrition. They examined temperature fluctuations, compared IMTA and polyculture approaches in earthen ponds, and tested binders for meagre feed for RAS.

Results: Under constant temperature, gilthead seabream achieved better feed conversion but showed higher cortisol levels, indicating increased stress than under variable conditions. The IMTA system improved oxygen stability (around 4.6% fluctuation in spring) and diversified production with fish and oysters, enhancing both economic value and ecological balance. In RAS for meagre, binder performance varied, with energy values between 82.5-88.1 kJ g^{-1} and protein digestibility from 89.3% to 92%.

Discussion and Conclusion: The trials highlight practical strategies to advance sustainable aquaculture in the Mediterranean. The use of solar heating, IMTA systems, and optimised feed binders enhances production efficiency, environmental performance, and diversification, contributing to progress toward the UN SDGs.

Acknowledgements: Interreg Euro-MED AZA4ICE (Euro-MED0200819) and INOVAQUA (MAR-021.1.3-FEAMPA-00004).

Fish brain-gut axis – brain responsiveness to intestinal inflammation

Rita Azeredo[1,*], Diogo Peixoto[1], Carla Teixeira[2], Paulo Rema[3], Jorge Dias[4], Benjamín Costas[1]

[1]CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Matosinhos, Portugal; [2]Riasearch, Lda., Murtosa, Portugal; [3]Departamento de Zootecnia, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal; [4]SPAROS Lda., Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal; *Presenting author.

Introduction

In fish, the intricate processes that are activated and/or regulate the brain-gut axis during intestinal inflammation are poorly documented. This preliminary approach intends to evaluate the outreach of a peripheral inflammatory process (enteritis) in activating central neuroendocrine mechanisms, focusing on gene expression changing patterns at both ends of the brain-gut axis.

Material and Methods

Juvenile rainbow trout (RT, 70.08 ± 12.15 g), *Oncorhynchus mykiss*, were either fed a commercial diet or the same diet supplemented with 1% dextran sodium sulfate (DSS) for 5 or 15 days. Gene expression was evaluated in the gut, hypothalamus and hypophysis. RNA-seq was performed in gut samples.

Results

Several immune- and neuroendocrine related genes (e.g. *cd3, cd8, ahr, il1β, serta, claudn, crh*) were upregulated in the gut of RT fed DSS-supplemented diets for 15 days. A 15-days feeding period more significantly affected gut transcriptomics (120 DEGs), with the biological function digestion being enriched by downregulated genes, while immune-related terms were enriched by upregulated genes.

Discussion

The upregulation of intestinal immune markers confirms a successful DSS-induced inflammation. Serotonin transporter expression also increased, suggesting peripheral serotonergic alterations that may trigger central neuroendocrine responses. Correspondingly, hypothalamic CRH expression rose, indicating brain sensitivity to intestinal inflammation. RNA sequencing of fish fed the DSS-supplemented diet for 15 days revealed immune activation and impaired digestion. Overall, DSS dietary administration appears to be a promising enteritis model in RT, with gut inflammation influencing brain gene expression despite limited serotonergic changes in sequencing data.

Acknowledgements

This work was supported by CIIMAR internal funding under the framework of “CIIMAR SEED projects”. RA, DP and BC, and were supported by FCT, Portugal (2022.03248.CEECIND**,** UI/BD/150900/2021 and 2020.00290.CEECIND, respectively).

Effects of protein hydrolysates on microdiet optimisation for turbot (*Scophthalmus maximus*) larvae

R. Barruncho[1,2*], A. Sharif[1], R. Teodósio[1], R. Colen[1], W. Pinto[3], A. Santos[3], C. Castro[4], R. Serradeiro[4], L.E.C. Conceição[3], S. Engrola[1]

[1]Centro de Ciências do Mar do Algarve (CCMAR/CIMAR-LA), Faro, Portugal. [2]Universidade do Algarve, Faro, Portugal. [3]SPAROS Lda., Olhão, Portugal. [4]FLATLANTIC - ACTIVIDADES PISCÍCOLAS, S.A., Praia de Mira, Portugal; *a64291@ualg.pt

Turbot (*Scophthalmus maximus*) is a high-value flatfish species widely farmed in Europe, particularly in Portugal and Spain. However, larval rearing remains a major bottleneck due to high mortality rates and nutritional constraints. Protein hydrolysates (PH) are functional dietary ingredients known to enhance digestibility, nutrient absorption, and larval robustness. This study evaluated how the molecular weight of PH influences key performance indicators in turbot larvae. Larvae were reared at CCMAR's experimental facilities under controlled Recirculating Aquaculture System conditions. Two microdiets were tested, containing either high molecular weight (High) or low molecular weight (Low) peptides at equal inclusion levels. Larvae were randomly distributed into six replicate tanks (n=3 per treatment) and reared between 3 and 27 days after hatching (DAH). Growth performance (dry weight, total length, condition factor, relative growth rate) and survival were analysed using t-tests or Mann–Whitney tests ($p < 0.05$). At 17 DAH, larvae fed the Low diet showed significantly higher dry weight and improved condition factor compared to those fed the High diet. However, no significant differences were observed in total length, relative growth rate, or survival at later stages (27 DAH). Mean survival was 9.17% (High) and 7.53% (Low), with no significant differences between treatments. These findings highlight the importance of stage-specific dietary formulations. Tailoring microdiets to larval nutritional physiology may enhance growth consistency and survival, contributing to more sustainable and efficient turbot aquaculture.

Acknowledgements

This work is part of project E!4876 FlatFIRST_1171, supported by EUROSTARS-3 program, and by Portugal and the European Union through ERDF, Algarve 2030, and COMPETE 2030, in the framework of Portugal 2030 and Portuguese national funds from FCT - Foundation for Science and Technology through contracts UID/04326/2025, UID/PRR/04326/2025 and LA/P/0101/2020 (DOI:10.54499/LA/P/0101/2020), and from the operational programmes CRESC Algarve 2020 and COMPETE 2020 through contract EMBRC.PT ALG-01-0145-FEDER-022121 to CCMAR.

In vitro characterisation of yellow mealworm and black soldier fly larvae oils: insights into their functional potential for aquafeed

Ana Basto[1,*], Andreia F. Silva[1], Daniel Acebes[1,2], Daniel Murta[3], Luisa M.P. Valente[1,2]

[1]CIIMAR/CIMAR LA, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal; [2]Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Porto, Portugal; [3]EntoGreen®, Ingredient Odyssey S.A., Santarém, Portugal; [*]Presenting author.

Extensive research has investigated the potential of yellow mealworm (YM; T*enebrio molitor*) and black soldier fly (BSF; *Hermetia illucens*) larvae meals as alternative protein sources for aquafeeds. However, their lipid fraction has remained largely overlooked. Advancing the understanding of its chemical composition and biological activities is crucial to unlocking its potential applications in fish nutrition. This study evaluated the fatty acid (FA) profile, tocopherol and polyphenolic compounds, lipophilic pigments, *in vitro* antioxidant activity, and antibacterial properties of YM and BSF larvae oils (-LO). The results revealed that YMLO was mainly composed of oleic acid (38% of oil) and linoleic acid (29% of oil), whereas BSFLO contained similar content of lauric and oleic acids (28% of oil). Both insect oils contained α-, β-, γ- and δ-tocopherols, with the total tocopherol content being higher in BSFLO than in YMLO (195 vs 128 µg/mL oil). The predominant tocopherol in both oils was β-tocopherol (100 µg/mL YMLO and 81 µg/mL BSFLO). None of the oils contained polyphenolic compounds such as tyrosol or hydroxytyrosol, but qualitative analysis indicated the presence of chlorophyll derivatives in both samples. Following *in vitro* evaluation of the effects of YMLO and BSFLO on the growth dynamics of nine fish pathogenic bacterial strains over 24 hours, both insect oils significantly reduced bacterial proliferation compared with the control group. YMLO exhibited significant inhibition of *Aeromonas salmonicida*, *Photobacterium damselae* subsp. *damselae*, *Tenacibaculum maritimum*, and *Vibrio vulnificus*, whereas BSFLO showed stronger antibacterial activity against *Aeromonas hydrophila*, *Listonella anguillarum*, *Vibrio harveyi*, and *Vibrio parahaemolyticus* relative to the control. YMLO and BSFLO demonstrated potential as functional ingredients for aquafeeds, offering valuable FAs, antioxidants, and antibacterial activity. Yet, future research should assess their *in vivo* effects and determine optimal inclusion levels to support fish growth and welfare.

Acknowledgements

This work has been supported by InsectERA Mobilising Agenda (PRR - InsectERA - The ERA of the insect industry), funded by Portugal and European Union - NextGenerationEU, with the identifier C644917393-00000032.

Automation of live feed identification and counting

Maxim Batalin, Zoltan Gorocs, Jessica Keszy, Dhruvin Naik, Maria Arredondo*, Lin Li, Andrew Jones

Lucendi, Inc., USA; *Presenting author

Introduction: Automation of live feed monitoring remains one of the least developed aspects of hatchery management despite advances in feeding and water control systems. Many hatcheries still rely on manual counting and characterisation of live feeds such as copepods, rotifers, and Artemia, which limits scalability, accuracy, and standardisation. Existing imaging solutions, such as lens-based flow cytometers, are costly, labour-intensive, and restricted to narrow organism size ranges. The objective of this study was to develop and validate an automated system for accurate, high-throughput quantification and identification of live feed organisms.

Materials and Methods: Lucendi developed the Aqusens platform, a compact holographic imaging system that operates without lenses and integrates a built-in pump capable of processing 100 mL/h to 5 L/h. The system illuminates flowing water samples with light pulses, capturing diffraction patterns that are reconstructed into colour intensity and phase images. The hardware requires no calibration or maintenance, enabling automated operation in laboratory, field, or hatchery settings. Captured images are analysed using a custom-trained AI model for organism detection, classification, and counting.

Results: The system was trained to identify and count *Parvocalanus crassirostris* eggs and N1/N2 nauplii using a manually labelled image library. Aqusens AI achieved 98% and 99% accuracy, respectively. It successfully excluded organisms in later development stages and contaminants such as rotifers and algae. Automated counts differed by less than 10% from manual counts by trained technicians, while providing far greater consistency and eliminating counting bias.

Discussion and Conclusion: Automated holographic imaging combined with AI analysis provides a robust, high-throughput alternative to manual microscopy for live feed monitoring. By eliminating human bias and increasing standardisation, the Aqusens platform enables continuous, real-time assessment of live feed cultures, improving hatchery efficiency and reducing operational costs. Beyond hatchery operations, this technology can be adapted for aquaculture R&D and environmental monitoring of planktonic organisms, including the early detection of Harmful Algal Blooms (HABs) and pathogens, which are critical for preventing culture losses and ensuring water quality.

Acknowledgements: This work was supported by Lucendi, Inc., with contributions from the Aqusens development and AI research teams.

Exploring the composition and activity of *Ulva*-associated microbial communities in natural and aquaculture environments

Britanny Beaudry*, Aschwin Hillebrand Engelen, Tânia Aires

Centro de Ciências do Mar do Algarve (CCMAR), University of Algarve, Faro, Portugal; *Presenting author.

Portugal has an extensive aquaculture sector and a rich macroalgae diversity which offers a unique setting to investigate host-microbe interactions in natural and cultivated environments. Within the framework of the AquaDiversify project, this study aims to characterise the composition and activity of *Ulva*-associated microbial communities in both natural and aquaculture settings. A further objective is to compare active (RNA-based) versus total (DNA-based) microbiomes across the day-night cycles to assess potential diel shifts in microbial function.

Samples of *Ulva* spp. will be collected from both the natural lagoon, Ria do Alvor, and the aquaculture facility, Aqualvor. DNA will be extracted for host barcoding and microbial community profiling, and DNA/RNA co-extraction (ZymoBIOMICS DNA/RNA miniprep kit) will be performed to assess transcriptional activity over day-night cycles. Sequencing data will be analysed through reference-based assembly (BLAST) to identify taxonomic and functional profiles of *Ulva*-associated microbiota.

Preliminary results are expected to reveal differences in microbial community composition between natural and cultivated sea lettuce populations, as well as diel variations in active microbial taxa. We also predict indications of environmental adaptation or aquaculture-induced shifts in microbial metabolism.

By integrating metagenomic and metatranscriptomic approaches, this study will provide new insights into how aquaculture practices and daily light cycles shape the structure and function of *Ulva*-associated microbiomes. Understanding these dynamics will contribute to optimising algal cultivation and improving sustainability within the Portuguese aquaculture sector.

This work is supported by the AquaDiversify project and the Centro de Ciências do Mar do Algarve (CCMAR). The authors thank the Aqualvor aquaculture facility for field support.

Algae-supplemented diets boost oxidative resilience in gilthead seabream

João Bolinhas[1,2,*], Rita Teodósio[1], Ana Teresa Gonçalves[3,4], Florbela Soares[5,6], Pedro Pousão-Ferreira[5,6], Sara Magalhães[7], Tiago Aires[7], Sofia Engrola[1]

[1]Centro de Ciências do Mar do Algarve (CCMAR/CIMAR LA), Faro, Portugal; [2]Universidade do Algarve, Faro, Portugal; [3]SPAROS Lda., Olhão, Portugal; [4]GreenCoLab, Faro, Portugal; [5]IPMA/EPPO, Olhão, Portugal; [6]S2AQUAcoLAB, Olhão, Portugal; [7]Sorgal, S.A., Ovar, Portugal; *a67915@ualg.pt

In aquaculture, fish are exposed to stressors such as transport, handling and crowding, which can compromise cellular function and lead to oxidative stress. Dietary supplementation with bioactive compounds may enhance fish resilience. This study evaluated the effects of an algae-supplemented diet on the antioxidant response of juvenile gilthead seabream after a short feeding trial and subsequent challenge. Fish (± 5.7 g) were fed for seven days with a commercial-like diet (CTRL) or the CTRL supplemented with *Tisochrysis lutea* biomass and *Gelidium* sp. extract (TisoG). After feeding, fish were exposed to standardised stress events and infection with *Photobacterium damselae* subsp. *piscicida*. At the end of feeding (S1), fish were weighed to assess growth and feed efficiency. Liver samples were taken at the end of feeding (S1) and six hours post infection (S2) to analyse oxidative stress biomarkers. The algae-supplemented diet had no negative effects on growth or feed efficiency. At S1, TisoG fish showed significantly lower superoxide dismutase (SOD) activity and total antioxidant status (TAS) compared to CTRL. However, after the challenge (S2), TAS significantly increased in TisoG group, indicating enhanced antioxidant response to stress and infection. Lipid peroxidation and catalase activity were unaffected by diet at either sampling point. Nevertheless, no differences in post-infection mortality were observed between the dietary groups. Overall, the TisoG diet improved antioxidant capacity under the challenge, suggesting an adaptive priming effect rather than an immediate regulation of the antioxidant system.

Acknowledgements

This work was financially supported by “Pacto da Bioeconomia Azul” (Project No C644915664-00000026) within the WP5 Algae Vertical, funded by Next Generation EU European Fund and the Portuguese Recovery and Resilience Plan (PRR), under the scope of the incentive line “Agendas for Business and Innovation” through the funding scheme C5 – Capitalization and Business Innovation; and by FCT through contracts UID/04326/2025, UID/PRR/04326/2025 and LA/P/0101/2020 (DOI:10.54499/LA/P/0101/2020) to CCMAR.

MicroRNA response to transport-induced acute stress in the skin mucus of Atlantic salmon, European seabass and rainbow trout

Tonka Buha[1,2,3,*], Bruno Reis[1], Gonçalo Themudo[1], Francisco Cunha[1], Christopher Pimentel[1,2], Paula Simó-Mirabet[6], Juan Antonio Martos-Sitcha[6], Paulo Rema[5], Rui Rocha[4], Benjamin Costas[1], Cláudia Raposo de Magalhães[3,4], Ana Teresa Gonçalves[3,4]

[1]Interdisciplinary Centre of Marine and Environmental Research of the University of Porto (CIIMAR), Matosinhos, Portugal; [2]Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto (UP), Porto, Portugal; [3]SPAROS Lda., Olhão, Portugal; [4]Riasearch Lda., Murtosa, Portugal; [5]University of Trás-os-Montes e Alto Douro, Vila Real, Portugal; [6]Instituto Universitario de Investigación Marina (INMAR), CEIMAR-Universidad de Cádiz, Cádiz, Spain; *Presenting author.

Current methods for assessing the immune and health status of farmed fish largely rely on invasive sampling procedures, such as tissue collection, highlighting the need for alternative, non-invasive approaches to effectively evaluate health and welfare. Given their stability in biofluids, such as mucus, miRNAs have great potential as non-invasive biomarkers. Although microRNAs (miRNAs) are increasingly recognised as key mediators of physiological and stress responses in vertebrates, their roles, especially in fish health and aquaculture, remain poorly understood. Improving our understanding of miRNAs is therefore essential for advancing welfare and sustainability of the aquaculture sector. The present study focused on three important aquaculture species, i.e., Atlantic salmon (*Salmo salar*), European seabass (*Dicentrarchus labrax*) and rainbow trout (*Oncorhynchus mykiss*), which were exposed to transport-induced acute stress. Skin mucus was sampled at three different time points of transport (0 h, immediately after transport, and following a 24 h recovery period). Small RNA data were processed using the nf-core/smrnaseq pipeline and analysed using differential expression analysis and target prediction. Results revealed key common, differentially expressed, miRNAs related to cell cycle regulation, DNA damage response, and stress modulation. This research improves the limited knowledge regarding miRNA expression patterns, functions, and regulatory networks under acute stress in fish, and highlights potential non-invasive biomarker candidates of acute stress.

Acknowledgements

Work Co funded by UKRI and by the European Union's Horizon Europe research and innovation programme (GA No 101084651 project IGNITION). T.B. received funds from FCT - Fundação para a Ciência e a Tecnologia through grant 2023.04651.BDANA.

Tetraselmis chui cryptides: unearthing their hidden potential for aquaculture

B. Carvalho[1,2], M. Kholany[1], J. Varela[1,2], H. Pereira[1,2], A.T. Gonçalves[1,3,4], R.A. Vitorino[1,2,*]

[1]GreenCoLab, Associação Oceano Verde (GCL), Universidade do Algarve, Faro, Portugal; [2]Centro de Ciências do Mar do Algarve (CCMAR), Universidade do Algarve, Faro, Portugal; [3]Sparos Lda, Nutrição em aquacultura (SPAROS), Olhão, Portugal; [4]Riasearch, aquacultura, nutrição, saúde (RIASEARCH), Murtosa, Portugal; *rafaelvitorino@greencolab.com

The expansion of aquaculture, imperative for meeting rising global food demands, is significantly hampered by factors compromising fish health. A promising strategy lies in proactively enhancing fish resilience against stressors by positively modulating the gut-microbiota complex. This complex serves as a protective barrier and strengthening it prior to stress exposure can safeguard against threats that can impair growth and health. This can be achieved through the incorporation of bioactive compounds into feed, which in turn can be acquired through targeted biorefinery methodologies of biomasses such as microalgae, enabling sustainable and efficient productions of functionally active ingredients. A particular ingredient of interest for this study are Cryptides, a group of small peptides known for their potential bioactive properties, remaining inactive while they are incorporated in larger protein structures, reliant on hydrolysis to release these smaller compounds to manifest their functional characteristics. In this study, we developed a downstream processing pipeline for *Tetraselmis chui,* aiming to produce ingredients enriched in cryptides. This pipeline involved an initial cellular disruption step via high pressure homogenization. The disrupted biomass was then processed through an enzymatic hydrolysis step consisting of an enzyme A (ingredient 1), an enzyme B (ingredient 2) and a sequential treatment with enzyme A, followed by enzyme B (ingredient 3). A fractionation method based on centrifugation was employed to isolate the compounds of interest in the supernatant. All produced ingredients were freeze-dried and had their bioactivity tested in an ex-vivo assay based on European seabass (*Dicentrarchus labrax*) intestinal explants. Here, ingredients' influence on the immune response (*TNFa* & *IL-1B*), antioxidant response (*GPx* & *CAT*), epithelium integrity (*OCL* & *TJP1*), antimicrobial peptide secretion (*PISC1* & *PISC4*), and epithelium regeneration (*MMP9*) was assessed through gene expression analysis. A low dosage of ingredient 1 increased by 4-fold the expression of genes linked to the epithelial regeneration. Meanwhile, high dosages of ingredient 2 elicited a widespread 2-fold increase response of antioxidant, immune, antimicrobial peptide secretion and epithelial regeneration genes. On the other hand, high dosages of ingredient 3 triggered a 5-fold increase response of epithelial regeneration, a 3-fold increase in immune genes and a 2-fold increase in antimicrobial peptide secretion genes. These results highlight the potential different enzymatic downstream processing methodologies have in generating a mixture of cryptides capable of eliciting differentiated responses, promoting the need to further research these hidden compounds and to discover their potential in functional nutrition practices in aquaculture.

Acknowledgements: This work was financially supported by "Pacto da Bioeconomia Azul" (Project No. C644915664-00000026) within the WP5 Algae Vertical, funded by Next Generation EU European Fund and the Portuguese Recovery and Resilience Plan (PRR), under the scope of the incentive line "Agendas for Business Innovation" through the funding scheme C5 - Capitalization and Business Innovation. Rafael Vitorino (Ref.2024.02936.BDANA) and Bernardo Carvalho (Ref.2024.02936.BDANA) are pleased to acknowledge their PhDs fellowships by FCT - Foundation for Science and Technology.

Theoretical integration of ESG metrics and SDG targets for biomass and bioactive compound optimisation in Mediterranean aquaculture systems

Michael Warren G. Ceballos[1,2], Sarah Ajala[1,2], Saniya Kadankandy Ramesh[1,2], Shahzad Malak[1,2,*], John Rey Rasgo[1,3], Marey R. De Jesus[4]

[1]Université de La Rochelle, Faculté des Sciences et Technologies, La Rochelle 17000, France; [2]Facultad de Ciencias Veterinarias y Experimentales, Universidad Católica de Valencia, Valencia 46001, Spain; [3]Odjel za ekologiju, agronomiju i akvakulturu, Sveučilište u Zadru, Zadar, Croatia; [4]College of Marine Sciences and Aquatic Resources, Romblon State University – Santa Fe, Philippines; *Presenting author.

Introduction: Global aquaculture faces the dual challenge of ensuring food security and achieving ecological sustainability. This study proposes a novel theoretical model for Mediterranean aquaculture that integrates Environmental, Social, and Governance (ESG) metrics with United Nations Sustainable Development Goal (SDG) targets. The main objective is to create a framework that simultaneously optimises biomass production and the extraction of high-value bioactive compounds within a circular bioeconomy approach.

Materials and Methods: The research developed a conceptual framework based on three interconnected pillars: (1) Multi-Trophic Synergy, using spatial modelling to design integrated aquaculture systems that minimise waste; (2) Climate-Adaptive Governance, conceptualising AI-driven tools for biomass forecasting under Mediterranean warming scenarios; and (3) Social License Innovation, developing metrics to link equitable value-chain participation to ecosystem service valuation. The methodology involved theoretical scenario analysis to demonstrate the model's potential.

Results: Theoretical scenarios from the model demonstrate that applying this precision aquaculture framework could reduce feed conversion ratios by 15–20% while simultaneously enhancing yields of bioactive compounds such as nutraceuticals and antioxidants. The model highlights the potential of endemic species like sea bream and microalgae to unlock niche markets for marine-derived pharmaceuticals, provided regenerative practices are implemented.

Discussion and Conclusion: This study concludes that the theoretical integration of ESG and SDGs provides a transformative pathway for Mediterranean aquaculture. The framework challenges traditional productivity metrics by advocating for SDG-linked Key Performance Indicators (KPIs) that quantify broader impacts like carbon sequestration, coastal employment, and nutrient recycling. The relevance of this work lies in its potential to align aquaculture growth with critical sustainability targets (SDGs 2, 8, 10, 12, 13, 14).

Copper(Cu) based antifouling toxicity to macroalgae and mussels in monoculture and in Integrated Multitrophic Aquaculture Systems

Rui Cereja[1,2,*], Alícia Pereira[3], Ana C. Brito[2,4], Mário S. Diniz[5,6], Carla Pires[1], Mário Mil-Homens[1,3], Pedro Brito[1,3], Pedro R. Costa[1,7], António Marques[1,3]

[1]IPMA, 1495-165 Lisboa, Portugal; [2]MARE, ARNET, Universidade de Lisboa, 1749-016 Lisboa, Portugal; [3]CIIMAR, University of Porto, Matosinhos, Portugal; [4]D. Biologia Vegetal, Fac. de Ciências, Univ. de Lisboa, 1749-016 Lisboa, Portugal; [5]UCIBIO, NOVA University Lisbon, 2819-516 Caparica, Portugal; [6]Associate Laboratory i4HB, NOVA University Lisbon, 2819-516 Caparica, Portugal; [7]CCMAR/CIMAR LA, University of Algarve, 8005-139 Faro, Portugal; *Presenting author.

Introduction: The co-cultivation of multiple species enables the diversification of production and increasing the protection against environmental stressors. Macroalgae are an important source of nutrients and bioactive compounds, and they can also remove contaminants from the environment, thereby protecting other species. This study aimed to assess the interaction between *Laminaria ochroleuca* and *Mytilus* sp. when cultured together (IMTA) or in monoculture and exposed to copper (Cu) contamination.

Methodology: Four different treatments were applied for each species: a) an isolated species on ropes without Cu (control), b) both species on ropes without Cu (control-IMTA), c) an isolated species on 50 cm nautical ropes painted with Cu antifouling paint, and d) both species on Cu-painted ropes (Cu-IMTA). During the 15-day exposure period, samples were collected for Cu quantification, analysis of photosynthetic pigment concentrations, and evaluation of bioactivity and oxidative stress biomarkers (SOD, CAT, GPX, GST/APX, & LPO).

Results: Macroalgae effectively reduced waterborne copper, accumulating this element at concentrations approximately one order of magnitude higher than those observed for mussels. Mussels in IMTA accumulated less Cu in the first 3 days. *L. ochroleuca* showed a high survival rate after 15 days of exposure, presenting only discolouration. Cu-exposed algae exhibited higher APX, CAT, and MDA levels as well as lower chlorophyll-a concentrations. By contrast, both IMTA and monoculture mussels showed alterations in antioxidant enzyme activities, as well as severe gill degeneration.

Discussion: *Laminaria ochroleuca* is highly Cu-absorbing, making it potentially useful for removing metal contamination from aquaculture sites and protecting co-cultured species. This macroalga is resistant to high concentrations of Cu, surviving at concentrations of up to 1 mg/g. Mussel's Cu induced mortality appears to be due to tissue degeneration, particularly in the gills.

Acknowledgement: This work was financially supported by Project No. C644915664-00000026, UIDP/04378/2020, UIDB/04378/2020, LA/P/0140/2020, UID/MAR/04292/2020 and LA/P/0069/2020.

Toxicity of copper-based antifouling paints to macroalgae and mussels subjected to salinity stress under mono- and multitrophic conditions

Rui Cereja[1,2*], Alícia Pereira[3], Ana C. Brito[2,4], Carla Pires[1], Mário S. Diniz[5,6], Tomás Chainho[1], Daniel Bolotas[1], Rita V.C. Gomes[1], Pedro R. Costa[1,7], António Marques[1,3]

[1]IPMA, 1495-165 Lisboa, Portugal; [2]MARE, ARNET, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal; [3]CIIMAR, University of Porto, Matosinhos, Portugal; [4]D. Biol. Vegetal, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal; [5]UCIBIO, School of Science and Technology, NOVA University Lisbon, 2819-516 Caparica, Portugal; [6]Associate Laboratory i4HB, NOVA University Lisbon, 2819-516 Caparica, Portugal; [7]CCMAR/CIMAR LA, University of Algarve, 8005-139 Faro, Portugal; *Presenting author.

Introduction: Copper-based antifoulings are the main tool to control fouling in marine infrastructures. Macroalgae can bioremediate environmental contaminants, including metals and organic pollutants, thereby protecting surrounding species. The present study aimed to evaluate the environmental toxicity effects of copper-based antifouling paints to the macroalga *Laminaria ochroleuca* and the bivalve *Mytilus* sp. under low-salinity events, when cultured in monoculture or in multitrophic conditions (IMTA).

Material and methods: *Laminaria ochroleuca* and *Mytilus* sp. were exposed for 15 days, either individually or together (IMTA system), to 20 µg/L of a commercial copper-based antifouling product and to reduced salinity conditions (25 PSU compared to 34 PSU in the control). Photosynthetic pigments, bioactivity, and stress biomarkers (SOD, CAT, GST, and LPO) were assessed in the macroalgae, while stress biomarkers were also evaluated in mussels. Samples were collected at days 0 and 7 for copper quantification, and at day 14 for all analysed parameters.

Results: In the mussels, the reduction in salinity led to an increase in copper accumulation and for *L. ochroleuca*, IMTA treatments revealed significantly lower copper concentrations than those in monoculture. *L. ochroleuca* in IMTA treatments also exhibited higher photosystem health in addition to higher copper and iron chelating capacities, while presenting lower DPPH and ABTS radical scavenging activities. In terms of stress biomarkers, *L. ochroleuca* exposed to copper, low salinity, or the combination of both stressors presented increased SOD and APX activities. Regarding oxidative stress in mussels, copper exposure led to a reduction in GST activity, without significant influence of either IMTA or salinity variations.

Acknowledgements: This work was financially supported by Project No. C644915664-00000026UIDP/04378/2020UIDB/04378/2020, LA/P/0140/2020, UID/MAR/04292/2020 and LA/P/0069/2020.

Swine protein hydrolysate in aquafeeds to mitigate acute thermal stress in gilthead seabream

Ana Claro[1,2,*], Micheli Zaminhan-Hassemer[1,3], Tiago Sá[1], André Almeida[4,5], Sara Magalhães[6], Jorge M.O. Fernandes[7], Luísa M.P. Valente[1,2], Marta Monteiro[1,2]

[1]Interdisciplinar Centre of Marine and Environmental Research (CIIMAR/CIMAR-LA), University of Porto, Matosinhos, Portugal; [2]School of Medicine and Biomedical Sciences (ICBAS), University of Porto, Porto, Portugal; [3]Universidade de Mogi das Cruzes, São Paulo, Brasil; [4]Indústria Transformadora de Subprodutos, S.A. (ITS), Coruche, Portugal; [5]Comércio e Indústria do Sebo, S.A. (SEBOL), Loures, Portugal; [6]Sociedade de Óleos e Rações S.A. (SORGAL), S. João Ovar, Portugal; [7]Institut de Ciències del Mar (ICM-CSIC), Barcelona, Spain; *Presenting author.

Climate change intensifies thermal stress in valuable farmed species, such as gilthead seabream (GSB), affecting performance and causing economical losses. Protein hydrolysates, rich in bioactive peptides, are emerging as functional aquafeed ingredients to mitigate stress. This study tested a swine hydrolysate (SH), produced from category 3 by-products obtained after thermal hydrolysis, to evaluate its potential to support growth, nutrient utilisation, and physiological responses of juvenile GSB under constant (22°C; CT) and fluctuating (22–15–22°C; FT) temperatures. Two experimental diets were compared: a control diet (CTRL; 20% fishmeal) and a SH3% diet, in which 3% of a commercial fish hydrolysate (CPSP90) was replaced by SH. Growth performance and nutrient utilisation were assessed after 12 weeks of feeding under either CT and FT conditions. To evaluate effects of thermal stress, blood and plasma samples were collected from fish immediately after the temperature drop to 15 °C and after 12 weeks under FT. FT reduced feed intake and final body weight regardless of diet. Following acute stress, SH3% increased haematocrit while decreasing white and red blood cell counts, suggesting improved oxygen transport capacity, and consistently elevated lactate, indicating enhanced glycolytic activity. After 12 weeks, most parameters returned to basal levels except lactate. Overall, SH effectively replaced CPSP90 in GSB diets, supporting growth while modulating haematological and metabolic responses under acute thermal stress.

Acknowledgements

This work was partially funded by the Blue Bioeconomy Innovation Pact (C644915664-00000026) and supported by the Portuguese Foundation for Science and Technology (FCT) through a grant to Ana Claro (2024.00449.BDANA).

GRINNAQUA project: building capacity for a more sustainable aquaculture

Benjamín Costas[1,*], Tânia Pereira[1], Rita Azeredo[1], Marina Machado[1], Lourenço Ramos-Pinto[1], Carolina Tafalla[2], Patricia Díaz-Rosales[2], Frank Nielsen[3], Aina-Cathrine Øvergård[3], Diego Robledo[4], Tim Bean[4], Sergio Fernández-Boo[1]

[1]Interdisciplinary Centre of Marine and Environmental Research of the University of Porto (CIIMAR), Matosinhos, Portugal; [2]Agencia Estatal Consejo Superior de Investigaciones Cientificas (INIA-CSIC), Madrid, Spain; [3]Universitetet i Bergen, Bergen, Norway; [4]The Roslin Institute, University of Edinburgh, Edinburgh, UK; *bcostas@ciimar.up.pt

The GRINNAQUA project, aligned with the EU's Farm-to-Fork Strategy, aims to strengthen CIIMAR's research capabilities and its leadership role in the aquaculture industry. In partnership with leading European institutions — Consejo Superior de Investigaciones Científicas (Spain), the University of Bergen (Norway), and the Roslin Institute at the University of Edinburgh (UK) —one of the project's focus is on enhancing expertise in fish health, genetics, and immunology. A key part of GRINNAQUA's approach falls on its training program, designed to help early-career researchers and professionals to build the skills needed to address pressing challenges in aquaculture. Through a series of hands-on workshops and summer schools, participants have gained valuable insights into cutting-edge techniques and tools, particularly useful for the Aquatic Animal Health field. The learning materials developed during these events include:

Workshop 1: Theoretical-practical workshop about omics tools in aquaculture – Led by the University of Edinburgh (UEDIN); **Workshop 2**: Theoretical-practical workshop on fish vaccination – Led by Consejo Superior de Investigaciones Científicas (CSIC); **Workshop 3**: Theoretical-practical workshop on fish health and welfare in industrial aquaculture – Led by the University of Bergen (UiB); **Summer School 1**: Genomic and genetic tools for the blue revolution – Led by UEDIN.

These materials — such as presentations, practical guides, and interactive exercises — were designed for easy access and long-term use. They provide essential resources for both academic programs and professional development in the aquaculture sector. This poster will highlight the key learning materials created throughout the project, showcasing how these resources support the ongoing development of aquaculture professionals and how these newfound skills can contribute to building a more sustainable and resilient aquaculture sector in Europe.

GRINNAQUA is co-funded by the European Union (GA 101079467) and the UK Research and Innovation (UKRI). Views and opinions expressed are however those of the authors only and do not necessarily reflect those of the European Union, the Research Executive Agency (REA) or the UKRI. Neither the European Union nor the granting authorities can be held responsible for them.

Exploring the role of probiotic and paraprobiotic supplements in the digestive function of gilthead seabream fed plant-based diets

Ricardo Couto[1,2,*], Rui Silva[1,2], Rafaela Santos[1,2], Paula Enes[1,2], Cláudia Serra[1,2], Aires Oliva-Teles[1,2], Inês Guerreiro[1,2]

[1]Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR), Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos, s/n, 4450-208, Matosinhos, Portugal; [2]Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, Ed. FC4, 4169-007 Porto, Portugal. *rcouto@ciimar.up.pt

Introduction

With the expansion of aquaculture and the pressure to reduce fishmeal use, plant-feedstuffs (PF) are increasingly used in diets, though their non-starch polysaccharides and other antinutritional factors can compromise diets digestibility and gut health. This study aimed to assess the effects of probiotic (bacteria and yeast) and paraprobiotic yeast in PF-based diets on gilthead seabream digestive function.

Material and Methods

Gilthead seabream (*Sparus aurata*, 173g) were fed 4 diets for 34 days: a control PF-based diet, and 3 similar diets supplemented with a probiotic bacterium, probiotic yeast, or paraprobiotic yeast. Each diet was fed to triplicate groups twice daily until visual satiation. Faeces were collected daily for digestibility determination. At the end of the trial, fish were weighed and intestines of 3 fish per tank were sampled for digestive enzymes (total alkaline proteases, lipase, α-amylase) activities.

Results

Growth performance, feed intake, feed efficiency, and digestive enzymes activities were not affected by the inclusion of the feed additives. Digestibility data are still being processed.

Discussion and Conclusion

Feed additive supplementation showed no major effects on the measured parameters. Digestibility data remain under analysis.

Acknowledgements

This project was funded by national funds through FCT – Foundation for Science and Technology under the project FEEDGUTBRAIN (2023.13660.PEX, DOI https://doi.org/10.54499/2023.13660.PEX).

Optimisation of *Ulva lacinulata* cultivation conditions for agriculture applications

Clarisse Eiras[1,2,*], Isabel Cunha[1,2], Aires M. Duarte[1,2], Isabel Costa[1,3]

[1]Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR-LA), Portugal; [2]Faculty of Sciences of University of Porto, University of Porto, Portugal; [3]ICBAS—Instituto de Ciências Biomédicas Abel Salazar, University of Porto, Portugal; *Presenting author.

Introduction: Seaweed extracts hold diverse bioactive compounds that may improve plant growth and health. Species of the *Ulva* genus are a valuable source of those compounds, which may be applied as biostimulants and plant protectors, an environmentally friendly method to increase agricultural production. To provide high yields of target compounds, tuning of biomass production may be achieved by controlling environmental factors, determinants for growth and composition. The goal of this Master thesis is the optimisation of *Ulva lacinulata* cultivation, in terms of nutrients and light availability, to customise its biomass for application in agriculture.

Materials and Methods: *Ulva lacinulata* from the LBC biobank was cultivated in a growth chamber using PES-enriched seawater at 18ºC and under 100 µmol photons m^{-2} s^{-1} and 16:8 (L:D), with weekly maintenance. Future work will be developed in two stages, using the grown biomass: 1) Cultivation optimization trials - test the effect of light and nutrients on the growth rate and content in phenolic compounds and polysaccharides of *U. lacinulata* (including stressing trials with suboptimal conditions); 2) Biochemical analyses - Phenolic compounds extraction will be performed using Natural Deep Eutectic Solvents (NADES); pigments, phenolic compounds, polysaccharides will be extracted. The antioxidant capacity of the biomass will be analysed.

Results: *U. lacinulata* showed high growth rates in the preliminary biomass growth assay. Further results: it is expected that nutrients concentration will have an impact on protein and polysaccharide contents, that light intensity/quality will have an impact on pigments and phenolic compounds contents, and that the efficiency of NADES for compounds extraction will be ascertained.

Discussion and Conclusion: Our own preliminary results, and the available literature, indicate that *U. lacinulata* has potential as a source of compounds with biostimulant properties, thanks to its fast growth and biochemical composition. This work will confirm this potential and will further establish the cultivation conditions for a tailored biomass for agricultural purposes.

Acknowledgements: We would like to thank Universidade do Porto and CIIMAR for institutional support. We would also like to thank the CIIMAR BYT+ funding program and the Amadeu Dias Foundation for funding this Dissertation.

Screening seaweeds from fish ponds as natural functional ingredients for European sea bass (*Dicentrarchus labrax*) health

Rute C. Félix[1,2], Maria João Xavier[1,2], Soraia Santos[1], Carolina Augusto[3], Leonardo Többen Vieira[3], Hugo Pereira[1,2], Ana Teresa Gonçalves[1,4]

[1]GreenCoLab – Associação Oceano Verde, Universidade do Algarve, Faro, Portugal; [2]Centro de Ciências do Mar do Algarve (CCMAR/CIMAR LA), Faro, Portugal; [3]Piscicultura do Vale da Lama, Alvor, Portugal; [4]SPAROS Lda., Área Empresarial de Marim Lote C, 8700-221 Olhão, Portugal.

Maintenance of optimal health and resilience in aquaculture species of utmost importance for the sector performance. Integrated Multi-Trophic Aquaculture (IMTA) offers a promising strategy to improve system sustainability and fish health through the integration of natural feed sources. In earth pond systems, naturally occurring algae are commonly ingested by fish and may provide functional benefits when properly integrated into IMTA systems. Our study aimed to investigate the functional potential of co-culturing the macroalgae *Ulva* sp. and *Gracilaria* sp. with European seabass (*Dicentrarchus labrax*), by evaluating their effects on gut physiological response. An optimised *ex vivo* gut mucosal screening model was employed, using anterior intestinal explants from locally raised adult seabass to assess the effects of low and high dosages of each macroalgae. Tissue responses were analysed by quantitative real-time PCR, targeting genes associated with three key physiological processes: immune response, antioxidant defence, and epithelial integrity. Gene relative expression levels were calculated and compared to determine algae-specific effects. The results revealed an overall enhancement of gut health transcriptional activity in response to macroalgae exposure. Both *Ulva* sp. and *Gracilaria* sp. upregulated immune-related genes, while *Ulva* sp. elicited a stronger stimulation of epithelial gene expression, suggesting a potential improvement in mucosal barrier function. These findings highlight the potential of IMTA systems integrating macroalgae and seabass to promote additional health benefits, contributing to more sustainable aquaculture practices.

Acknowledgements

This work was cofinanced by the Innovation and Digital Transition Program - COMPETE 2030, by the regional programs Centro 2030 and Algarve 2030, and by the European Union within the scope of the project AQUADIVERSIFY - Diversification and sustainable production in Integrated Multitrophic Aquaculture, COMPETE2030-FEDER-01454800 and ALGARVE-FEDER-01454800.

AQUATECHinn 4.0: the interactive way to learn aquaculture

Leonor Ferrão[1,*], Elsa Cabrita[1], Pedro Rodrigues[1], Margarida Ribau Teixeira[2], Gonçalo Brito[1], Fábio Barroso[3], João Saraiva[3], Mariana Sá[4], Isidro Blanquet[4], Rita Abecasis[1]

[1]Centro de Ciências do Mar do Algarve (CCMAR/ CIMAR-LA), Universidade do Algarve, Faro; [2]Centro de Investigação em Ambiente e Sustentabilidade (CENSE), Universidade do Algarve, Faro; [3]Associação FishEthoGroup, Faro, Portugal; [4]Associação Portuguesa de Aquacultores (APA), Lagos, Portugal;
*Presenting author.

AQUATECHinn 4.0 is a cutting-edge training platform designed to transform the aquaculture sector by integrating the latest technological and productivity innovations within the Industry 4.0 framework. Responding to the growing need for skilled professionals and rapid technological progress, the program includes three specialised modules: (1) New Technologies in Fish and Shellfish Aquaculture, (2) Sustainability Management in Aquaculture, and (3) Animal Health Management and Biosecurity, Welfare, Nutrition, and Reproduction. Each module is organised into submodules that combine theoretical knowledge with practical applications, supported by real case studies and troubleshooting scenarios. Topics range from fundamental biology, such as "Nutrition, Reproduction and Fish Welfare", to advanced technology, including "Omics technologies" and "Digitalization, semi-automation and automation, use of drones and robots in farming and harvesting processes". Designed for both **aquaculture technicians and students,** AQUATECHinn 4.0 offers a flexible and engaging learning experience that bridges academic knowledge with professional practice. Delivered through an innovative e-learning environment, **AQUATECHinn 4.0** employs advanced training methods, including digital blended learning, immersive Virtual Reality simulations, gamified learning experiences, engaging instructional videos, and open educational resources (MOOCs), equipping learners with adaptable skills to address real-world aquaculture challenges. To ensure accessibility across Europe, AQUATECHinn 4.0 platform will be available in seven different languages, enabling aquaculture professionals and educational institutions from across the European aquaculture community to benefit from the program's resources and knowledge. Alongside other European partners, AQUATECHinn 4.0 was developed through collaboration among leading Portuguese institutions, including APA (Associação Portuguesa de Aquacultores), UAlg (Universidade do Algarve), CCMAR (Centro de Ciências do Mar), and FishEthoGroup, to strengthen aquaculture innovation and knowledge exchange without borders. **Acknowledgements:** the project is funded by CCMAR strategic program (UIDB/04326/2020, UIDP/04326/2020, LA/P/0101/2020 – https://doi.org/10.54499/LA/P/0101/2020) and AQUATECHinn 4.0 (Ref HORIZON-ERASMUS-EDU-2022-PI-ALL-INNO-101108913) Digital learning in aquaculture 4.0 for the technological training of fish farm technicians ERASMUSEDU-2022-PI-ALL-INNO).

Advanced qPCR solutions for reliable identification of *Tenebrio molitor* and *Hermetia illucens* in aquafeed and food products

Andreia Filipa-Silva[1,*], Thaise Martins[1,2], Maria J. Mota[3,4], André Almeida[5,6], Daniel Murta[7,8], Luísa M.P. Valente[1,2], Sónia Gomes[1,2]

[1]CIIMAR/CIMAR-LA, Centro Interdisciplinar de Investigação Marinha e Ambiental, Matosinhos, Portugal; [2]ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal; [3]SORGAL, Sociedade de Óleos e Rações, S.A., S. João de Ovar, Portugal; [4]SAVINOR - Sociedade Avícola do Norte S.A., Trofa, Portugal; [5]SEBOL, Comércio e Indústria do Sebo, S.A., Loures, Portugal; [6]ITS, Indústria Transformadora de Subprodutos, S.A., Coruche, Portugal; [7]Thunder Foods Lda., Ingredient Odyssey S.A., Santarém, Portugal; [8]EntoGreen, Ingredient Odyssey S.A., Santarém, Portugal. *Presenting author.

The increasing demand for alternative protein sources has heightened the need for high-throughput authentication methods for insect-based feed and food products in the agrifood sector. This study focuses on developing and validating two species-specific qPCR-based assays to detect and quantify DNA two commercially significant insect species: *Tenebrio molitor* (yellow mealworm) and *Hermetia illucens* (black soldier fly). Using primers targeting the mitochondrial cytochrome b gene for *T. molitor* and NADH dehydrogenase subunit for *H. illucens*, the assays demonstrated high sensitivity, detecting target DNA concentrations as low as 2 pg/µL for *T. molitor* and 0.2 pg/µL for *H. illucens* in complex food matrices. A key innovation is the harmonised thermal cycling conditions allowing simultaneous detection of both species in a single run, saving time and resources. The method´s robustness was validated across various commercial meals, hydrolysates, and animal feed products, accurately identifying both target species in mixtures at concentrations as low as 0.24% inclusion. No significant cross-reactivity was observed with other tested species or matrices. This robust, cost-effective approach supports regulatory compliance, ensures authenticity, and upholds safety standards in the insect-based protein supply chain, highlighting the need for advanced detection. These novel protocols set a foundation for future monitoring and quality assurance measures in food and feed industries.

Acknowledgements

This research was funded by the Blue Bioeconomy Pact (C644915664–00000026), for the exercise of activities in the WP6 FEED (Pep4Fish project) through international funds provided by the European Union, and supported by the strategic funding UIDB/04423/2020 and UIDP/04423/2020, and LA/P/0101/2020 (https://doi.org/10.5 4499/LA/P/0101/2020) through national funds provided by FCT – Fundação para a Ciência e a Tecnologia.

Cyanotoxins on the move - Freshwater origins with marine consequences: a systematic review of global changes and emerging trends

Marta Fraga[1,*], Catarina Churro[2,3], José Leão-Martins[4], Alisa Rudnitskaya[5], Maria João Botelho[1, 6]

[1]Laboratory of Marine Biotoxins, Department of the Sea and Marine Resources, IPMA-Portuguese Institute for the Sea and Atmosphere, Av. Alfredo Magalhães Ramalho 6, 1495-165, Algés, Portugal; [2]Laboratory of Phytoplankton, Department of the Sea and Marine Resources, IPMA - Portuguese Institute for the Sea and Atmosphere, Av. Alfredo Magalhães Ramalho 6, 1495-165, Algés, Portugal; [3]Blue Biotechnology, Environment and Health, CIIMAR- Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto, Av. General Norton de Matos s/n, 4450-208, Matosinhos, Portugal; [4]Department of Analytical and Food Chemistry, Faculty of Chemistry, University of Vigo, University Campus of Vigo, As Lagoas Marcosende, 36310 Vigo, Spain; [5]CESAM- Centre for Environmental and Marine Studies and Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal; [6]Contaminant Pathways in Marine Environment, CIIMAR-Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto, Av. General Norton de Matos s/n, 4450-208, Matosinhos, Portugal;

*Presenting author.

Introduction: The increasing occurrence of toxic freshwater cyanobacteria blooms in marine or brackish waters, coupled with elevated cyanotoxin concentrations in marine life, poses an emerging threat to seafood production and human health. These events are often associated with temperature, salinity and the eutrophication of affected areas, however global changes causing extreme events can cause rapid shifts in their dynamics and subsequent propagation. This systematic review presents reports from 2010 to 2024 where the main objectives were to describe (i) worldwide occurrence of freshwater cyanobacteria toxins in transitional waters; (ii) global changes and shifts in cyanobacteria dynamics and (iii) patterns of the cyanotoxins microcystins, nodularins, saxitoxins, cylindrospermopsin and anatoxins in marine organisms.

Materials and Methods: PRISMA ("Preferred Reporting Items for Systematic Reviews and MetaAnalyses") protocol was used, and literature search was done using two databases (PubMed and Web of Science) to summarise the research outcomes.

Results: The higher number of events was reported in the USA (west coast and east coast), followed by the Baltic Sea. Both Mediterranean and Atlantic coasts of Europe, including Portugal, had experienced the severe impact of these events in coastal and brackish environments. Despite the lack of consensus for cyanotoxin guidelines, the highest values of the most common cyanotoxins accumulated in marine organisms were in bivalves (microcystins) and in fish (nodularins), with values largely exceeding the existing guideline of 51 µg cyanotoxins.kg^{-1} body weight.

Acknowledgements: Thanks are due for financial support to Vigo University, CESAM by FCT/MCTES (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020), to CIIMAR (UIDB/04423/2020 + UIDP/04423/2020) and to the Programme Mar, through the project SNMB-Monit IV (grant number 02.01.02-FEAMP-0177).

Beyond harvest: ecological benefits of bivalve aquaculture – The AQUABENEFIT project

Vânia Freitas[1,*], Sandra Ramos[1,2], Marina Dolbeth[1], Ester Dias[1], André Dias[3]

[1]Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR-LA), University of Porto, Matosinhos, Portugal; 2 Biology Department, Faculty of Science of University of Porto; 3 Institute for Systems and Computer Engineering, Technology and Science (INESC TEC), Porto, Portugal; *Presenting author.

Introduction: Bivalve aquaculture plays a crucial role in global food security and coastal economies, yet its ecological benefits such as habitat provision, biodiversity enhancement, and ecosystem services remain poorly understood and undervalued. The AQUABENEFIT project aims to develop an integrated approach to assess these ecological benefits by combining ecological knowledge, robust monitoring methods, and artificial intelligence (AI)-based data processing, in close collaboration with producers and other stakeholders.

Materials and Methods: An off-bottom oyster farm in the Lima estuary (NW Portugal) was selected as a case study to evaluate farming structures as artificial substrates and as refuge, nursery, and foraging habitats for fish and mobile invertebrates. Biodiversity and habitat use will be assessed through a complementary suite of methods, including traditional ecological sampling, molecular tools (eDNA) methods, and underwater video monitoring. Deep-learning algorithms will be developed to automate species identification from video imagery, while stable isotope analysis will trace food web linkages. Monitoring spans a complete production cycle to capture temporal variation and identify practices that maximise ecological benefits.

Results: The project is currently in its initial phase, focused on baseline field surveys, stakeholder consultations, and method development. Comparative protocols between eDNA and traditional survey methods are being established, and prototype AI models for automatic image processing are under design.

Discussion and Conclusion: AQUABENEFIT bridges ecological research and technological innovation to deliver a practical and transferable framework to quantify the habitat benefits of bivalve aquaculture. The project will generate scientific knowledge to inform management, improve the sustainability and public perception of shellfish farming, and support policy decisions toward recognising aquaculture as a nature-based solution that aligns food production with coastal ecosystem health.

Acknowledgements: This work is funded by ERDF through COMPETE 2030 – Operational Programme for Competitiveness and Internationalization (project COMPETE2030-FEDER-00787300 / nº 16692).

Physiological and oxidative responses of Senegalese sole postlarvae to algae-supplemented diets

C. Freitas de Oliveira[1,2,*], A. Sharif[1,3], R. Colen[1], R. Teodósio[1], P. Santos[4,6], B. Costas[4], A. T. Gonçalves[5,6], W. Pinto[5], L.E.C. Conceição[5], S. Engrola[1]

[1]Centro de Ciências do Mar do Algarve (CCMAR/CIMAR-LA), Faro, Portugal; [2]Universidade do Algarve, Faro, Portugal; [3]University of Cadiz, Cadiz, Spain; [4]Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR-LA), Matosinhos, Portugal; [5]SPAROS Lda., Olhão, Portugal; [6]GreenCoLab, Faro, Portugal.
*a82249@ualg.pt

The Senegalese sole is a key aquaculture species in Southern Europe. As production intensifies, diets must support growth and physiological resilience under hatchery stressors such as transport and handling. Micro- and macroalgae, rich in bioactive compounds with antioxidant and immunomodulatory potential, represent promising functional ingredients to enhance larval robustness. This study investigated how algae-supplemented microdiet affects growth performance and postlarval resilience in sole, focusing on the modulation of oxidative balance, intestinal integrity, and immune responses. A feeding trial was conducted during the benthic phase (18–45 days after hatching, DAH). Two diets were tested in triplicate: a commercial-like control (CTRL) and one supplemented with *Ulva* sp. and *Pavlova gyrans* (ULPAVL). Growth performance, physiological, and molecular markers were assessed at 38 DAH (S1). From 38 to 43 DAH, all fish were fed a common diet supplemented with *Skeletonema costatum* extract (BOOST). Fish were then transported for 24 h and challenged with *Tenacibaculum maritimum*. Responses to infection were evaluated at 45 DAH (S2). After 20 days of feeding, ULPAVL fish showed lower body weight than CTRL, while condition factor and survival remained similar. Antioxidant enzyme activities were not significantly affected by diet; however, ULPAVL fish exhibited reduced lipid peroxidation, indicating lower oxidative damage. Following the bacterial challenge, no significant differences were detected between diets in immune or oxidative responses. Overall, the algae-supplemented diet mitigated oxidative damage, but this was at the expense of growth performance and had no apparent impact on post-stress resilience. These findings underscore the challenge of formulating stage-specific diets that enhance both performance and robustness in marine fish larvae. **Acknowledgements:** This work was financially supported by "Pacto da Bioeconomia Azul" (Project No C644915664-00000026) within the WP5 Algae Vertical, funded by Next Generation EU European Fund and the Portuguese Recovery and Resilience Plan (PRR), under the scope of the incentive line "Agendas for Business and Innovation" through the funding scheme C5 – Capitalization and Business Innovation; and by FCT through contracts UID/04326/2025, UID/PRR/04326/2025 and LA/P/0101/2020 (DOI:10.54499/LA/P/0101/2020) to CCMAR. The authors thank the company Necton SA for supplying the microalgae.

Optimising co-feeding protocols for Senegalese sole larvae: impacts of Artemia reduction on growth performance

Tiago Garcia[1,2,*], Aqsa Sharif[1], Rita Colen[1], Wilson Pinto[3], André Santos[3], Carolina Castro[4], Renata Serradeiro[4], Luís E. C. Conceição[3], Sofia Engrola[1]

[1]Centro de Ciências do Mar do Algarve (CCMAR/CIMAR LA), Faro, Portugal; [2]Universidade do Algarve, Faro, Portugal; [3]SPAROS Lda, Olhão, Portugal; [4]FLATLANTIC - Actividades piscícolas, S.A., Praia de Mira, Portugal;
*a90112@ualg.pt

The rearing of Senegalese sole remains constrained by its reliance on live feeds, particularly *Artemia*, which are nutritionally unbalanced and substantially increase production costs. Developing high-quality microdiets is critical to promote robust and resilient larvae. This study evaluated the performance of larvae reared under a co-feeding regime from mouth opening, in which *Artemia* supply was reduced by 50%. Larvae were reared in triplicate tanks from 2 to 27 days after hatching (DAH) under a recirculating aquaculture system. Two feeding regimes were tested: a control (CTRL) co-feeding protocol combining *Artemia* and microdiet at standard *Artemia* rations, and an experimental group (CTRL-50%) where *Artemia* supply was reduced by 50% while maintaining the same microdiet level. The reduction period extended from 7 to 17 DAH, after which, all groups were fed a common commercial diet until 27 DAH. Feeding regime effects were evaluated through performance indicators (dry weight, total length, condition factor, relative growth rate, and growth homogeneity) measured at 7, 17, and 27 DAH, along with final survival. The reduction of *Artemia* negatively affected larval weight and length at the end of the pelagic phase. However, relative growth rate and survival were not significantly affected. By the end of the benthic phase, body weight converged between treatments, indicating that early growth setbacks were compensated once fish were fed exclusively with inert diets. These results demonstrate that substantial live feed reduction can be achieved in *Senegalese sole* co-feeding protocols without compromising key performance indicators, offering a promising approach to enhance hatchery sustainability and reduce operational costs.

Acknowledgements

This work is part of project E!4876 FlatFIRST_1171, supported by EUROSTARS-3 program, and by Portugal and the European Union through ERDF, Algarve 2030, and COMPETE 2030, in the framework of Portugal 2030 and Portuguese national funds from FCT - Foundation for Science and Technology through contracts UID/04326/2025, UID/PRR/04326/2025 and LA/P/0101/2020 (DOI:10.54499/LA/P/0101/2020), and from the operational programmes CRESC Algarve 2020 and COMPETE 2020 through contract EMBRC.PT ALG-01-0145-FEDER-022121 to CCMAR.

Beneficial effects of *Skeletonema costatum* on fish skeletal development

Paulo Gavaia[1,*], Alessio Carletti[1], Vincent Laizé[1], Patricia Diogo[2]

[1]Centro de Ciências do Mar do Algarve (CCMAR/CIMAR-LA), Universidade do Algarve, Faro, Portugal; [2]Necton, S.A., Belamandil, Olhão, Portugal; *Presenting author.

Incidence of skeletal malformations affect productivity, growth, survival and increase stress and susceptibility to pathogens in farmed fish. Malformations also downgrade the quality of the produced fish, reducing profitability of aquaculture. Since skeletal malformations have a multifactorial origin and represent one of the main constraints in fish farming, strategies are required to reduce and mitigate their incidence during larval stages. Farmed fish like gilthead seabream (*Sparus aurata*), European seabass (*Dicentrachus labrax*) and Senegalese sole (*Solea senegalensis*) often present incidences of malformations above 50% during larval and juvenile stages, and the industry can benefit greatly from technical improvements that promote a reduction of these malformations. Nutritional imbalances are major factors affecting bone development and the incidence of skeletal deformities, which are acknowledged to constitute one of the major constraints in the production of high-quality fish. *Skeletonema costatum* is a source of antioxidants and essential nutrients such as vitamins, minerals, polyunsaturated fatty acids, and pro-osteogenic bioactive molecules. *S. costatum* was supplemented as extract in larval diets for zebrafish (*Danio rerio*) at 2.5%, or as whole biomass at 25% in *Artemia* enrichement for Senegalese sole. *S. costatum* dietary supplementation improved fish overall quality and allowed a reduction of skeletal malformations in both species. In *D. rerio*, *S. costatum* extract improved skeletogenesis and mineralisation by promoting osteoblastic and decreasing osteoclastic activity. In *S. senegalensis*, the larvae fed *S. costatum* supplemented *Artemia* had a better growth in length and weight, but more importantly, a reduction of 20% in the incidence of malformations and a marked reduction of severe malformations. Also, the charge of malformations was greatly reduced from 4.3 in control to 3 in the treatment group. Overall our experimental dietary treatments showed that *S. costatum* induces a protective effect on the development of skeletal deformities during larval stages of both *D. rerio* and *S. senegalensis*. This translated into juvenile fish with greater quality and less prone to develop severe deformities like vertebral curvatures (lordosis-kyphosis and scoliosis), that significantly deteriorate fish quality. Inclusion of *S. costatum* in larval diets have the potential to improve larval quality and promote the sustainability of the aquaculture sector.

Study funded by Prémio Inovação Expo Fish Portugal funded by Docapesca - Portos e Lotas, S.A. and by Portuguese national funds from Foundation for Science and Technology - FCT through contracts UID/04326/2025, UID/PRR/04326/2025 and LA/P/0101/2020 (DOI: 10.54499/LA/P/0101/2020).

Capacitating the aquaculture industry for a just transition

Renata Gonçalves[1,2,*], Cátia Marques[1,3], Pedro Pousão-Ferreira[1,3], Joana Rosa[1,2]

[1]S2AQUA – Collaborative Laboratory Association for Sustainable and Smart Aquaculture, 8700-194 Olhão, Portugal; [2]CCMAR – Centre of Marine Sciences, 8005-139 Faro, Portugal; [3]IPMA – Instituto Português do Mar e da Atmosfera, 8700-194 Olhão, Portugal; *Presenting author.

Introduction: European aquaculture plays a key role in the transition toward sustainable food systems. Its growing technological base and environmental focus make it a potential platform for integrating cellular agriculture, particularly cultivated seafood (CSF). This study compares aquaculture businesses in Northern and Southern Europe, identifying regional opportunities and barriers to integrating CSF into existing production models.

Methodology: The analysis combined quantitative and qualitative data from interviews with 26 aquaculture companies across Europe. Companies were grouped by region and size to identify structural patterns including production systems and resources, partnerships, market orientation, financing models, innovation and investment capacity. Comparative analysis focused on key challenges and opportunities, identifying enabling conditions for integrating CSF into conventional aquaculture operations.

Results: Clear regional contrasts were identified. In the South, the sector consists mostly of small, family-run enterprises with limited financing but strong market orientation and regional identity. Main challenges include difficulty attracting specialised workers and competition with non-EU imports. In the North, aquaculture is dominated by large and medium-sized firms with strong investment capacity, advanced technology, and collaboration with research institutions. These conditions support innovation and potential for CSF integration. Main challenges include strict legislation and low public perception of aquaculture.

Conclusion: Integrating CSF into aquaculture could address current challenges by enhancing production efficiency, both in terms of time and space, and by promoting product diversification. Northern companies are best placed to pioneer integration. Northern Europe's aquaculture sector therefore provides a strong foundation for advancing CSF in a sustainable and inclusive way.

Acknowledgements: This study was funded under the HORIZON-CL6-2023-FARM2FORK-01 call, grant agreement no. 101136749.

Evaluation of microalgae-derived extracts as functional ingredients in larval diets of gilthead seabream and meagre

Renata Gonçalves[1,2,*], Íris A.L. Silva[1], Michael Viegas[1,2], Joana Rosa[1,2], Bárbara Requeijo[1], Mafalda Rocha[1], Inês Freitas[1], Rui Sousa[1], Ana Mendes[3], Sara Castanho[3], Marisa Barata[3], Florbela Soares[1,3], Cátia Marques[1,3], Pedro Pousão-Ferreira[1,3]

[1]S2AQUA – Collaborative Laboratory Association for Sustainable and Smart Aquaculture, 8700-194 Olhão, Portugal, [2]CCMAR – Centre of Marine Sciences, 8005-139 Faro, [3]IPMA – Instituto Português do Mar e da Atmosfera, 8700-194 Olhão; *Presenting author.

Introduction: The circular use of agricultural effluents for the production of microalgae offers a sustainable route toward generating bioactive feed ingredients for aquaculture. This study evaluated the safety and functional potential of *Tetradesmus* spp extracts, obtained from effluent-grown biomass, when incorporated into larval diets of gilthead seabream (*Sparus aurata*) and meagre (*Argyrosomus regius*).

Methodology: Seabream larvae (27–52 days after hatching, DAH; 25 days) and meagre (21–36 DAH; 15 days) were fed diets supplemented with *Tetradesmus* spp. retentates (0.2 µm) or permeates (0.6 µm) at 0.2% or 0.4–0.5%. Growth was assessed at mid- and end-trial. At the end, larvae were subjected to a 3 h transport stress followed by *Photobacterium damselae* subsp. *piscicida* challenge. Survival and skeletal anomalies were evaluated. In seabream, post-trial antioxidant enzymes and stress-related gene expression were analysed. A 15-day post-feeding follow-up under a commercial diet was also conducted.

Results: Growth and survival were high in both species (seabream: 0.3 to 1.8–2.5 mg; 69–78% survival; meagre: 40 to 158–190 mg; 87–92% survival), with no significant dietary effects. Stress and bacterial challenge outcomes were similar across treatments, and neither species showed a significant dietary effect on the incidence of skeletal anomalies. Additionally, no effects were detected on seabream antioxidant enzymes or stress-related gene expression.

Conclusion: *Tetradesmus* spp. extracts at 0.2–0.5% were safe for marine fish larvae but did not elicit measurable improvements in growth, stress resilience, or immune response under the tested conditions. These results support their suitability as neutral feed ingredients and indicate that higher inclusion levels or extract refinement may be required to achieve functional effects.

Acknowledgements: This study was funded by the European Union, grant agreement ID: 101060991.

Soft-shell crab production to boost aquaculture economic sector in the Mediterranean region: the SOFTCRAB project

Renata Gonçalves[1,2,*], Joana Cruz[2,3], Vânia Baptista[2,3], João Encarnação[2], João N. Monteiro[2,3], Francisco Leitão[2,3], Dora Agapito[3,4], Jennifer Elston[3,4], Pedro Gonçalves[3], Clarisse Vilarinho[1], Inês Nazaré[1], Cátia Marques[1,6], André Cabrita[5], Pedro Pousão-Ferreira[1,6], M. Alexandra Teodósio[2,3]

[1]S2AQUA – Collaborative Laboratory Association for Sustainable and Smart Aquaculture, 8700-194 Olhão, Portugal; [2]CCMAR/CIMAR-LA – Centre of Marine Sciences, 8005-139 Faro; [3]Universidade do Algarve, 8005-139 Faro; [4]CinTurs – Research Centre for Touris, Sustainability and Well-Being, 8005-139 Faro; [5]Atlantik Fish – Pescado de Maré, 8950-124 Castro Marim; [6]IPMA – Instituto Português do Mar e da Atmosfera, 8700-194 Olhão; *Presenting author.

Introduction: Crustaceans are among the most valuable seafood commodities, with soft-shell crab production representing a high-value niche market traditionally dominated by the blue crab (*Callinectes sapidus*). Its recent establishment along the Algarve coast raises both commercial opportunities and ecological concerns. In parallel, the native green crab (*Carcinus maenas*), currently of low economic value and mainly used as bait in the local octopus fishery, remains underexploited. The SOFTCRAB project evaluates the feasibility of soft-shell production from both species, aiming to promote sustainable valorisation of an invasive stock while creating higher-value uses for a native resource.

Methodology: The project comprises four tasks: T1 – management; T2 – optimisation of soft-shell production; T3 – development of aquaculture solutions; and T4 – product acceptance and dissemination. Work includes laboratory trials assessing temperature and feeding effects on moulting, and pilot tests on cage deployment in tidal pond systems and crab–fish co-culture.

Preliminary results: A green crab feeding trial tested three dietary treatments: no feeding, mussel meat and seabass pellets. The no-feeding treatment led to rising mortality, especially after 40 days, with no moulting. Mussel meat and pellets supported survival above 60%, with moulting occurring only after day 35 and at low frequency (~20%).

Preliminary conclusions: Soft-shell production from green crab is not feasible without feeding. Both tested diets supported survival and induced moulting, although at delayed timing and limited frequency. Further optimisation of feeding and rearing conditions is required to improve production efficiency.

Acknowledgements: Funded by PITD Algarve, Fundo FEDER, within Portugal 2030, Compete 2030 and Algarve 2030, co-financed by the European Union.

From diagnosis to action: health solutions for aquaculture

Sílvia F. Gregório[1,*], Rui Sousa[1], Ana Carolina Matias[2], Marcelo Livramento[1], Maria Carolina Ribeiro[1,2], Iris Silva[1], Pedro Pousão-Ferreira[1,2], Cátia Lourenço-Marques[1,2], Florbela Soares[1,2]

[1]Collaborative Laboratory, Association for a Sustainable and Smart Aquaculture (S2AQUA), Olhão, Portugal; [2]Portuguese Institute for the Ocean and Atmosphere/Aquaculture Research Station (IPMA/EPPO), Olhão, Portugal; [*]silvia.gregorio@s2aquacolab.pt

S2AQUAcoLAB's main mission is to help transform aquaculture into a more sustainable and intelligent sector. Together with its associates and team, the organisation works daily to provide producers with better tools and increased productive capacity by bridging scientific research with industry needs. Its goals include promoting sustainable and smart aquaculture, optimising marine organism production, and positioning aquaculture as a key driver of economic growth. In Portugal, bivalve aquaculture plays a central role in exports, and S2AQUAcoLAB is committed to supporting its continued growth through the study and prevention of diseases. S2AQUAcoLAB integrates a multidisciplinary team of specialists in pathology, microbiology, molecular biology, ecology, and functional biology, dedicated to supporting aquaculture producers through advanced diagnostic solutions and comprehensive health management strategies. S2AQUAcoLAB's Pathology Laboratory provides advanced diagnostic and analytical services to support aquaculture health and sustainability. Our expertise includes in vivo and in vitro pathogen challenge tests, biochemical and haematological analyses, immunological and stress assessments, and training in aquatic pathology. We perform pathological examinations, identify bacteria and parasites, and enable early pathogen detection through cutting-edge molecular biology techniques in collaboration with specialised partners. Equipped to handle bacteria, parasites, and viruses, our adaptable protocols cover multiple species. These services ensure timely diagnosis, healthy stock selection, best aquaculture practices, and product quality. Committed to research and prevention, we partner with producers to promote welfare and drive sustainable growth in the aquaculture industry.

Acknowledgements

This work was funded by Missão Interface and co-funded by PRR – Plano de Recuperação e Resiliência (operation code 01/C05-i02/2022.P148), Portuguese Blue Biobank under the Blue Economy Pact (Project Nº C644915664-00000026), and SAUDE&AQUA-II (operation code MAR-021.1.3-FEAMPA-00018), supported by MAR 2030, Portugal 2030 and the European Union.

Functional diets enriched with microalgae and macroalgae to enhance immune competence in Senegalese sole (*Solea senegalensis*) larvae

Mariana Hinzmann[1,*], Paulo Santos[1,4,5], Francisca Brito[1], André Cunha[1,4,5], Carolina Oliveira[2], Rita Teodósio[2], Luís E.C. Conceição[3], Ana T. Gonçalves[3,4], Sofia Engrola[2], Benjamín Costas[1,5]

[1]Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR-LA); University of Porto, Matosinhos, Portugal; [2]Centro de Ciências do Mar do Algarve (CCMAR/CIMAR-LA), Faro, Portugal; [3]SPAROS Lda., Olhão, Portugal; [4]Associação Oceano Verde (GreenCoLab), Faro, Portugal; [5]Instituto de Ciências Biomédicas Abel Salazar (ICBAS-UP), University of Porto, Porto, Portugal; *Presenting author.

Portugal stands as a leading producer of Senegalese sole in Southern Europe. Sustainable production depends on implementing strategies that enhance stress tolerance and optimise nutritional balance, crucial during larval development, impacting growth, health, and survival. Macro and microalgae have been recommended as good supplements for aquafeeds, as sustainable sources of bioactive compounds. This study aimed to evaluate the effects of dietary supplementation with bioactive-rich macro and microalgae, tested their synergistic potential on the immune response and stress resilience of Senegalese sole (*Solea senegalensis*) larvae. Experimental diets were formulated by incorporating a selected macroalgae (*Ulva* sp.) into a control commercial diet, combined with one of three microalgae (*Tetraselmis striata*, *Tisocrysis lutea*, and *Pavlova gyrans*), all at the same incorporation level. Larvae at 18 days after hatching (DAH) were fed the experimental diets for 20 days (S1). After this period, they were exposed to transport-induced stress and later challenged with *Tenacibaculum maritimum* (S2) to monitor larval survival and immune response. Immunological biomarkers, including lysozyme, antibacterial activity, peroxidase, and immune-related genes, were analysed in larvae after the growth phase (S1) and the challenge (S2). Larvae fed algae-supplemented diets showed different survival rates for *T. maritimum* infections. Specific combinations of macroalgae and microalgae had different synergistic effects on immune modulation. The most promising was *Ulva* sp. with *T. striata*, which showed higher larval resilience and immune competence against disease, though further studies are needed. These findings highlight the potential of using marine products as functional feed ingredients for early stages of Senegalese sole. This supports animal welfare and circular bioeconomy strategies.

Acknowledgements: This study was funded by “Pacto da Bioeconomia Azul” (Project No. C644915664-00000026) within the WP5 Algae Vertical, both co-funded by Next Generation EU European Fund and the Portuguese Recovery and Resilience Plan (PRR), under the scope of the incentive line “Agendas for Business Innovation” through the funding scheme C5 - Capitalization and Business Innovation; and by FCT contracts UID/04326/2025, UID/PRR/04326/2025 and LA/P/0101/2020 to CCMAR. MH supported by doi.org/10.54499/2023.07575.CEECIND/CP2848/CT0014.

Mantle immune response of the Mediterranean mussel (*Mytilus galloprovincialis*) to bacterial challenges

Hanne Hoornaert, Jennifer Mc Shane, Tatiana Alberto, Maoxiao Peng, Deborah M Power, João CR Cardoso*

Centro de Ciências do Mar do Algarve (CCMAR/CIMAR-LA), Campus de Gambelas, Universidade do Algarve, 8005-139 Faro, Portugal; *Presenting author

Production of marine bivalves is expanding globally but shifting environmental conditions and microbiota are an increasing challenge to growth and sustainability. Marine bacteria, *Vibrio spp*., are a diverse group containing well-known pathogens, that cause disease outbreaks in shellfish and can cause major losses in production. Bivalves, as filter feeders accumulate both non-pathogenic and pathogenic bacteria- including *Vibrio* - when exposed to contaminated waters. Dysbiosis and disease emergence are the outcome of the complex interaction between virulence factors in pathogens and the hosts immune system. In bivalves the response to pathogens depends on innate immunity but better characterisation of cellular and molecular immunity is needed to support the development of mitigation strategies that can prevent or reduce disease outbreaks. This study evaluated how *Vibrio* spp., diversity modified the immune response in the Mediterranean mussel (*Mytillus galloprovincialis*), the second most economically valuable farmed bivalve species worldwide. Mussels were injected in the adductor muscle with *Vibrio* spp., (10^7 cfu/ml) and changes in gene expression and the microbiota were assessed 16h (for molecular) and 96h (for microbiota) post-priming by looking at the mantle, the shell-building organ, and an emerging immune barrier. Mantle microbiome analysis revealed that immune challenges did not modify bacterial community diversity but increased their abundance towards protective bacteria genera. Mantle transcriptomes suggest that *Vibrio* injections modified expression of immune-related genes but high individual variability was found and that bacterial challenges likely down-regulated immune-related genes but stimulated expression of stress-response related genes.

Acknowledgements: This study received Portuguese national funds from FCT – Foundation for Science and Technology through contracts UID/04326/2025, UID/PRR/04326/2025 and LA/P/0101/2020 (DOI: 10.54499/LA/P/0101/2020), from the INNATEMEM project (DOI:10.54499/2022.08563.PTDC) and FCT, IP and Aga Khan Development Network (AKDN) FCT/AKDN/541666287/2019 HealthyBi4Namibe project. Thanks to Manuel Manchado (IFAPA, Spain) for providing the bacterial strains.

Tetrodotoxins in the Algarve: current evidence and potential implications for aquaculture

Sandra Lage

Centro de Ciências do Mar do Algarve (CCMAR/CIMAR LA), University of Algarve, Faro, Portugal

Introduction

Tetrodotoxin (TTX) is a potent neurotoxin, first identified in fish from the Tetraodontidae family, but also detected in marine invertebrates. A Human poisoning episode after consumption of trumpet shell *Charonia lampas*, likely caught off the Portuguese mainland southern coast – Algarve, together with the increasing reports of TTX in European waters, led the European Food Safety Authority (EFSA) to recommend maximum safe limits for Human consumption of shellfish meat. However, data on temporal and species incidence of TTX are lacking.

Objectives and methods

This presentation will summarise recent studies conducted at CCMAR aimed at evaluating potential vectors, tissue distribution, and seasonal variability of TTX and its analogues in marine organisms from Algarve. TTXs were analysed using liquid chromatography–high resolution mass spectrometry (LC-HRMS) in trumpet shells, sea stars (*Astropecten aranciacus*), and intertidal crabs (*Afruca tangeri* and *Carcinus maenas*).

Discussion and conclusions

Trumpet shell represents a potential public health risk due to its capacity to accumulate TTX and analogues while being consumed as a gourmet food. Although these toxins are mainly concentrated in non-edible tissues, unsafe levels may be transferred to edible parts, emphasising the need for European regulations to monitor TTX in seafood and to inform consumers about safe preparation practices. The origin of TTX in trumpet shells remains uncertain, but the detection of TTX and its analogues in their prey, the sea star, supports an exogenous acquisition pathway through the food web. Additionally, the occurrence of TTX analogues in crab species suggests the presence of multiple potential vectors along the Algarve coast, raising further questions about the risk of contamination in other commercially relevant marine species and the ecological dynamics of TTX transfer.

Acknowledgements

This work was supported by the European Union's Horizon 2020 research and innovation program through a Marie Skłodowska-Curie Individual Widening Fellowship (No. 101003376), the "la Caixa" Foundation (ID 100010434) through a Junior Leader Retaining Fellowship (LCF/BQ/PR23/11980049), and Portuguese national funds from FCT—Foundation for Science and Technology through projects UIDB/04326/2020, UIDP/04326/2020, and LA/P/0101/2020; the operational programs CRESC Algarve 2020; COMPETE 2020 through project EMBRC.PT ALG-01-0145-FEDER-022121.

Seasonal dynamics of parasites in gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) reared in earthen ponds in southern Portugal

Marcelo Livramento[1], Sónia Rocha[2,3], Luís Filipe Rangel[4], Rui Sousa[1], Carolina Ribeiro[1,5], Ivo Monteiro[5], Pedro Pousão-Ferreira[1,5], Cátia Lourenço-Marques[1,5], Florbela Soares[1,5,*]

[1]Collaborative Laboratory, Association for a Sustainable and Smart Aquaculture (S2AQUA), Olhão, Portugal;
[2]School of Medicine and Biomedical Sciences (ICBAS), University of Porto, Portugal;
[3]Institute for Research and Innovation in Health (i3S), University of Porto, Portugal; [4]CIIMAR/CIMAR LA, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Porto, Portugal;
[5]Portuguese Institute for the Ocean and Atmosphere/Aquaculture Research Station (IPMA/EPPO), Olhão, Portugal; [*]fsoares@ipma.pt

This study compared the prevalence and seasonality of internal and gill parasites of seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) cultured in earthen ponds supplied with seawater from the Ria Formosa, southern Portugal. Parasitic infections were monitored in forty specimens of each species, sampled throughout the four seasons, over one year. Biometrics and parasite prevalence were recorded for the parasitological screening. In *D. labrax*, *Amyloodinium ocellatum* reached 100% prevalence in summer and was absent in the other seasons, whereas *Diplectanum* sp. showed higher prevalence at temperatures below 20 °C. Internal parasite analysis revealed a higher prevalence of *Kudoa* sp. in winter (70%) and spring (60%), with infection disappearing during warmer periods. A similar trend was observed in *S. aurata*, with 80% prevalence of *A. ocellatum* infection in summer and 100% prevalence of *Lamellodiscus* sp. infection at temperatures below 20 °C. Myxosporean parasites (*Kudoa* sp., *Ceratomyxa* sp. and *Sphaerospora* sp.) were detected at low prevalence, with *Ceratomyxa* sp. infections occurring only in summer (40%). These results reveal evident seasonal patterns of parasitism and highlight the importance of continuous monitoring to improve fish health and promote sustainable aquaculture.

Acknowledgements

This study was funded by SAÚDE&AQUA II (MAR-021.1.3-FEAMPA-00018), INOVAQUA (MAR-021.1.3-FEAMPA-00004) and by FEDER and national funds (FCT) under operation no. 16118, operation code COMPETE2030-FEDER-00726900, in the scope of the Call for Applications No. MPr-2023-12.

Precision feeding: species-specific microdiet strategies for seabream and seabass

Cátia Lourenço-Marques[1,2,*], Inês Freitas[1], Morgana Angelo[1], Ana Candeias-Mendes[2], Renata Gonçalves[1], Bárbara Requeijo[1], Luís Conceição[3], Wilson Pinto[3], Pedro Pousão-Ferreira[1,2], Iris A. L. Silva[1]

[1]S2AQUA, Olhão, Portugal; [2]IPMA/EPPO, Olhão, Portugal; [3]SPAROS,Lda, Olhão, Portugal; *Presenting author.

Introduction

Early larval nutrition is fundamental for the growth, survival, and development of marine fish species. Optimising microdiet formulations to match species-specific digestive and physiological capacities is crucial to improve aquaculture performance and sustainability. This study comprised four experimental trials designed to assess the nutritional impact of innovative microdiets on growth and stress resilience in gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*).

Materials and Methods

Trials 1 and 2 examined how peptide size and hydrolysis level in microdiets affected larval growth and growth-related gene expression in seabream and seabass. Trials 3 and 4 assessed how different functional feeds influenced stress resilience and stress-related gene expression following acute stress.

Results

In Trial 1 and 2, seabream larvae fed the HIGH diet showed significantly greater total length and dry weight, whereas seabass fed the LOW diet achieved higher survival. Although differences in gene expression were not statistically significant, trends indicated diet-dependent modulation of growth-related genes. In Trial 3 and 4, seabass exhibited diet-specific responses to stress, with CTRL and DIET1 groups showing higher post-stress expression of stress-related genes, while DIET2–4 promoted lower expression changes. In seabream, no change was observed between diets.

Discussion and Conclusion

Together, these trials demonstrate that early nutritional programming through peptide profile optimisation and functional feed ingredients can significantly influence larval growth and molecular stress responses in a species-specific manner.

Acknowledgements

This study had the support of the projects “Pacto da Bioeconomia Azul” (Project No. C644915664-00000026) within the WP5 Algae Vertical, co-funded by Next Generation EU European Fund and the Portuguese Recovery and Resilience Plan (PRR), under the scope of the incentive line “Agendas for Business Innovation” through the funding scheme C5 Capitalization and Business Innovation, and Interface Mission cofinanced by PRR - Plano de Recuperação e Resiliência by the European Union (operation code 01/C05-i02/2022.P148).

Impact of macroalgae integration on seabream health: monitoring monogenean parasites in a recirculating aquaculture system

Ana C. Matias[1], João Araújo[1], Ana C. Carvalho[1], M. Carolina Ribeiro[1], Marisa Barata[1], Pedro Pousão-Ferreira[1,2], Florbela Soares[1,2,*]

[1]Portuguese Institute for the Ocean and Atmosphere/Aquaculture Research Station, Olhão, Portugal (IPMA/EPPO); [2]Laboratory, Association for a Sustainable and Smart Aquaculture, Olhão, Portugal (S2AQUA)
[*]fsoares@ipma.pt

Introduction: Integrating *Ulva ohnoi* into multitrophic recirculating aquaculture systems has been proposed as a sustainable approach to improve water quality, valorise by-products, and improve fish health. The health and welfare challenges posed by parasites in cultured fish have driven research towards sustainable solutions, such as bioactive compounds derived from macroalgae, namely *U. ohnoi*. This study evaluated the effects of macroalgae presence on monogenean parasite occurrence in *S. aurata* reared in systems with and without *U. ohnoi*.

Materials and Methods: Two identical recirculating aquaculture systems (RAS) were used to integrate *U. ohnoi* cultivation with *S. aurata* rearing. Fish were reared in systems with (A) and without (B) macroalgae to evaluate monogenean infection. Gill parasite prevalence was monitored, and parasite egg deposition was determined by placing cotton strips near the tank outlets. Environmental parameters were recorded.

Results: No significant differences were identified in fish growth between systems. Egg deposition on cotton strips was lower in the macroalgae system (A), particularly at temperatures between 20–21 °C. At lower temperatures (16–20 °C), egg counts were minimal in both systems, but as temperatures rose to around 20 °C, deposition increased markedly, with significantly higher counts in system B.

Discussion and Conclusion: Monogenean eggs deposition was significantly lower in the *U. ohnoi* system (A) at 20–21 °C. This suggests that the macroalgae may exert a potential inhibitory effect on monogenean reproduction or eggs accumulation, potentially contributing to the moderation of parasite cycles in RAS. Further long-term research is needed to confirm these results.

Acknowledgements: This work was financially supported by “Pacto da Bioeconomia Azul” (Project No. C644915664-00000026) within the WP5 Algae Vertical, funded by Next Generation EU European Fund and the Portuguese Recovery and Resilience Plan (PRR), under the scope of the incentive line “Agendas for Business Innovation” through the funding scheme C5 - Capitalization and Business Innovation, supported by the projects: SAÚDE&AQUA II (MAR-021.1.3-FEAMPA-00018); INOVAQUA (MAR-021.1.3-FEAMPA-00004) and AQUAFISH0.0 (funded by ERDF through the INTERREG Atlantic Area 2021-2027 programme (EAPA_0062/2022).

LiRRIEs in action: preliminary insights from the AZA4ICE experience in the Ria Formosa

Ana Catarina Matias[1,*], Laura Ribeiro[2], Joana Patrício Rodrigues[1], Florbela Soares[1], Domitília Matias[3], Pedro Pousão-Ferreira[1], Athanasios Koukounaris[4], Konstantina Marousi[4]

[1]Estação Piloto de Piscicultura de Olhão (EPPO), Portuguese Institute of the Sea and Atmosphere (IPMA), Av. do Parque Natural da Ria Formosa s/n, 8700-194 Olhão, Portugal; [2]Faculty of Sciences and Technology, University of Algarve, Campus de Gambelas, 8005-139, Faro, Portugal; [3]IPMA, Portuguese Institute for Sea and Atmosphere, Av. 5 de Outubro, 8700-305 Olhão, Portugal; [4]Department of Civil Engineering, School of Engineering, University of Patras, 26500 Patras, Greece; *Presenting author.

Introduction: Aquaculture remains a key economic sector in Portugal but faces multiple challenges, including limited public-sector capacity, outdated business models, complex regulations and weak stakeholder coordination. The AZA4ICE project addresses these issues by promoting sustainable aquaculture in close-to-coast and inland waters through a participatory methodology that engages stakeholders, fosters dialogue and knowledge exchange and supports the co-development of circular aquaculture strategies in the Ria Formosa Lagoon.

Materials and Methods: The LiRRIEs (Living Responsible Research and Innovation Ecosystems) methodology, developed under the AZA4ICE project, engages stakeholders from the Quintuple Helix model (academia, industry, government, civil society and the environment) in a participatory, multi-phase process to support the transition toward circular aquaculture. It involves stakeholder mapping, definition challenges, collaborative workshops and joint strategy development, with qualitative data analysed to assess participation, representativeness and stakeholder satisfaction.

Results: Two LiRRIEs meetings were held in Portugal under the AZA4ICE project to encourage stakeholder dialogue on circular aquaculture in the Ria Formosa Lagoon. In total, 28 participants attended, mostly from the government sector. The second meeting included 70% new participants and 30% returning ones, showing both outreach progress and ongoing engagement. Participants particularly valued the interactive discussions, though low attendance was identified as a key challenge.

Discussion and Conclusion: The LiRRIEs meetings in the Ria Formosa Lagoon effectively promoted dialogue and learning on circular aquaculture. Although attendance was limited, participants were highly engaged and increased their awareness of sustainable practices. The insights gained will guide the 3rd meeting, where stakeholders will validate results and draft an Action Plan to strengthen collaboration and circular initiatives.

Acknowledgements: This work was funded by the Interreg Euro-MED AZA4ICE (Euro-MED0200819).

Diet composition modulates the effects of swine protein hydrolysate on European seabass intestinal integrity and stress response while ensuring growth performance

M. Monteiro[1,2], D. Marques[1], A. Claro[1,2], T. Sá[1], B. Pinho[1], A. Almeida[3,4], L.M.P. Valente[1,2]

[1]CIIMAR/CIMAR LA, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Av. General Norton de Matos S/N, 4450-208 Matosinhos, Portugal; [2]Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal; [3]ETSA, Empresa Transformadora de Subprodutos, 2660-119 Loures, Portugal; [4]SEBOL, Comércio e Indústria do Sebo, S.A. (ETSA Group), 2660-119 Loures, Portugal; *Presenting author.

With the aquaculture industry's growing demand for sustainable protein sources, protein hydrolysates derived from by-products have emerged as promising functional ingredients for stress mitigation, due to their bioactive properties. As aquafeed formulations become increasingly complex, understanding the influence of diet composition on the efficacy of such functional ingredients is essential. This study evaluated the effects of including a swine protein hydrolysate (SH;1.5% and 3%) in plant-based (VEG) or processed animal protein-based (PAP) diets on growth performance, nutrient utilisation, intestinal integrity and short-term acute stress response in European seabass (*Dicentrarchus labrax*).

Results showed that VEG diets enhanced protein and energy digestibility and reduced phosphorus losses, but growth performance remained similar to PAP diets. Inclusion of SH did not affect growth performance; however, at 3%, basal metabolism was altered, intestinal lamina propria thickness decreased, and differences in acid goblet cell counts between CTRL PAP and CTRL VEG were mitigated. Following acute stress, haematological parameters remained stable, but metabolic and antioxidant responses differed between diets. PAP-fed fish showed higher plasma glucose and triglyceride levels, with SH1.5 reducing cortisol concentrations, whereas VEG-fed fish exhibited enhanced antioxidant activity, indicated by elevated glutathione peroxidase and a dose-dependent increase in catalase activity. Collectively, these findings indicate that the efficacy of hydrolysates is strongly influenced by both diet composition and inclusion level, with low doses supporting nutrient utilisation and stress adaptation, while higher doses modulate intestinal architecture and basal metabolic processes.

Acknowledgements

This work was partially funded by the Blue Bioeconomy Innovation Pact (C644915664-00000026) and supported by the Portuguese Foundation for Science and Technology (FCT) through a grant to Ana Claro (2024.00449.BDANA).

From animal by-products to functional protein hydrolysates: circular feed solutions for Mediterranean aquaculture

M. Monteiro[1,2,*], A. Alves de Oliveira[1,2], L. Rodrigues-dos-Santos[1-4], A. Claro[1,2], D. Marques[1], A. Marques, T. Sá[1], A. Almeida[3,4], L.M.P. Valente[1,2]

[1]CIIMAR/CIMAR LA, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Av. General Norton de Matos S/N, 4450-208 Matosinhos, Portugal; [2]Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal; [3]ETSA, Empresa Transformadora de Subprodutos, 2660-119 Loures, Portugal; [4]SEBOL, Comércio e Indústria do Sebo, S.A. (ETSA Group), 2660-119 Loures, Portugal; *Presenting author.

Aquaculture has become a key solution to meet the growing global demand for protein, supplying over half of the fish consumed worldwide. However, its continued expansion faces challenges, particularly regarding the availability and quality of available feed ingredients. As fishmeal becomes an increasingly strategic ingredient, the industry must explore alternative strategies that ensure both nutritional adequacy and fish robustness under challenging feed formulation scenarios.

The Pep4Fish project highlights the potential of protein hydrolysates (PHs) derived from animal by-products, as functional feed ingredients in aquaculture. This project focused on the development of highly digestible protein sources rich in bioactive peptides, while contributing to circular economy principles in feed production. These hydrolysates were tested in four experimental trials using key Mediterranean species, European seabass (*Dicentrarchus labrax*) and Gilthead seabream (*Sparus aurata*), with dietary formulations that reflect current aquaculture practices.

Among the tested hydrolysates, swine hydrolysate demonstrated strong potential by maintaining feed intake, macronutrient digestibility, and whole-body composition, even when replacing high-quality fishmeal and a commercially valuable fish hydrolysate. However, its impact on growth performance and somatic indexes varied depending on dietary formulation, suggesting alterations in lipid metabolism as a key metabolic response. These findings reinforce the viability of hydrolysed animal by-products as functional ingredients in aquafeeds, supporting both waste valorisation and circular feed solutions to reduce pressure on marine resources while offering alternatives.

Acknowledgements: This work was supported by the Blue Bioeconomy Pact (C644915664-00000026), for the exercise of activities in the WP6 FEED (Pep4Fish project) through international funds provided by the European Union. AAO, AC and LRS acknowledge FCT for their PhD grants (2023.02611.BD; 2023.00947.BDANA; 2024.00449.BDANA).

Assessing the resilience of European flat oysters (*Ostrea edulis*) under challenging environmental conditions

Cláudia Moura[1], Simão Correira[1], Helena Barbosa[1], Inês Ferreira[1], Sandra Ramos[1,2], Benjamin Costas[1], Vânia Freitas[1], Sérgio Fernández-Boo[1]

[1]Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR LA), University of Porto, Matosinhos, Portugal; [2]Biology Department, Sciences Faculty, Porto University, Rua do Campo Alegre, 687, 4169-007 Porto, Portugal.

Introduction: The European flat oyster (*Ostrea edulis*), a species of high ecological and economic value, has largely vanished from European coasts due to overexploitation, disease, and habitat loss. Its reintroduction faces challenges, particularly under climate change (e.g., rising temperatures, salinity stress, and pathogens), that affects its resilience. This project evaluates the feasibility of reintroducing *O. edulis* as a native species to support sustainable aquaculture and ecosystem restoration.

Materials and Methods: Juvenile oysters from two distinct origins, Mediterranean Sea (Sardinia, Italy) and Northern Sea (Koster Islands, Sweden), were deployed in February 2025 in a commercial oyster farm in Lima estuary (NW Portugal), characterised by strong salinity and temperature variations. Monthly assessments included survival, growth, pathogen prevalence (*Bonamia ostreae*, *Marteilia refringens*), and microplastic contamination, as well as continuous environmental monitoring to detect stress events like flooding and heat waves. Genomic analysis of surviving oysters will identify resilience markers against pathogens and environmental stressors.

Results: Preliminary results show significant survival differences by origin (log-rank test, $p<0.0001$), with Italian oysters having lower mortality (5%) than Swedish oysters (45%) after 7 months. Swedish oysters showed slow growth in shell length and weight (≤0.12 mm day^{-1} and ≤0.03 g day^{-1}), peaking in early summer (June–July). By contrast, the Italian stock exhibited delayed growth, becoming apparent only after grading in late July, with maximum daily growths of ≤0.12 mm day^{-1} and ≤0.02 g day^{-1}. All oysters analysed so far contained microplastics, with higher concentrations in the Italian stock and during winter (March) compared to summer (August).

Discussion and Conclusion: Both oyster stocks can survive under challenging estuarine conditions, although the Atlantic stock showed poorer survival. Ongoing monitoring will determine long-term growth performance and resilience, and genomic analysis will help identify the best genotype for Lima's estuary commercial production and restoration purposes.

Acknowledgements: This work is developed within the project ShellFishBoost (SBEP/0009/2023), financed by Sustainable Blue Partnership, European Union and FCT.

Effects of spirulina and black soldier fly meal on Nile tilapia under acute thermal stress

Sara Moutinho[1,*], Diogo Filipe[1], Paulo Rema[1,2], Nabil Ibrahim[3,4], Deogratias Mulokozi[5], Leonor Nunes[1], António Marques[1,6], Luisa M.P. Valente[1,7]

[1]CIIMAR/CIMAR LA, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto; [2]UTAD, Universidade de Trás-os-Montes e Alto Douro; [3]CLAR, Central Lab for Aquaculture Research, Abou Hammad, Sharkia, Egypt; [4]MSA, University of Modern Science and Arts, Egypt; [5]TAFIRI, Tanzania Fisheries Research Institute, Tanzania; [6]IPMA, Instituto Português do Mar e da Atmosfera, Divisão de Aquacultura, Valorização e Bioprospeção; [7]ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto. *Presenting author.

African aquaculture is rapidly expanding, providing protein and supporting economic stability. To promote sustainable production, the INNOECOFOOD project evaluates local feed alternatives, such as spirulina (SPIR) and black soldier fly larvae meal (BSF), within ECOHUBS. Nile tilapia (*Oreochromis niloticus*), valued for fast growth, is highly sensitive to temperature fluctuations, which are increasingly exacerbated by climate change. This study aimed to assess the effects of SPIR and BSF inclusion on juvenile tilapia responses to an acute cold challenge. Five diets (35% protein, 8% lipids) were tested: a control with 5% fishmeal (FM; diet CTRL); partial FM replacement (25%) with BSF (BSF25) or spirulina (SPIR25); and total FM replacement with either 75% BSF + 25% spirulina (BSF75_SPIR25) or 100% BSF (BSF100). Fish (initial weight 8.2 ± 0.03 g) were fed for 71 days at 27°C (thermoregulated RAS system), then temperature was reduced overnight to 15 °C. Liver antioxidant status and plasma metabolites profile were assessed. Under normal growing conditions, SPIR-diets lead to lower superoxide dismutase and glutathione S-transferase activities than BSF25, and SPIR25 showed lower glutathione peroxidase (GPX) activity than diet BSF25. After the cold stress, fish fed diet BSF100 showed increased GPX activity compared to the control. Regarding plasma metabolites, under normal growing conditions, the inclusion of 25% SPIR (SPIR25 and BSF75_SPIR25) increased plasma glucose compared to BSF25 and triglycerides relative to the CTRL. Plasma cholesterol was also higher than in fish fed BSF100. Furthermore, albumin (ALB), globulin (GLOB), and total protein (TP) were higher in SPIR25-fed fish under normal conditions, whereas after cold stress, BSF25-fed fish showed higher TP and GLOB, and BSF-only diets showed a lower ALB/GLOB ratio compared to the CTRL. In conclusion, while SPIR influenced plasma metabolites under normal conditions, BSF-containing diets were more effective in enhancing antioxidant and immune responses during acute stress. These results highlight the potential of these alternative ingredients, especially BSF, for improving Nila tilapia performance. **Acknowledgements**: This project has received funding from the European Union's Horizon Innovation action under Grant Agreement no. 101136739 (INNOECOFOOD). Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union, nor the granting authority can be held responsible for them.

Effects of environmental enrichment on inhibitory control in juvenile gilthead seabream (*Sparus aurata*): a cognitive study using the cylinder task

Inês Neves[2,*], João L. Saraiva[1,2], Maria Cabrera-Álvarez[1, 2]

[1]FishEthoGroup Association, 8005-226 Faro, Portugal; [2]Fish Ethology and Welfare Group, Centre of Marine Sciences (CCMAR/CIMAR LA), University of Algarve, 8005-139 Faro, Portugal; *a69028@ualg.pt

As global demand for seafood increases, aquaculture has become the fastest-growing food sector, yet intensive farming practices often compromise fish welfare. Recent research highlights that fish are sentient and possess complex cognitive abilities, highlighting the need to align farming conditions with their behavioural and mental needs. Environmental enrichment (EE) has emerged as a promising tool to enhance welfare by stimulating natural behaviours and cognition. This study aims to evaluate the cognitive abilities of gilthead seabream (*Sparus aurata*) through the Cylinder Task and to assess whether environmental enrichment influences their cognitive performance and welfare. The study was conducted at CCMAR within the ManyFishes project, a collaborative initiative dedicating to assessing fish cognition across species. Forty juvenile gilthead seabream were housed in enriched (EE) and non-enriched (NE) tanks under to evaluate the effects of environmental enrichment on cognitive performance. Fish underwent a four-phase experimental protocol based on the Cylinder Task: plate training, cylinder familiarisation, forced cylinder, and an inhibitory control test. Fish from enriched environments showed significant improvement in task performance across training trials, indicating learning and engagement. Although EE did not significantly affect overall success rates in the inhibitory control test, these fish displayed higher behavioural engagement and effort levels compared to non-enriched fish. Individual analyses revealed substantial variability in learning performance, with four distinct behavioural clusters reflecting differences in learning rate, latency, and exploratory activity. These findings suggest that environmental enrichment enhances motivation and engagement, while individual behavioural traits play a key role in cognitive performance. Larger fish and proactive, exploratory individuals consistently performed better, highlighting the role of individual differences in shaping task outcomes. These results emphasise that while EE can improve motivation and welfare in captive fish, its benefits for complex cognitive tasks may depend on individual characteristics.

A macroalgae aquaculture study case in the digital twin of the ocean

Gabriela Oliveira[1,*], Irene Martins[1], Martinho Marta-Almeida[2], Isabel Sousa Pinto[1]

[1]Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR LA), University of Porto, Matosinhos, Portugal; [2]Centre for Environmental and Marine Studies (CESAM), University of Aveiro, Aveiro, Portugal; *Presenting author.

Introduction

DTO-BioFlow project (Integration of biodiversity monitoring data into the Digital Twin Ocean) supports the EU Mission to restore oceans and waters by 2030, activating access to hidden biodiversity data and integrating processed data flows into the EU Digital Twin Ocean. By linking models, algorithms, and continuous data streams, the biological component of the DTO is strengthened to support evidence-based ecosystem protection and restoration. A case study on macroalgae mariculture has been developed within the DTO-BioFlow project, aiming to develop an integrated dynamic and spatial ecological model of kelp aquaculture (*Laminaria ochroleuca* and *Saccorhiza polyschides*) along the southern Portuguese coast.

Materials and Methods

A simulation of kelp growth and biomass using mechanistic dynamic models under current and future climate scenarios has been developed for the south of Portugal. The model incorporates grazing equations to quantify biomass loss from fish herbivory and also considers seaweeds' biomass interactions, assessing biodiversity effects. A map of habitat suitability and species distribution models (GAM/Niche-GAM/MAXENT) will be applied to identify optimal sites for aquaculture. These data and models will be implemented into the DTO-BioFlow framework to create a replicable approach for sustainable ocean management.

Results

Preliminary results for growth and biomass production under current and future climate scenarios can be accessed.

Discussion and Conclusion

Connecting data streams, models and algorithms, it strengthens the DTO's biological foundation for evidence-based ecosystem management.

Acknowledgements: This work integrates the DTO-BioFlow Project, funded by the European Union. The institutional support of CIIMAR, The Coastal Biodiversity Lab and The Marine Ecosystem Modelling Group. Special thanks to Irene Martins and Martinho Marta-Almeida for the collaborations.

The effect of pain on gilthead seabream (*Sparus aurata*) behaviour

Gonçalo Oliveira[1,2,*], Jose Carlos Campos-Sánchez[2,3], Maria J. Cabrera-Álvarez[1,2], João L. Saraiva[1,2]

[1]FishEthoGroup Association, 8005-226 Faro, Portugal; [2]Fish Ethology and Welfare Group, Centre of Marine Sciences (CCMAR/CIMAR LA), University of Algarve, 8005-139 Faro, Portugal; [3]Immunobiology for Aquaculture Group, Department of Cell Biology and Histology, University of Murcia, 30100 Murcia, Spain;

*Presenting author.

Introduction

Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Electrophysiological and neuroanatomical studies found that in fish the nociceptors accumulate predominantly in the lips. The objective of this study was to evaluate the behavioural indicators of pain of gilthead seabream (*Sparus aurata*) after inducing a noxious stimulus through a subcutaneous injection of acetic acid (AA) into the lips.

Materials and Methods

Forty specimens were individually placed in tanks and recorded for 30 minutes. The fish were then sedated and pierced subcutaneously in the lips (sham group), injected with PBS (control group), or 10% of AA. Recordings of 15 minutes were made at different times after injection, and potential alterations in behaviour were analysed with BORIS software in a blinded test.

Results

Sham and control groups suffered no variation over time in any of the observed behavioural indicators, while fish injected with 10% AA showed a decrease in the time spent active. Additionally, fish injected with 10% AA increased the time spent at the bottom of the tanks when compared to other experimental groups. Regarding fish activity, individuals injected with 10% AA decreased the number of movements to the top of the tank at all experimental times when compared to the control group.

Discussion and Conclusion

The painful experience of AA at a concentration of 10% evokes clear and distinctive behavioural changes. As there are potential painful stimuli under seabream aquaculture production, future studies on nociceptive mechanisms are imperative to prevent negative consequences and promote greater awareness for fish welfare in aquaculture.

Acknowledgements

This project was funded by FCT – Foundation for Science and Technology (UIDB/04326/2020 and LA/P/0101/2020) and “Programa para la recualificación del sistema universitario español para 2021-2023 - Margarita Salas Modality (R-1593/2022)”.

Development of an *in vitro* model for European seabass: from immune cell isolation to single-cell characterisation

S. Oliveira[1,2], M. Ferreira[3], P. Costa[4], A. Correia[5,6], L.M.P. Valente[1,2], V. Kiron, S. Gomes[1,2,*]

[1]CIIMAR/CIMAR LA, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Matosinhos, Portugal; [2]ICBAS-Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, Porto, Portugal; [3]Faculty of Biosciences and Aquaculture, Nord University, Bodø, Norway; [4]BioFabics, Porto, Portugal; [5]i3S Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal; [6]IBMC, Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal; *Presenting author.

Introduction: The aquaculture industry requires advanced in vitro models to reduce live-animal testing and study host-microbe interactions. European seabass (*Dicentrarchus labrax*) is economically important in Mediterranean aquaculture, yet comprehensive immune cell atlases and defined protocols for studying its immune-microbiota axis in vitro remain scarce. This work aimed to develop an in vitro model by optimising immune cell isolation protocols and characterising immune cell populations through single-cell RNA sequencing.

Materials and Methods: The head kidney (HK) were collected, and tissues were mechanically dissociated through cell strainers, and cell suspensions were layered on a Percoll gradient. The intermediate band containing enriched leucocyte populations was collected. For immune cell characterisation, single-cell RNA sequencing (scRNA-seq) was performed on HK-derived cells to generate a high-resolution immune cell atlas.

Results: The optimised isolation protocol yielded heterogeneous cell populations. The scRNA-seq identified all major leucocyte lineages, including B cells, T cells, macrophages, erythrocytes, and natural killer-like cells. Identity validation was achieved using conserved marker genes, providing a comprehensive picture of immune cell diversity in seabass.

Discussion and Conclusion. This work provides critical tools for understanding the immune-microbiota axis in seabass. The cell isolation protocol and scRNA-seq offer unique resolution of immune cell populations in this species. This work represents a critical first step towards understanding the immune-microbiota axis in seabass.

Acknowledgements: This project is funded by BASSinCELLS (http://doi.org/10.54499/2022.08942.PTDC), PRR-Innovation Pact, supported by European Commission (nº02/C-05-i01/2022), by the strategic funding UIDB/04423/2020 and UIDP/04423/2020 and LA/P/0101/2020 (https://doi.org/10.54499/LA/P/0101/2020), and through the grant awarded to Santos, S. (2024.02006.BDANA) through national funds provided by FCT.

Harmful algae grazing fear: a tool to predict bivalve biotoxin contamination

Maria F. Pais

Centro de Ciências do Mar do Algarve (CCMAR/CIMAR LA), University of Algarve, Faro, Portugal

Harmful Algal Blooms (HABs) threaten coastal ecosystems and aquaculture by contaminating seafood with biotoxins, leading to economic, environmental, and public health risks. Current methods for predicting biotoxin accumulation in bivalve molluscs are limited. This project proposes an innovative approach to predict biotoxin accumulation in bivalve molluscs by integrating insights from predator-prey chemical interactions, specifically through copepodamides (polar lipids produced and released by copepods) that induce defensive traits (e.g., toxin production and bioluminescence) in harmful algae. We intend to (1) determine the copepodamides profile of copepods from the NE Atlantic and evaluate the effects of feeding and temperature on their production by copepods; (2) isolate copepodamides from natural populations; (3) assess copepodamides toxin and bioluminescence induction in dinoflagellates and if these inductions are strain- and/or temperature-dependent; (4) measure copepodamides concentrations in bivalve molluscs and passive samplers, alongside harmful algae cell counts and oceanographic data, to optimize bivalve molluscs biotoxin contamination predictive models. The present project will improve the accuracy of predictions regarding bivalve molluscs contamination by biotoxins. This will help determine optimal harvesting periods, preventing unnecessary harvesting costs and the destruction of contaminated products. The outcomes will have significant socio-economic benefits for the bivalve molluscs industry and play a crucial role in preventing shellfish poisoning. This abstract presents a granted PhD research plan. Although the project has already begun, no experimental results are available at this stage.

Acknowledgements

This project has received funding from the “la Caixa” Foundation (ID 100010434) through a Junior Leader Retaining Fellowship (LCF/BQ/PR23/11980049), and Portuguese national funds from FCT—Foundation for Science and Technology through projects UIDB/04326/2020, UIDP/04326/2020, LA/P/0101/2020 and FCT PhD Studentship in a Non-Academic Environment (2025.00661.BDANA); the operational programs CRESC Algarve 2020; COMPETE 2020 through project EMBRC.PT ALG-01-0145-FEDER-022121.

Replacement of fish meal with crustacean meals in diets for long-snouted seahorse, *Hippocampus guttulatus*: digestibility and growth performance

Jorge Palma[1], Miguel Correia[2,3], José Pedro Andrade[1], Dominique Bureau[4]

[1]Centro de Ciências do Mar do Algarve (CCMAR/CIMAR LA), University of Algarve, Faro, Portugal; [2]Seahorse, Pipefish, and Seadragon Specialist Group, IUCN Species Survival Commission, Gland, Switzerland; [3]Project Seahorse, Institute for the Oceans and Fisheries, The University of British Columbia, Vancouver, Canada; [4]UG/OMNR Fish Nutrition Research Laboratory, Department of Animal and Poultry Science, University of Guelph, Guelph, Canada

In marine aquaria, seahorses (*Hippocampus* spp.) are highly valued for their distinctive morphology and unique reproductive behaviour. Although adult seahorses can be maintained on frozen natural diets, these can be nutritionally enhanced to better meet their dietary requirements. This study evaluated the effects of partially replacing fish meal with krill and copepod meals in inert diets, co-fed with shrimp, on the growth and nutrient digestibility of the long-snout seahorse (*Hippocampus guttulatus*). A control diet (Diet 1) containing raw starch and four experimental diets with comparable protein (≈44.8%) and energy (≈15.1 MJ/kg) levels were tested. Diet 2 included fish meal as the sole protein source, whereas in Diets 3–5, 44% of the fish meal was replaced with krill and copepod meals. Seahorses co-fed shrimp and Diets 2–5 exhibited significantly higher growth rates ($p < 0.05$) than those receiving shrimp + Diet 1, with no significant differences among Diets 2–5. Apparent digestibility of dry matter (46.1–72.2%), lipids (73.3–85.5%), crude protein (89.8–95.8%), energy (82.0–92.2%), and phosphorus (28.7–64.4%) varied across diets and was consistently lowest in shrimp + Diet 1. These results demonstrate that supplementing shrimp with nutritionally balanced inert diets enhances growth and nutrient utilisation in *H. guttulatus*. Moreover, despite their agastric physiology, seahorses effectively digest key nutrients and energy, with digestibility reflecting diet quality. This co-feeding approach is suitable for medium- to large-sized *Hippocampus* species capable of ingesting larger prey; adaptations are needed for smaller species. Overall, crustacean meals represent viable and sustainable alternatives to fish meal in inert diets, supporting more efficient and environmentally responsible seahorse husbandry.

Acknowledgements: This study was funded by the project HIPPONUTRE—"Cultivo do cavalo marinho de focinho comprido, *Hippocampus guttulatus*: Optimização zootécnica e avaliação de requisitos nutricionais" (Programa Operacional MAR2020, 16-02-01-FMP-54) and received Portuguese national funds from FCT - Foundation for Science and Technology through contracts UID/04326/2025, UID/PRR/04326/2025 and LA/P/0101/2020 (DOI:10.54499/LA/P/0101/2020).

INNOECOFOOD Project: ensuring food safety through agroecological aquaculture and smart EcoHubs in Africa

Mariana Palma[1,*], Marta Dias[1], Alícia Pereira[1], Sara Moutinho[1], Cheila Almeida[1,2], Amparo Gonçalves[1,2], Carla Pires[1,2], Luísa M. P. Valente[1,3], Leonor Nunes[1], António Marques[1,2]

[1]CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, Porto, Portugal; [2]IPMA - Portuguese Institute for the Sea and Atmosphere, Algés, Portugal; [3]ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal; *Presenting author.

Introduction: The INNOECOFOOD project purposes to establish sustainable food systems by developing innovative, circular production ECOHUBs and enhance aquaculture farms in lakes across four African countries: Kenya, Tanzania, Ghana, and Egypt. This action aims to promote food production under sustainable agroecological principles and improve food and nutrition security in these countries. The ECOHUBs are facilities where local communities will produce and process nutritious foods: fish (catfish and tilapia), spirulina and insects (black soldier fly and crickets), as products specially developed in balance with the local ecosystems and cultures.

Material and Methods: This farm to fork approach will combine technological innovation with sustainable practices to enhance food safety across the production chain. Sensors and AI-controlled systems will monitor critical production parameters to reduce contamination risks and optimise production and post-harvest processing. In parallel, solar-powered drying, cooling, and extrusion equipment will be used to process products. All production and processing procedures will be designed to produce products under Good Manufacturing Practice and Good Hygiene Practice, while ensuring microbiological and chemical safety. Life Cycle Assessment and digital certification tools will validate nutritional quality, environmental sustainability, and compliance with food safety standards, enabling secure trade within AU and EU markets. Circular practices upcycle by-products (e.g. fish waste, insect residue) into animal feed, fertilisers, or new food products, minimising food loss and environmental impact. To improve local capacity, 120 women and 120 youth will receive in-person training in food safety, quality control, and sustainable production practices within the ECOHUBs. Additionally, over 5000 community members will access training materials digitally.

Results: The INNOECOFOOD project is now halfway through, the four agroecological ECOHUBs are being established, as well as the processing units for fish, spirulina and insects, and have already delivered promising results

Acknowledgement: This work was supported by project “INNOECOFOOD”, under the EU Horizon 2024 Research and Innovation program, grant agreement No.101136739, and the UKRI grant No. 10110792.

NMR-Metabolomics in aquaculture - case studies from farmed fish species

Mariana Palma[1,2,*], Ludgero C. Tavares[3], Margarida Coelho[4,5,6], John G. Jones[5,6], Ivan Viegas[1]

[1]Centre for Functional Ecology, Associate Laboratory TERRA, Department of Life Sciences, University of Coimbra, Coimbra, Portugal; [2]current address: Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR LA), University of Porto, Matosinhos, Portugal; [3]CIVG - Vasco da Gama Research Center / EUVG - Vasco da Gama University School, Lordemão Campus, Coimbra, Portugal; [4]Department of Life Sciences, University of Coimbra, Coimbra, Portugal; [5]Center for Neuroscience and Cell Biology (CNC-UC), Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal; [6]Center for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, Coimbra, Portugal; *Presenting author.

Introduction: Nuclear Magnetic Resonance (NMR)-Metabolomics is a powerful approach to assess metabolome in several aquaculture species, providing an expeditious tool for metabolite profiling and to evaluate responses to various production conditions, such as nutritional or environmental challenges. It can also be applied to several different tissues (muscle, liver, intestine, plasma), while providing complementary information to other Omics approaches. In recent years, we have applied NMR-Metabolomics in diverse studies thus contributing to an integrated analysis of biological systems within real aquaculture production scenarios/challenges. **Materials and Methods:** In all studies, well-established metabolomics workflows were followed: tissue aqueous fraction extraction, NMR spectra acquisition, spectra processing, and data analyses by multivariate analysis. **Results:** The NMR-Metabolomics approach was applied to: i) Assess the effects of dietary glycerol supplementation on the muscle and liver metabolome of rainbow trout (*Oncorhynchus mykiss*) and European seabass (*Dicentrarchus labrax*), aiming to spare dietary protein for growth; ii) To profile and evaluate the effects of a high digestible starch content diet on the muscle and liver metabolome of barramundi (*Lates calcarifer*) as a complementary approach to the assessment of the hepatic glycogen synthesis; iii) To evaluate the metabolic effects of tributyrin supplementation (known to reduce the negative effects of high inclusion levels of plant protein) in rainbow trout (*O. mykiss*) fed a plant-based diet, combined with the hepatic and intestinal mRNA analysis to assess tissue-specific responses; and iv) To analyze the metabolomic profiles of plasma and digesta in 1 selected and 2 commercial strains of rainbow trout (*O. mykiss*) fed plant- or fishmeal-based diets to support histological data on the onset of enteritis. **Conclusions:** NMR-based metabolomics proved to be a suitable tool to investigate the effects of alternative diets and disease onset in aquaculture species, contributing to more sustainable and resilient aquaculture production systems. **Acknowledgements:** NMR data was collected at the UC-NMR facility which is supported in part by FEDER – European Regional Development Fund through the COMPETE Programme (Operational Programme for Competitiveness) and by National Funds through FCT RECI/QEQ-QFI/0168/2012, CENTRO-07-CT62-FEDER-002012, and also through support to Rede Nacional de Ressonância Magnética Nuclear (RNRMN) and to Coimbra Chemistry Centre through grant UID/QUI/00313/2019. Supported by FCT and co-funded by FEDER) through the Operational Programme for Competitiveness and Internationalization (POCI/POFC) and structural funds Centre for Functional Ecology UIDB/04004/2020; Associate Laboratory TERRA LA/P/0092/2020.

Modulation of mucosal immunity and neuroendocrine responses by dietary tryptophan supplementation in dip-vaccinated European seabass (*Dicentrarchus labrax*)

Diogo Peixoto[1,2,*], Inês Carvalho[1,2], André Cunha[1,2], Rita Azeredo[1,2], Benjamín Costas[1,2]

[1]CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Matosinhos, Portugal; [2]ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal. *dpeixoto@ciimar.up.pt

This study evaluates the combined effects of dietary tryptophan supplementation and dip vaccination on skin and intestinal mucosal immunity of European seabass, as well as the complex interaction between neuroendocrine-immune systems.

European seabass juveniles (26.23 ± 7.22g) were randomly assigned to 2 recirculating seawater systems. In a completely randomised design, fish received either a control diet (CTRL) or CTRL-supplemented diets with tryptophan (TRP1 - 0.05% and TRP2 - 0.5% DM), each in triplicate tanks *per* system. After 3 days of feeding, three fish *per* treatment from system 1 were euthanised for sampling: skin mucus and blood (immune status), brain (hypothalamus), pituitary, head kidney (qPCR), and intestine (shotgun proteomics). Remaining fish were dip-vaccinated and sampled at 1h, 6h, and 21 days post-vaccination. They continued on the same diets for 3 days post-vaccination, then switched to the CTRL diet until day 21. Fish in system 2 followed the same feeding regime but were not disturbed. Short-term tryptophan supplementation before vaccination had limited systemic effects but moderately influenced early mucosal immunity, notably by increasing IgM and metabolic reprogramming. The lower dose more effectively modulated the immune-neuroendocrine axis, reducing cortisol, enhancing antioxidant capacity, and promoting coordinated local and systemic immune activation. In contrast, the higher dose induced persistent stress responses with elevated cortisol and failed to achieve immune-endocrine balance, indicating metabolic-immune misalignment. Long-term, TRP1 supported post-vaccination homeostasis similar to non-vaccinated controls, whereas TRP2 resembled control-vaccinated fish, showing signs of metabolic strain and immune suppression.
These results highlight the importance of dosage and timing in functional feeds, with TRP1 emerging as a promising option to boost vaccine response without increasing stress.

This work was supported by IMMUNAA project (PTDC/CVT-CVT/7741/2020), financed through FCT. DP, IC, BC, and RA were supported by FCT (UI/BD/150900/2021, 2021.04867.BD, 2020.00290.CEECIND and 2022.03248.CEECIND, respectively).

GRINNAQUA insights from stakeholder engagement: addressing challenges and opportunities in aquaculture

T. Pereira[1,*], I. Oliveira[2], Â. Pécurto[3], F. Avelelas[4], A. Correia[5], T. Aires[6], P. Nabais[7], R. Ribeiro[8], M.T. Dinis[9], R. Mamede[10], S. Gomes[1], A. Jardim[11], P. Abreu[12], R. Nunes[12], R. Oliveira[13], A. Pereira[14], T. Batista[15], J. Fernandes[16], N. Leite[17], T. Poletto[18], I. Viegas[19], P. Vinagre[20], A. Marques[21], A.L. Maulvault[21], P. Anacleto[21], N. Bandarra[21], A. Faria[22], M. Palma[1], B. Costas[1], H. Oliveira[21], L. Ramos Pinto[1], M.L. Nunes[1], N. Lourenço[23]

[1]Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR); [2]Algaplus; [3]Associação Nacional de Comerciantes e Industriais de Produtos Alimentares (ANCIPA); [4]Associação Portuguesa de Aquacultores (APA); [5]Aquanostra; [6]Aquasoja; [7]Autoridade de Segurança Alimentar e Económica; [8]Blue Bioeconomy CoLAB (B2E); [9]Centro de Ciências do Mar (CCMAR); [10]Centro de Estudos do Ambiente e do Mar (CESAM); [11]Direção Geral de Alimentação e Veterinária (DGAV); [12]Direção Geral de Politica do Mar; [13]Direção-Geral de Recursos Naturais, Segurança e Serviços Marítimos; [14]Instituto de Conservação da Natureza e das Florestas (ICNF); [15]Instituto Politécnico de Leiria (IPL); [16]Oceano Fresco; [17]Seaentia; [18]SPAROS; [19]Universidade de Coimbra; [20]WAVEC; [21]Instituto Português do Mar e da Atmosfera; [22]AirCentre; [23]CoLab + Atlantico; *tania.pereira@ciimar.up.pt

The GRINNAQUA project is dedicated to enhancing sustainable aquaculture practices through research, collaboration, and innovation. As part of this initiative, an event was organised, in collaboration with the MycoFish (*PTDC/CVT-CVT/2660/2021*) and AquaClimAdapt (*PTDC/CTA-AMB/0592/2021*) projects to gather together academia, aquaculture industry stakeholders, and policymakers, in equal representation. This event provided a platform for open discussions on both the broad challenges facing the aquaculture sector and the specific topics that are crucial for its future. The conversations covered a range of topics, from the use of artificial intelligence in aquaculture and the role of functional feeds in fish health to concerns about food safety and animal welfare. Beyond these focused discussions, participants also tackled larger, industry-wide challenges such as sustainability, regulatory pressures, market access, and the need for innovation. A key point of focus was how different policies—from environmentally protective measures to more liberal market approaches—could influence the sector's growth and sustainability, either presenting opportunities or posing barriers. The findings from these discussions served as the basis for developing position papers and policy briefs, which aim to guide future research and inform policy decisions. This poster summarises the key takeaways from these events, focusing on the challenges identified, the policy recommendations proposed, and the strategies suggested to help the aquaculture sector grow in a sustainable and competitive way. *GRINNAQUA is co-funded by the European Union (GA 101079467) and the UK Research and Innovation (UKRI). Views and opinions expressed are however those of the authors only and do not necessarily reflect those of the European Union, the Research Executive Agency (REA) or the UKRI. Neither the European Union nor the granting authorities can be held responsible for them.*

Chronic thermal stress compromises innate immunity in juvenile rainbow trout (*Oncorhynchus mykiss*), a comparative analysis of plasma and molecular markers

Christopher Pimentel[1,2,*], Bruno Reis[1], Francisco Cunha[1], Rita Azeredo[1], Benjamín Costas[1,2]

[1]Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Universidade do Porto, Avenida General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal; [2]Instituto de Ciências Biomédicas Abel Salazar (ICBAS-UP), Universidade do Porto, Rua de Jorge Viterbo Ferreira nº 228, 4050-313 Porto, Portugal; *cpimentel@ciimar.up.pt

Climate change-induced stressors like rising water temperatures induce a state of chronic stress in fish compromising growth, immune function, and survivability by imposing a heavy physiological load. This study focuses on how chronic stress induced by a simulated heat wave affects rainbow trout (*Oncorhynchus mykiss*), with particular focus on its impact on the immune system. Juvenile rainbow trout (approx. 11 g) were placed in control (16 °C), moderate (19 °C) and high temperature conditions (22 °C) to mimic an environmental heat wave for a duration of 4 weeks and then sampled. Blood and plasma samples were used for immune parameters and metabolites, whereas liver was sampled for the analysis of metabolites and oxidative stress. Fish endocrine and immune responses were analysed in the head-kidney according to molecular markers analysis. Results showed, after 4 weeks, the lowest levels of anti-protease activity at the control and moderate temperatures, and an increase in lysozyme coupled with a decrease in peroxidase at the high temperature. Oxidative stress analysis showed the lowest hepatic LPO and CAT activity and the highest tGSH in trout reared at the moderate temperature in comparison to control and high temperature, and unchanged tGSH and low hepatic CAT activity at the high temperature compared to control. Regarding gene expression, transcript levels of *gr1*, *arg2* and *il34* were lowest at the control temperature and highest at the high temperature. Preliminary results indicate that 19 °C do not seem to be detrimental for rainbow trout immunity. In contrast, 22 °C appears to induce a chronic stress response in rainbow trout according to molecular markers. More in-depth studies should be considered in order to have a more holistic picture of trout juveniles' immunity when reared under high water temperature.

Acknowledgements: The present work is supported by IGNITION (co-funded by the European Union, with the GA 101084651, and the UKRI https://ignition-project.eu/), financed by national funds, through Fundação para a Ciência e a Tecnologia (FCT, Portugal). Christopher Pimentel benefits from grant from FCT (2024. 06159.BDANA).

Aquaculture-based approaches to enhance kelp forest restoration

Tomás F. Pinheiro*, Silvia Chemello, Isabel Sousa-Pinto, Tânia R. Pereira

Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR LA), University of Porto, Matosinhos, Portugal; *Presenting author.

Introduction

Widespread declines of kelp forests highlight the need for scalable and cost-effective restoration strategies. The green gravel technique—adapted from aquaculture propagation—offers a promising, low-impact approach to reforest degraded habitats by seeding gametophytes onto small rocks and cultivating recruits before out-planting. This study optimises green gravel production for the golden kelp *Laminaria ochroleuca*, evaluating how substrate type, light intensity, and seeding density influence recruitment, early growth, and overall efficiency.

Materials and Methods

Three complementary laboratory trials were conducted under controlled conditions (15 °C, standardised light and nutrients). (1) Substrate trial: gametophytes were seeded on granite, limestone, quartz, and schist to assess growth and recruitment. (2) Seeding-density trial: two gametophyte densities were tested to examine propagule use and survival trade-offs. (3) Light-intensity trial: kelp recruits were cultured under 40 and 130 $\mu mol\ m^{-2}\ s^{-1}$ to evaluate effects on growth and algal contamination.

Results

All substrates supported adhesion and development, though performance varied. Quartz and limestone yielded the highest recruit growth, while granite and schist performed moderately. High seeding densities increased early growth but led to greater mortality, indicating diminishing returns. Higher light intensity enhanced growth and surface coverage but also favoured algal contamination; lower light reduced growth but maintained cleaner cultures.

Discussion and Conclusion

Optimising substrate, light, and density is essential for efficient green gravel production. Limestone and quartz offer cost-effective, biologically favourable substrates; moderate seeding densities and a two-phase light regime (low for settlement, higher for growth) maximise output while limiting contamination. These refinements strengthen the link between aquaculture and restoration, enhancing the scalability of kelp reforestation efforts.

Acknowledgements

The authors gratefully acknowledge the support of CIIMAR/CIMAR LA and the Laboratory of Coastal Biodiversity (LBC) for providing research facilities and technical assistance.

Strengthening the science-industry interface in aquaculture: the RIASEARCH–CESAM chair

Lourenço Ramos-Pinto[1,2*], Jorge Dias[2,3], Amadeu M.V.M. Soares[1,4], Rui Rocha[1,2,3,4]

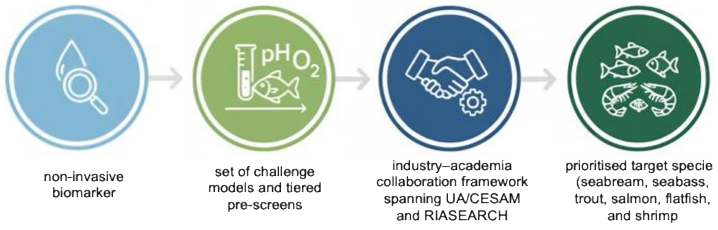
[1]Centre for Environmental and Marine Studies (CESAM), University of Aveiro, Aveiro, Portugal; [2]Riasearch Lda, Cais da Ribeira de Pardelhas n°21, Murtosa, Portugal; [3]SPAROS Lda, Área Empresarial de Marim, Lote C, Olhão, Portugal; [4]Department of Biology, University of Aveiro, Aveiro, Portugal; *Presenting author

Introduction

Aquaculture is central to sustainable protein supply but faces rising demands on environmental performance, product quality, and animal welfare under One Health lens. SME-dominated ecosystems like Portugal's are particularly exposed to shocks and therefore require science that can be rapidly translated into practice. To address this, RIASEARCH Lda. and CESAM–University of Aveiro created an Aquaculture Chair coupling immunonutrition, non-lethal welfare diagnostics, and farm-ready SOPs. Aligned with Aquaculture 4.0, we also aim to advance non-invasive water/skin-mucus biomarkers for digital monitoring to accelerate sustainable aquaculture.

Approach

(1) Pre-screening (*ex vivo/in vitro*) of functional ingredients with 3Rs priority. **(2) Proof-of-concept** (*in vivo*): trials with key fish species and shrimp to resolve dose–response and feasibility of non-lethal sampling under different scenarios. **(3) Pilot validation** (RIASEARCH + partners): deploy Standard Operating Procedures (SOPs) and decision thresholds; assess scalability, uptake, data fidelity.



Conclusion

An industry-anchored pipeline that mainly integrates **non-lethal diagnostics** and **immunonutrition** is feasible at CESAM and will accelerate technology transfer while maintaining scientific rigour. The programme maps to CESAM thematic priorities, including climate and One Health, environmental stressors, and circular bioeconomy innovation, and leverages RIASEARCH as a real-world living lab to translate research into rapid, scalable deployment.

Acknowledgements

Supported by the FCT – Tenure Funding Programme (1st Edition, 2023), Ref. 2023.15265.TENURE.004, which assume the RIASEARCH × CESAM Chair in Aquaculture; governed by UA Regulation no. 609/2015 and the Statute of the Scientific Research Career.

Circularity baseline assessment of the aquaculture sector in Mediterranean regions

Joana Patrício Rodrigues[1,*], Laura Ribeiro[2], Florbela Soares[1], Domitília Matias[3], Pedro Pousão-Ferreira[1], Konstantina Marousi[4], Athanasios Koukounaris[4], Ana Catarina Matias[1]

[1]Estação Piloto de Piscicultura de Olhão (EPPO), Portuguese Institute of the Sea and Atmosphere (IPMA, I.P.), Av. do Parque Natural da Ria Formosa s/n, 8700-194, Olhão, Portugal; [2]Faculty of Sciences and Technology, University of Algarve, Campus de Gambelas, 8005-139, Faro, Portugal; [3]IPMA, Portuguese Institute for Sea and Atmosphere, Av. 5 de Outubro, 8700-305, Olhão, Portugal; [4]Department of Civil Engineering, School of Engineering, University of Patras, 26500, Patras, Greece; *Presenting author.

Introduction

In the Aquaculture activity, Circular Economy (CE) represents a necessary transition model that emphasises the elimination of waste, the continued use of products and materials, and the regeneration of natural systems. Assessing the generalised level of investment in CE in the Mediterranean region is essential for informing strategies that drive the transition toward circularity and, consequently, enhance aquaculture sustainability, which is the aim of the AZA4ICE project.

Materials and Methods

The BLUEfasma Self-Assessment Circularity Tool was used to evaluate the level of circularity in organisations operating within the aquaculture sector. This evaluation is based on the application of CE principles that have been adopted across the participating stakeholders among eight target territories operating in close-to-coast and inland waters: (1) Étang de Thau, France (2) Varna Bay, Bulgaria (3) Cabras Lagoon, Italy (4) Estuary of River Krka, Croatia (5) Šasko Lake, Montenegro (6) Ria Formosa Lagoon, Portugal (7) Bay of Cádiz, Spain (8) Paleo Lagoon, Greece.

Results

From the survey of 65 businesses across the eight Euro-MED territories, the following key metrics, quantified using the BLUEfasma tool, were obtained: CE Readiness Index (CERI) of 1.9; Willingness to InveSt in CE (WISE) of 3.5. Notably, the metrics for the Ria Formosa Lagoon were consistent with this range. These results demonstrated a clear orientation toward the green economy and an eco-thinking mindset of aquaculture businesses, placing them only in the second position of the CE ladder ($1.0 < \text{CERI} < 4.0$).

Discussion and Conclusion

From this baseline analysis, it was identified a low operational maturity in the Mediterranean, but with demonstration of an encouraging starting point for further improvement. Hence, policy-driven interventions and incentives are required to promote faster industrial progress.

Acknowledgements: This work was funded by the Interreg Euro-MED AZA4ICE (Euro-MED0200819).

Effect of nursery-to-farm transportation on the quality and physiology of kelp *Laminaria ochroleuca* seedlings

Maria Francisca Sá[1,*], Isabel Sousa-Pinto[1,2], Gonçalo Silva Marinho[1]

[1]CIIMAR/CIMAR LA, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, 4450-208 Matosinhos, Portugal; [2]Department of Biology, Faculty of Sciences, University of Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal; *Presenting author**.**

Introduction: The transportation of seaweed seedlings from nursery to cultivation sites is a critical phase in aquaculture, directly affecting seedling viability and farming success. *Laminaria ochroleuca*, a kelp species native to the Portuguese coast and of high ecological and commercial value, is a strong candidate for large-scale off-shore cultivation. However, nursery-to-farm transport—typically carried out in cool, humid conditions without seawater—can expose seedlings to desiccation and thermal stress.

Materials and Methods: This study assessed the impact of simulated transport on the physiological condition and survival of *L. ochroleuca* seedlings. Seeded spools were stored in isothermal containers with ice packs for 0, 6, 12 and 24 hours, with temperature and humidity continuously monitored. Photosynthetic efficiency (Fv/Fm) was measured using a Junior-PAM fluorometer as an indicator of physiological status, biomass colour was also recorded, and seedlings were returned to culture tanks for one week to evaluate recovery.

Results: Fv/Fm values remained stable for 6 hours (0.77 ± 0.04 to 0.75 ± 0.06) but declined significantly after 12 (0.56 ± 0.3) and 24 hours (0.35 ± 0.3). The proportion of healthy individuals decreased from 97% to 63% and 43%, while colour shifted from bright brown to greenish tones. Recovery was limited after extended transport (12-24 hours), indicating limited reversibility of transport-induced stress.

Discussion and Conclusion: *L. ochroleuca* seedlings tolerate up to six hours of transport without major physiological impairment, whereas longer durations substantially reduce viability. Transport, under the tested conditions, should therefore be completed within six hours to ensure seedling quality and optimise offshore yields.

Acknowledgements: This work was financially supported by "Pacto da Bioeconomia Azul" (Project No. C644915664-00000026) within the WP5 Algae Vertical, funded by Next Generation EU European Fund and the Portuguese Recovery and Resilience Plan (PRR), under the scope of the incentive line "Agendas for Business Innovation" through the funding scheme C5 - Capitalization and Business Innovation.

FishEUTrust: science and innovation to promote the consumption of aquaculture products

Joana Sá Couto[1,*], Narcisa Bandarra[1], Carlos Cardoso[1], Florbela Soares[2], Pedro Pousão-Ferreira[2]

[1]Divisão de Aquacultura, Valorização e Bioprospeção, Instituto Português do Mar e da Atmosfera, Algés, Lisboa, Portugal; [2]Estação Piloto de Piscicultura de Olhão, Instituto Português do Mar e da Atmosfera, Olhão, Algarve, Portugal; *Presenting author.

Given Portugal's high fish consumption and cultural connection to the seas, sustainable seafood production is essential in the face of climate change as well as in terms of nutrition and food security. Aquaculture fish and bivalve production has been increasing in Portugal, however, consumer distrust of these products remain.

In this context, the FishEUTrust project's central objective is to increase consumer confidence in aquaculture products, promoting greater consumption and reducing the burden on wild stocks of certain species most sought after and consumed in the national market, such as sea bream or sea bass. To this end, IPMA has collaborated with several European partners on a series of innovations and nutritional analyses of sea bass samples from various sources, yielding important results that reflect on the importance and potential of aquaculture. This paper will highlight the main tasks of IPMA in the context of the FishEUTrust, in particular the nutritional and lipidomic findings. The *living lab* as a co-created methodology will also be presented and discussed regarding the importance of stakeholder engagement and co-creation of knowledge, as well as its sustainability plan.

Multidisciplinary citizen science is an important tool for scientific and social innovation. When it comes to conservation of marine ecosystems facing degradation due to climate change and overfishing, IPMA's aquaculture living lab *Aquatrust* is a tool for social, economic and environmental development.

The FishEUTrust project is funded by the European Union through HORIZON, Grant ID 101060712.

Different combinations of marine algae biorefined extracts as boosting strategies to improve European seabass *Dicentrarchus labrax* juveniles immunity

Paulo Santos[1,2,3], Francisca Silva-Brito[1*], André Cunha[1,2,3], Mariana Hinzmann[1], Rafael Vitorino[3], Sara Magalhães[4], Tiago Aires[4], Luis E.C. Conceição[5], Rita Azeredo[1,2], Ana Teresa Gonçalves[3,5], Benjamin Costas[1,2]

[1]Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR-LA); [2]Instituto de Ciências Biomédicas Abel Salazar (ICBAS-UP), University of Porto, Porto, Portugal; [3]Associação Oceano Verde (GreenCoLab), Faro, Portugal; [4]SORGAL S.A., Ovar, Portugal; [5]SPAROS Lda., Olhão, Portugal; *Presenting author.

Algae and microalgae are increasingly recognised as sustainable sources of bioactive compounds. However, their potential to improve fish robustness and welfare in aquaculture has not yet been fully explored. Therefore, the present study aims to evaluate the innate immune defences and disease resistance of European seabass (*Dicentrarchus labrax*) juveniles (12.12 ± 1.01 g) fed diets supplemented with different combinations of biorefined algae-derived compounds.

Five experimental diets were tested, consisting of a commercial diet (CTRL), a commercial diet with an immunestimulant (CTRL+), and three CTRL-based diets enriched with a mixture of *Ulva* extract plus different algae broken cells: Diet1 (*Ulva* spp.), Diet2 (*Gracilaria gracilis*), and Diet3 (*Euglena gracilis*). These diets were evaluated as nutritional boosting strategies using two temporal approaches, 5 or 14 days of feeding, prior to a bacterial challenge with *Photobacterium damselae* subsp. *piscicida*, to assess immune response indicators, redox balance, and intestinal microbiome and proteome profiles both before and after the challenge. The most relevant finding concerns intestinal integrity, which was positively modulated by Diet3, particularly after 5 days of feeding, as evidenced by the upregulation of cell adhesion–related genes. Further studies may confirm the potential role of *Ulva* extract and *Euglena gracilis* broken cells in enhancing gut stability and disease resilience, which could ultimately contribute to improved fish health and welfare and promoting sustainable aquaculture.

Acknowledgements

This study was funded by “Pacto da Bioeconomia Azul” (Project No. C644915664-00000026) within the WP5 Algae Vertical, both co-funded by Next Generation EU European Fund and the Portuguese Recovery and Resilience Plan (PRR), under the scope of the incentive line “Agendas for Business Innovation” through the funding scheme C5 - Capitalization and Business Innovation; PS and AC were supported by Fundação para a Ciência e Tecnologia (2022.13449.BD and 2022.12761.BD respectively). MH supported by doi.org/10.54499/2023.07575.CEECIND/CP2848/CT0014.

Microbiome atlas of Portuguese aquaculture fish (MAPAfish)

Felipe Schoninger, Maria João Peixoto, Benjamín Costas, Gonçalo Espregueira Themudo*

CIIMAR / UP - Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Matosinhos, Portugal; *Presenting author.

A critical aspect of sustainable aquaculture is reducing disease burden in farmed fish. This issue is particularly important for larvae, when health assessment is difficult and problems are often detected too late to prevent losses. Non-invasive or minimally invasive techniques for disease prediction could revolutionise the sector by enabling prophylactic interventions, reducing financial loss and enhancing sustainability, animal health, and welfare.

Imbalances in microbial flora are linked to numerous pathologies, yet knowledge of fish microbiome lags behind that of humans and other mammals. Advances in sequencing technologies now enable rapid and affordable characterisation of microbial populations. This has the potential to inform on taxonomic diversity, which can serve as the basis of prophylactic measures. Such progress offers an opportunity to accelerate fish microbiome research, providing insights into taxonomic diversity and informing strategies such as dietary supplements to improve inflammatory responses. By comparing microbiomes of animals reared in optimal versus suboptimal conditions, researchers can identify profiles associated with greater disease resilience.

We will create an open repository of metagenomic data for key fish species in Portuguese aquaculture, including trout, seabass and seabream. MAPAfish will characterise the metagenomes of multiple tissues in fish reared under optimal conditions and those exposed to environmental and biological stressors. By integrating publicly available data, MAPAfish aims to leverage metagenomics as a tool for early disease diagnosis and improve aquaculture management.

Acknowledgements

This work has been supported by FCT – Fundação para a Ciência e a Tecnologia, I.P., within project 2024.15445.PEX.

Detecting pathogens before they spread

Iris A. L. Silva[1], Inês Freitas[1,*], Morgana Angelo[1], Rui Sousa[1], Marcelo Livramento[1], Pedro Pousão-Ferreira[1,2], Florbela Soares[1,2], Cátia Lourenço-Marques[1,2]

[1]S2AQUA, Olhão, Portugal; [2]IPMA/EPPO, Olhão, Portugal; *Presenting author.

Introduction

Molecular tools have revolutionised disease diagnosis in aquaculture, offering rapid and precise detection and identification of fish and bivalve pathogens, often before clinical signs emerge. This enhances the accuracy of diagnosis, improves disease management and supports proactive monitoring of environmental pathogen loads, increasing product biosecurity.

Results

S2AQUA has developed and optimised molecular diagnosis services for marine and freshwater parasites – including *Amoebas spp.*, *Amyloodinium ocellatum*, Myxozoa such as *Tetracapsuloides bryosalmonae*, *Kudoa spp*, Microsporidia, and Monogenea – and bacteria - as *Photobacterium damselae* subsp. *damselae* and *Phd subs*. *piscicida*, *Tenacibaculum maritimum, T. soleae* and *T. discolor*, and multiple Vibrio species. In bivalves it was also possible to optimise the detection of *Vibrio mediterranei, V. bivalvicida, and V. cyclitrophicus*, which are increasingly recognised for their pathogenic potential in shellfish farming. Additionally, protocols were established in S2AQUA for the detection and quantification of Nodavirus (VNN) in fish and Norovirus (NoV) in bivalves. To enable rapid detection of species within the same genus, multiplex PCR protocols were developed to allow simultaneous identification of several pathogens in one reaction – streamlining up to five analyses at once, reducing even more the time and costs.

Discussion and Conclusions

In aquaculture, molecular tools form a vital early-warning system to intercept disease before it spreads. These technologies allow for rapid and accurate detection of parasites, bacteria, and viruses, enabling producers to act swiftly and decisively to outbreaks and strengthen the biological defence of fish and bivalve farming systems. By integrating these advanced diagnostic solutions, S2AQUA positions itself as a strategic partner to enhance disease diagnosis, strengthen preventive management, and ensure product biosecurity across the aquaculture sector.

Acknowledgements

This study had the support of the projects WP9- Portuguese Blue Biobank under the Blue Economy Pact - Project Nº. C644915664-00000026, Interface Mission cofinanced by PRR - Plano de Recuperação e Resiliência by the European Union (operation code 01/C05-i02/2022.P148) and SAUDE&AQUA II (MAR-021.1.3-FEAMPA-00018).

Gene expression solutions for aquaculture species

Iris A.L. Silva[1,*], Morgana Angelo[1], Inês Freitas[1], Pedro Pousão-Ferreira[1,2], Cátia Lourenço-Marques[1,2]

[1]S2AQUA, Olhão, Portugal; [2]EPPO/IPMA, Olhão, Portugal; *Presenting author.

Introduction

Relative gene expression analysis provides critical insights into the molecular mechanisms that regulate physiological and adaptive processes in living organisms. In S2AQUA we optimised biomarker panels for different key pathways as growth and metabolism, stress response, immune regulation and inflammation in several marine fish species as gilthead seabream, European seabass, meagre, Senegalese sole, turbot, blue and green crab, bivalves and crustaceans, etc. Tailored biomarkers and pathways are also available for other species.

Materials and Methods

For these analyses, total RNA is extracted from target tissues such as liver, gills, intestine or muscle, followed by gene expression quantification by quantitative real-time PCR (qPCR) using gene-specific primers. Expression data is normalised against reference genes and statistically analysed to assess differential regulation among target genes.

Results

S2AQUA has applied this approach in several research projects, highlighting the importance of gene expression analysis in understanding physiological responses of fish to environmental and nutritional stressors. For instance, in the AquaCLIMADAPT project stress- and immune-related biomarkers were evaluated in the head kidney of *Diplodus sargus*, revealing that fish receiving algae-supplemented diets showed lower gene expression levels compared to the CTRL group. Similarly, in the NoviFEED project, hypoxia- and stress-related biomarkers were analysed in *Argyrosomus regius* subjected to two levels of hypoxia under chronic stress, demonstrating that fish exposed to hypoxia exhibited higher expression levels than the CTL group. S2AQUA is also involved in numerous other projects encompassing different fish species and a broad range of physiological biomarkers.

Discussion and Conclusion

By identifying key molecular markers, our approach provides actionable insights to optimise feed formulations, improve animal welfare, and enhance productivity in aquaculture systems. Our customizable gene expression services combine scientific precision with practical application, enabling researchers, feed developers, and producers to make data-driven decisions that support innovation and sustainability across the aquaculture sector.

Acknowledgements: This study had the support of the projects Interface Mission cofinanced by PRR - Plano de Recuperação e Resiliência by the European Union (operation code 01/C05-i02/2022.P148).

Unravelling the genomic basis of disease resistance in Senegalese sole (*Solea senegalensis*): insights from the BetterFLAT project

Iris A.L. Silva[1,*], Inês Freitas[1], Morgana Angelo[1], Silvia Gregório[1], Rui Sousa[1], Bárbara Requeijo[1], Pedro Pousão-Ferreira[1,2], Diogo Medina[3], Carolina Castro[3], Florbela Soares[1,2], Cátia Lourenço-Marques[1,2]

[1]S2AQUA, Olhão, Portugal; [2]IPMA/EPPO, Olhão, Portugal; [3]FLATLANTIC Lta., Mira, Portugal; *Presenting author.

Introduction

The growing global demand for seafood calls for sustainable and resilient aquaculture systems. The Senegalese sole (*Solea senegalensis*) is a high-value species contributing to diversification and competitiveness in European aquaculture. However, its benthic lifestyle increases exposure to sediment-associated pathogens, making disease management a key production bottleneck. The EU Aquaculture Strategy 2030 emphasises the need for environmentally friendly strategies to mitigate disease. The BetterFLAT project addresses this challenge by developing genomic tools to support selective breeding of more disease-resistant Senegalese sole.

Materials and Methods

BetterFLAT integrates functional genomics, transcriptomics, and marker discovery to identify genetic signatures linked to resistance against major pathogens in Senegalese sole. The project unites a complementary, multidisciplinary team: FLATLANTIC, IPMA, and S2AQUA. Together, they combine aquaculture, pathology, and molecular expertise to advance more sustainable fish production.

Results

A preliminary pre-test determined the optimal bacterial concentration for in vivo challenge trials by estimating the median (LD_{50}) and 75% (LD_{75}) lethal doses of selected isolates. Fish were exposed to varying bacterial concentrations, and mortality was monitored to assess virulence. Molecular identification and virulence gene profiling confirmed species identity and pathogenic potential, enabling selection of the most virulent and well-characterised strain for subsequent trials.

Discussion and Conclusion

By combining multi-omics data and pathogen-challenge trials, BetterFLAT provides the foundation for establishing disease-resilient broodstock lines. This approach supports healthier, more robust juveniles and contributes to a more sustainable and economically viable aquaculture industry.

Acknowledgements

The operation BetterFLAT was developed with funding from Programmes COMPETE2030, Algarve2030, by Portugal2030 and the European Union, Interface Mission cofinanced by PRR - Plano de Recuperação e Resiliência by the European Union (operation code 01/C05-i02/2022.P148) and SAUDE&AQUA II (MAR-021.1.3-FEAMPA-00018).

Exploring the role of probiotic and paraprobiotic supplements in the oxidative status of gilthead seabream fed plant-based diets

Rui Silva[1,2,*], Ricardo Couto[1,2], Rafaela Santos[1,2], Paula Enes[1,2], Cláudia Serra[1,2], Aires Oliva-Teles[1,2], Inês Guerreiro[1,2]

[1]Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR), Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos, s/n, 4450-208, Matosinhos, Portugal; [2]Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, Ed. FC4, 4169-007 Porto, Portugal; *E-mail: up202108444@fc.up.pt

Introduction

With the expansion of aquaculture and the pressure to reduce fishmeal use, plant-feedstuffs (PF) are increasingly used in diets, though their non-starch polysaccharides and other antinutritional factors can induce oxidative stress and affect gut health. This study assessed the effects of probiotic (bacteria and yeast) and paraprobiotic yeast in PF-based diets on gilthead seabream intestine oxidative status.

Material and Methods

Gilthead seabream (*Sparus aurata*, 173g) were fed 4 PF-based diets for 34 days: a control and 3 supplemented with probiotics (bacteria or yeast), or paraprobiotic yeast. Each diet was fed to triplicate groups twice daily to visual satiation. At the end of the trial, intestines of 3 fish per tank were collected to measure antioxidant enzymes activities (SOD, CAT, GPX, and GR), lipid peroxidation (LPO), and expression of stress-related genes (*hsp70, cat, sod, gpx*).

Results

Antioxidant enzymes activities and LPO were not affected by the inclusion of the feed additives. Gene expression data are still being processed.

Discussion and Conclusion

Feed additive supplementation showed no major effects on the measured antioxidant enzymes and LPO. Gene expression data remain under analysis.

Acknowledgements

This project was funded by national funds through FCT – Foundation for Science and Technology under the project FEEDGUTBRAIN (2023.13660.PEX, DOI https://doi.org/10.54499/2023.13660.PEX).

AQUA4ALL - Turning waste into value: microbial insights from recirculating aquaculture systems

Rui Sousa[1,*], Ana Carolina Matias[2], Sílvia F. Gregório[1], Ravi L. Araújo[2], Pedro Pousão-Ferreira[1,2], Florbela Soares[1,2], Cátia Lourenço-Marques[1,2]

[1]Collaborative Laboratory, Association for a Sustainable and Smart Aquaculture, Olhão, Portugal (S2AQUA);
[2]Portuguese Institute for the Ocean and Atmosphere/Aquaculture Research Station, Olhão, Portugal (IPMA/EPPO); [*]rui.sousa@s2aquacolab.pt

Introduction

The aquaculture sector is essential for global food security, but its sustainable development relies on efficient resource use and reduced environmental impact. Recirculating aquaculture systems (RAS) have emerged as innovative land-based solutions that enable water reuse and waste minimisation through advanced filtration and life-support technologies. The AQUA4ALL project applies a circular economy approach to close water and nutrient cycles in RAS, promoting sustainable aquaculture and improving surrounding marine ecosystems. By converting nutrient-rich effluents into microalgae biomass for industrial use, the project reduces environmental impact, enhances resource recovery, and transforms waste into value.

Materials and Methods

Water samples were collected from four RAS systems: rotifers, turbot (*Psetta maxima*), sole (*Solea senegalensis*), and gilthead seabream (*Sparus aurata*), at multiple system points.

Results and Discussion

Preliminary results revealed distinct bacterial dynamics across species and sampling zones, with notable differences in total marine bacterial load and *Vibrionaceae* levels, reflecting the influence of system design and biological activity. Rotifer system showed higher bacterial loads in culture tanks, while biofilter and skimmer remained stable with low *Vibrionaceae* levels. Turbot RAS samples revealed temporal fluctuations, especially in *Vibrionaceae*. Sole RAS samples showed microbiological stability, with higher *Vibrionaceae* proportion in the drum filter. For seabream RAS system, bacterial load increased in the skimmer, while *Vibrionaceae* levels remained stable across sampling points. These results give insights into bacterial dynamics on effluents of different RAS systems, providing essential information for promoting sustainable solutions for aquaculture.

Acknowledgements

AQUA4ALL was supported by Algarve 2030, by Portugal 2030 and by the European Union; Missão Interface, project co-funded by PRR - Plano de Recuperação e Resiliência pela União Europeia (operation code 01/C05-i02/2022.P148) and SAUDE&AQUA-II (MAR-021.1.3-FEAMPA-00018).

Arginine requirement in plant-based diets: impacts on zootechnical performance and metabolic response in *Colossoma macropomum* (Cuvier, 1818)

Samuel Oliveira Teixeira[1,*], Ivan Viegas[2], Luis André L. Barbas[3], Daniel Abreu Vasconcelos Campelo[4]

[1]Federal University of Pará, Graduate Program in Animal Science, Brazilian Agricultural Research Corporation – EMBRAPA, and Federal Rural University of the Amazon – UFRA, Castanhal, Pará, Brazil; [2]University of Coimbra, Life Sciences Department, Centre for Functional Ecology, Associate Laboratory TERRA, Coimbra, Portugal; [3]Federal Institute of Education, Science and Technology of Pará Castanhal, Brazil; [4]Federal University of Pará, Institute of Coastal Studies, School of Fisheries Engineering, Bragança-PA, Brazil; *Presenting author.

Introduction: With the growth of inland aquaculture, it has become essential to understand the nutritional requirements of fish, such as tambaqui, the most widely farmed native species in Brazil. The increasing use of plant-based ingredients as partial or total substitutes for animal protein offers economic and sustainability benefits but may limit essential amino acids, highlighting the importance of establishing precise nutritional requirements for each species.

Materials and Methods: The 60-day trial was carried out with 300 juvenile tambaqui distributed in 15 tanks under a completely randomised design (five treatments, three replicates). Isoproteic and isoenergetic diets were supplemented with graded levels of L-arginine (10.8–29.1 g/kg) to determine its requirement. Fish were fed six times daily (5% biomass). Growth performance, biochemical, and haematological parameters were assessed, and liver and muscle samples were collected for metabolomic analyses to evaluate the metabolic effects and identify biomarkers related to arginine supplementation.

Results: Intermediate dietary levels of arginine promoted weight gain and protein efficiency in juvenile tambaqui, with the highest gains observed at 19.8 g/kg. No effects were observed on specific growth rate, feed conversion, or survival. Morphometric indices were similar among treatments, although VSI tended to increase at higher arginine levels. The estimated arginine requirement, based on weight gain and protein efficiency, was 18.9–19.2 g/kg of diet, corresponding to approximately 6.3% of crude protein, indicating that intermediate levels optimise growth and protein utilisation.

Conclusions: Arginine supplementation in plant-based diets improves weight gain and protein efficiency in juvenile tambaqui without affecting survival or morphometric indices. The estimated requirement is 18.9–19.2 g/kg of diet (≈6.3% of crude protein), indicating that adequate arginine levels optimise growth and feed efficiency in an economical and sustainable way.

Acknowledgements: We thank CAPES (Brazil) for financial support, Cargill for donating materials used in feed formulation, and Professor Andresa Carla Feihrmann (UEM) for performing the amino acid analyses essential to this research.

Multiomics of European seabass (*Dicentrarchus labrax*) exposed to acute stress by transport

Gonçalo Espregueira Themudo[1,*], Bruno Reis[1], Felipe Schoninger[1], Francisco Cunha[1], Christopher Pimentel[1], Paula Simó-Mirabet[2], Juan A. Martos-Sitcha[2], Benjamín Costas[1]

[1]CIIMAR / UP - Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Matosinhos, Portugal; [2]Universidad de Cádiz, Cádiz, Spain; *Presenting author.

Unravelling new knowledge on biomolecular response to acute stress in farmed fish is essential for better monitoring, improve animal welfare and health, and reduce mortality. This study aimed to evaluate physiologic and metabolic effects of acute stress induced by transport in European seabass (*Dicentrarchus labrax*). Trials were conducted at University of Cádiz facilities. European seabass juveniles were randomly divided into four groups of fish. One group was immediately sampled as a non-stressed initial control, whereas two groups were transferred to three oxygen saturated containers and transported for 4 hours. Upon arrival, half were immediately sampled while the other were put in the original system and sampled 24h post-arrival as recovery group. A fourth group was kept under control conditions and sampled at 24h (n=12 per sampling time). Total RNA was extracted from skin tissue and pooled (n=4 samples per treatment) for RNAseq on an Illumina sequencer. Transcripts were quantified using kallisto. Differentially expressed genes (DEGs) were determined using R/DESEQ2. Skin mucus samples from the same fish were processed for proteomic analysis. Raw peptide data hits were filtered using coverage above 30%, unique peptides above 2 and a SEQUEST HT score greater than 100. Differentially expressed peptides were determined using R/DEP. Data showed genes regulated during transport induced acute stress. The European seabass transcriptome returned to base level expression by 24h, in contrast with the proteome. This indicates that while the effect of the stress may no longer be detectable at the gene transcript level, the physiological response continued, as peptide levels were still modulated. Gene Ontology (GO) terms upregulated in the transcriptome during stress include genes implicated in circadian rhythm, negative regulation of cellular processes and downregulated were genes related to nervous system development, DNA-binding and transcription. At the proteomic level, differentially expressed proteins are associated with GO terms regarding the activation of the immune response and wound healing/coagulation. These results elucidate the cascade of events that occur after acute stress in seabass. Further ongoing analyses with other types of stress will enable us to generalise these results and more robust stress related biomarkers for European seabass.

Acknowledgements: Work Co-funded by UKRI and by the European Union's Horizon Europe research and innovation programme (GA No. 101084651- project IGNITION).

Methionine dietary supplementation improves antiviral response of rainbow trout (*Oncorhynchus mykiss*) against viral haemorrhagic septicaemia virus (VHSV) a transcriptomic approach

Mariana Vaz[1,2], Gonçalo Espregueira Themudo[1], Carolina Tafalla[3], Patricia Díaz-Rosales[3], Diego Robledo[4,5], Benjamín Costas[1,6], Marina Machado[1,*]

[1]Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIIMAR-LA), University of Porto, Matosinhos, Portugal; [2]Faculty of Mathematics and Natural Sciences, University of Bergen, Bergen, Norway; [3]Animal Health Research Centre (CISA), National Institute for Agricultural and Food Research and Technology (INIA), Spanish Research Council (CSIC), Madrid, Spain; [4]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, UK; [5]Department of Genetics, University of Santiago de Compostela, Santiago de Compostela, Spain; [6]Instituto de Ciências Biomédicas Abel Salazar (ICBAS-UP), University of Porto, Porto, Portugal; *Presenting author.

Several studies have shown that dietary methionine enhances immune function, modulates inflammation, and improves bacterial resistance in fish through pathways such as DNA methylation, aminopropylation, and transsulfuration. However, its role in antiviral immunity remains unclear. This study examined the effects of dietary methionine supplementation on immune modulation and resistance to viral haemorrhagic septicaemia virus (VHSV) in rainbow trout (*Oncorhynchus mykiss*). Juvenile trout (5.9 ± 0.9 g) were fed for four weeks with either a control diet (CTRL) or a methionine-supplemented diet (MET; 1% L-methionine). After feeding, fish were bath-challenged with VHSV (10^5 $TCID_{50}$/mL), and tissues (gills, skin, liver, head kidney, spleen) were collected at 0, 24, 72, and 120 hours post-infection. Peak viral replication was observed at 72 hours post-infection, coinciding with the highest number of differentially expressed genes (DEGs) in skin and gill tissues. RNA sequencing performed at this time point revealed MET-specific DEGs enriched in pathogen recognition and signalling pathways, including TLR3, MYD88, TRAF2, NF-κB, STING, IRF3, and IRF7. Gene ontology analysis further indicated upregulation of antiviral, and inflammatory processes involving genes such as VIG1, caspases, and TNF. Notably, viperin—an antiviral protein encoded by VIG1—was significantly upregulated in the gills of MET-fed fish, suggesting methionine enhances viperin-mediated antiviral defence. In summary, dietary methionine supplementation enhances the antiviral immune response of trout against VHSV infection, with viperin emerging as a central effector. These findings suggest that methionine supplementation represents a promising nutritional strategy to strengthen antiviral immunity and improve disease resilience in aquaculture. Work funded by the European Union's Horizon Europe research and innovation programme (GA No.101079467 project GRINNAQUA). BC and MM were supported by FCT, Portugal (2020.00290.CEECIND and 2022.03304.CEECIND, respectively).

Farming in heavy water – lessons from tracer studies in aquaculture

Ivan Viegas[1,*], Mariana Palma[1,2], Ludgero C. Tavares[3], Nick Wade[4,5], John G. Jones[6,7]

[1]University of Coimbra, Life Sciences Department, Centre for Functional Ecology, Associate Laboratory TERRA, Coimbra, Portugal; [2]current address: Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR LA), University of Porto, Matosinhos, Portugal; [3]CIVG – Vasco da Gama Research Center / EUVG –Vasco da Gama University School, Coimbra, Portugal; [4]CSIRO Agriculture and Food, Queensland Bioscience Precinct, St Lucia, QLD, 4067, Australia; [5]The Roslin Institute, The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, EH25 9RG, Scotland; [6]Center for Neuroscience and Cell Biology (CNC-UC), Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal; [7]CIBB - Center for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, Coimbra, Portugal; *Presenting author.

Introduction: Heavy water is a common term referring to deuterated water (^2H_2O), an incredibly practical tracer that is ideally suited for aquatic organisms since it can be incorporated directly into the tank water for an indefinite period. During residence in ^2H_2O, ^2H-enrichment rapidly equilibrates with the organism´s body water and homogeneously distributes within tissues. ^2H is then incorporated into a diversity of biosynthetic pathways including gluconeogenesis, lipogenesis and protein synthesis following well-defined metabolic steps. Positional isotopic analysis by ^2H-NMR effectively informs its fractional biosynthetic rate, and in some cases the precursor sources. Throughout the last 15 years, this has been our tool for aquaculture. **Materials and Methods:** Tank water is ^2H-enriched to 4-5% by the addition of 99%-enriched ^2H_2O to system water where the respective growth trial took place. Individuals are allowed to recover and feed as during the growth trial for 3-6 days. Studies included dietary manipulations of source and quantities of protein and carbohydrates, supplementation of glycerol and effects of tributyrin, among other. **Results:** Tank water ^2H-enrichment is reached within body water in less than 4-6 hour in a variety of species: freshwater like rainbow trout (*Oncorhynchus mykiss*); marine European seabass (*Dicentrarchus labrax*); flatfish Senegalese sole (*Solea senegalensis*); and black tiger shrimp (*Penaeus monodon*). Studies included alterations in levels and source of carbohydrates and protein, supplements and novel ingredients. Enrichments were obtained in blood glucose, liver glycogen, muscle alanine, and triacylglycerols (TAG). Regarding the latter, fatty acids and TAG-bound glycerol are labelled differently allowing for separate analysis. TAG analysis in barramundi (*Lates calcarifer*) liver, muscle and perivisceral fat allowed for an integrated flux within tissues where adiposity can differ in relevance. **Conclusions:** Deuterated water has systematically provided reliable estimates for metabolic flux, building upon other existing tools frequently employed in aquaculture studies. **Acknowledgements:** NMR data was collected at the UC-NMR facility which is supported in part by FEDER – European Regional Development Fund through the COMPETE (Operational Programme for Competitiveness) and by National Funds (FCT RECI/QEQ-QFI/0168/2012, CENTRO-07-CT62-FEDER-002012), and also Rede Nacional de Ressonância Magnética Nuclear (RNRMN) and Coimbra Chemistry Centre (UID/QUI/00313/2019). Supported by FCT and co-funded by FEDER through the Operational Programme for Competitiveness and Internationalization (POCI/POFC) and structural funds Centre for Functional Ecology UIDB/04004/2020; Associate Laboratory TERRA LA/P/0092/2020.

Health-promoting potential of *Chlorella* sp. co-cultures on European seabass gut mucosa

Maria João Xavier[1,2], Soraia Santos[1], Rute Félix[1,2], Veronica Rossetto[2], Tamára Santos[2], Mariana Carneiro[3], Hugo Pereira[1,2], Ana Teresa Gonçalves[1,4]

[1]Fundação Oceano Verde (GreenCoLab), Universidade do Algarve, Faro, Portugal; [2]Centro de Ciências do Mar do Algarve (CCMAR/CIMAR LA), Universidade do Algarve, Faro, Portugal; [3]NECTON, S.A., Belamandil s/n, Olhão, Portugal; [4]SPAROS, Área Empresarial de Marim, Portugal.

Chlorella sp. is one of the most widely used microalgae in aquafeeds, recognised for its high nutritional value and beneficial effects on fish performance and health. Co-cultivation of *Chlorella* sp. with selected bacteria improves growth and culture robustness. Moreover, bacterial co-cultures may modulate the nutritional composition of the algae and potentiate its health-promoting properties. Therefore, this study aimed to unveil the functional properties of *Chlorella* sp. co-cultured with different bacteria consortia on gut mucosal health of European seabass (*Dicentrarchus labrax*) through an ex-vivo model. Biomass and supernatants from axenic *Chlorella* sp. co-cultured with three different bacterial consortia (Mix1, Mix2, and Mix3) were evaluated as potential ingredients in seabass intestine explant, under standardised conditions. The intestinal explants were tested in triplicate and incubated with the different ingredients at low and high inclusion dosages. Tissue response was evaluated by transcriptional analysis of a panel of genes related to innate immune response, wound healing capacity, antioxidant defence, and epithelial integrity and permeability. Overall, the results showed that both the supernatant and biomass of the *Chlorella* sp. co-culture with different bacterial consortia can modulate key immune biomarkers related to innate response and wound healing pathways of the fish gut mucosa. These findings highlight that co-cultivation with beneficial bacteria not only optimises *Chlorella* sp. production but also enhances its bioactivity, supporting its potential as a functional ingredient to promote gut health in aquaculture species.

Acknowledgements

Funded by the European Union (Grant agreement ID: 101060991, REALM). Views and opinions expressed are however those of the authors only and do not necessarily reflect those of the European Union or the European Research Executive Agency (REA). Neither the European Union nor the granting authority can be held responsible for them.

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