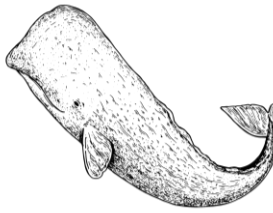
BLUE THINK CONFERENCE

BTC 2026

BOOK OF ABSTRACTS

FARO

3 AND 4 SEPTEMBER 2026



BLUE THINK CONFERENCE

BTC 2026

Book of Abstracts Blue Think Conference 2026

Editors: Alizé Deguette, Carolin Franzl, Daniela Castro, Diana Gonçalves, João Monteiro, Sari Ponnet

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Students Meeting CIMAR LA – BTC 26

3 and 4 September 2026

Faro, Portugal

Welcome to Faro!

Welcome to the 7th edition of the Blue Think Conference!

It is with great enthusiasm that we welcome you to the University of Algarve for this year's Blue Think Conference (BTC). For the first time, BTC is relocating from Matosinhos to Faro, in a symbolic gesture that reflects the strengthening collaboration within CIMAR-LA, the Associated Laboratory that brings together CIIMAR and CCMAR, and their respective PhD student committees.

This conference represents more than just a change of scenery. It is a recognition that marine science thrives on diversity of perspectives, approaches, and places. By alternating between the northern and southern coasts of Portugal, we celebrate the richness of our shared research community while honoring the distinct identities of our two research centres.

As early-career researchers, you are the heartbeat of our research centres and of this conference. Whether you are presenting your first poster, delivering an oral presentation, or simply engaging in the conversations that unfold during coffee breaks, you are contributing to something larger than yourself. You are shaping the future of marine science. The challenges we face—biodiversity loss, climate change, the sustainable use of ocean resources—are complex and urgent. They demand interdisciplinary thinking, bold ideas, and collaborative spirit. What better space to cultivate these qualities than a conference designed by and for students?

We are honored to host an inspiring lineup of keynote speakers, each bringing a unique perspective to our three thematic sessions. From fish nutrition to drug delivery, from multiple stressors to ecosystem services, their journeys remind us that scientific excellence is built on curiosity, perseverance, and the willingness to cross disciplinary boundaries.

We also extend our gratitude to the institutions, partners and sponsors who made this event possible. To CCMAR and the University of Algarve for hosting the conference, to CIIMAR for supporting the travel and accommodation of its students, and to all our remaining sponsors.

Over the next two days, we hope you will not only share your knowledge but also build connections that extend far beyond this event. In the finest tradition of the Blue Think Conference, may this be a space where ideas are challenged, friendships are forged, and the future of marine science is built.

Welcome to BTC2026. Welcome to Faro. Let science and the connections begin!

The organizer

CIMAR-LA is an Associated Laboratory that unites two of Portugal's premier marine research centres—CCMAR (University of Algarve) and CIIMAR (University of Porto)—in a strategic partnership designed to advance ocean science and promote sustainable development. Founded in 2002, the institution operates through a governance model that fosters internal and external collaboration across five core activity areas: advanced training, policy and society, business and innovation, internationalization, and research infrastructure.

The laboratory's mission is to generate scientific knowledge that benefits society while contributing to regional, national, and international public policy development in marine science and economics. Its research focuses on two main thematic lines: global change, ecosystem services and conservation; and the sustainable exploitation of marine resources through aquaculture and blue biotechnology.

Within these themes, CIMAR-LA pursues key objectives: understanding global changes and predicting their repercussions; identifying threats to ecosystem services; developing *in situ* solutions for conservation and restoration; encouraging the transition to circular economic models; creating new resources from marine organisms; improving processes from production to consumption; and adapting human activities to future challenges.

As a reference entity in international marine research, CIMAR-LA actively works to attract and retain top-tier researchers, promote knowledge exchange across disciplines, and strengthen Portugal's capacity to secure international funding, talent, and collaborations—fulfilling its role as an Associated Laboratory supporting the country's scientific and technological development.

Committees

Organization Committee

CIIMAR: Afonso Mota, André Ferreira, Francisca Espincho, Inês Leal, Leonor Pizarro, Mena Khalaf, Nicola Gambardella e Pedro Cruz.

CCMAR: Alizé Deguette, Aqsa Sharif, Brigitta Varga, Carlos Alemán, Carolin Franzl, Daniela Castro, Diana Gonçalves, Fanourios Michalakis, Federico Clementoni, Gabriela Ribeiro, Giulia Zeri, Inês Cerveira, João Monteiro, Maria Pais, Miguel Pinto, Myrsini Lymperaki, Rita Camacho, Rui Peres dos Santos e Sari Ponnet.

Scientific Committee

Topic 1: Biology, Aquaculture & Seafood Quality

Chair: Rita Teodósio (CCMAR)

Ana Filipe Basto, Ana Rato, Diogo Peixoto, Helena Fernandes, João Monteiro, Leonor Ferrão, Marta Monteiro, Miguel Gandra, Nuno Sales Henriques, Rafaela Santos, Renata Gonçalves, e Sara Moutinho.

Topic 2: Marine Biotechnology, Biomedicine & Biochemistry

Chair: Davide Liberti (CCMAR)

Diogo Alexandrino, Eunice Ferreira, Francisca Felix Azeredo, Gil Martins, Henrique Sousa, Ines Funico Guerra, Mariana Carneiro, Mariana Girão, Pedro Moñino Fernández, Rafaela Mendes, Raquel Chaves, Riccardo Trentin e Tiago Ribeiro.

Topic 3: Global Changes, Ecosystem Conservation and Services & Human Impacts

Chair: Sofia Graça Aranha (CCMAR)

Ana Matos, Caio Ribeiro, Duarte Frade, Eliza Fragkopoulou, Francisco Pascoal, José Queirós, Marlene Pinheiro, Monika Jadwiga Szynaka, Pedro Rodrigues, Rui Peres dos Santos, Sabrina Rodrigues, Sebastian Kraft.

Sponsors

We would like to express our sincere gratitude to our sponsors for their generous support, which has made this conference possible.

Moreover, 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, UID/PRR/04326/2025, UID/PRR/04423/2025 and LA/P/0101/2020.

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Scientific Program

TIME	THU, 3 SEPT	FRI, 4 SEPT
08:30.	**Registrations**	
08:45.		
09:00	**Opening Session**	**Topic 3** **Keynote Talk** Tasman Crowe
09:15		
09:30	**Topic 1** **Keynote Talk** Sadasivam Kaushik	
09:45		**Topic 3** **Students Oral Presentations**
10:00		
10:15	**Coffee Break**	
10:30		
10:45	**Topic 1** **Students Oral Presentations**	
11:00		**Coffee Break**
11:15		
11:30		**Topic 3** **Poster Session**
11:45		
12:00	**Lunch**	
12:15		
12:30		**Closing Session**
12:45		
13:00		**Lunch**
13:15	**Topic 2** **Keynote Talk** Ana Grenha	
13:30		
13:45		
14:00	**Topic 2** **Students Oral Presentations**	**Visit to CCMAR Facilities**
14:15		
14:30		
14:45		**Social Activity on the beach** **(Kayak/Volleyball)**
15:00		
15:15	**Coffee Break**	
15:30		
15:45	**Marine Protected Areas:** Patrícia Nogueira AIMM Portugal	
16:00		
16:15	**Round Table:** Jorge Gonçaves, Alberto Queiruga, Miguel Rodrigues	
16:30		
16:45		
17:00		
17:15		
17:30	**Topic 1 & 2** **Poster Session**	
17:45		
18:00		
18:15		

Detailed programme

Wednesday, September 2, 2026

Time	Event
18:00 – 20:30	Social Icebreaking – Madalena Bar

Thursday, September 3, 2026

Time	Event
09:00 – 09:30	Opening Ceremony - Welcoming speech from CCMAR and University
09:30 – 10:15	Keynote talk - Strengths and limitations of “Bluefood” production – Sadasivam Kaushik
10:15 – 10:45	Coffee Break
10:45 – 12:00	Topic 1 – Students Oral Presentations
10:45 – 11:00	Transgenerational effects of thermal and nutrient stress priming in the golden kelp *Laminaria ochroleuca* – *Benjamim Portugal*
11:00 – 11:15	Exploring the microbiome- thermal resilience relationship in *Laminaria ochroleuca* gametophytes – *Júlia Soares Aguiar*
11:15 – 11:30	Yeast β-glucans as functional feed additives to improve European seabass resilience against *Tenacibaculum maritimum* infection – *Miguel Cabano*
11:30 – 11:45	Response of European seabass to a hypoosmotic challenge and potential for modulation with diets containing insect meal – *Rafaela Silva Cosa*
11:45 – 12:00	Tetrodotoxin trail: how prey composition and gut microbiome predict contamination in *Charonia lampas - Smilla Adler*
12:00 – 13:15	Lunch
13:15 – 14:00	Keynote talk - New therapies growing on the basis of natural (marine) materials – *Ana Grenha*
14:00 – 15:15	Topic 2 – Students Oral Presentations
14:00 – 14:15	New broad-host range plasmids to heterologously express cyanobacterial biosynthetic pathways – *Diana Sousa*
14:15 – 14:30	Bioengineering GRP-EVs: A Human Cell Production Platform – *Joana Carreira*
14:30 – 14:45	CDKL2-Mediated Functional Rescue in a Zebrafish Model of CDKL5 Deficiency Disorder- *Kelly Sandoval*
14:45 – 15:00	Leveraging Composting Microbiomes and Cellulolytic Phenotypes for Sustainable Textile Waste Management – *Liliana Almeida*
15:00 – 15:15	Modeling ankylosing spondylitis in zebrafish - CRISPR/Cas9-mediated erap1a/b deficiency – *Max Domingues*

15:15 – 15:45	Coffee Break
15:45 – 16:15	Keynote Talk - Marine Protected Areas - *Oceano Azul Foundation*
16:15 – 17:30	Round Table - Marine Protected Areas (MPAs): focus on the process of creating the Algarve Reef Marine Natural Park – *Nuno Sales Henriques; Jorge Gonçalves, Miguel Rodrigues and* Brendal Townsend
17:30 – 18:30	Science & Cocktails — Topic 1 and 2 - Poster Session
18:30 – 19:30	Icebreaking activity (Lab terrace/Game over)
19:30 – 21:30	Conference Dinner – Restaurant Sem Tempo

Friday, September 4, 2026

Time	Event
9:00 – 9:45	Keynote talk - Expanding Ireland's network of Marine Protected Areas – an evidence-led participatory approach – *Tasman Crowe*
9:45 – 11:00	Topic 3 – Students Oral Presentations
9:45 – 10:00	Spatial and Temporal Patterns of Deep-Sea Elasmobranch Bycatch in Commercial Crustacean Trawls off Southern Portugal - *Cátia Matos*
10:00 – 10:15	Mixed or fixed: genetic composition and connectivity of green sea turtles foraging in West Africa - *Celine Travella*
10:15 – 10:30	Population genomics for the conservation of the red gorgonian, *Paramuricea clavata,* in the Parc National des Calanques (France) - *Inês Ramos*
10:30 – 10:45	Multi-species seagrass transplantation in salt-production lagoons: lessons for design, cultivation and restoration – *Sari Ponnet*
10:45 – 11:00	Cyanobacteria and Cyanotoxins in Portugal: Distribution Patterns and Emerging Risks in the Alqueva Multipurpose Project – *Rui Barbosa*
11:00 – 11:30	Coffee Break
11:30 – 12:30	Topic 3 – Poster Presentations
12:30 – 13:00	Closing Ceremony - Speech from CIIMAR and Announcement of best oral and poster presentations
13:00 – 14:00	Lunch
14:00 – 15:30	Visit to CCMAR facilities
14:30 – 19:00	Social Activity (Kayak/SUP/Volleyball) – Praia de Faro
19:00 – 21:30	Sunset on the beach – Praia de Faro

Keynote Speakers

Topic 1: Biology, Aquaculture & Seafood Quality

Sadasivam Kaushik

University of Las Palmas, Spain

Thursday, 3rd September

9:30-10:15 a.m.

Presentation Title: Strengths and limitations of "Bluefood" production

Abstract of the presentation: Over the recent past, there is much interest towards "Blue Economy", which involves several sectors and activities, of which "Bluefood" production is but a small portion. The term "Bluefood" covers all food products derived from the aquatic environment, be it from inland waters or from the seas around us, and be it farmed or otherwise, contributing to the human food basket. In terms of volumes, half of world aquaculture production is represented by seaweeds and microalgae. Globally, aquaculture's contribution in the provision of food for the humans is becoming more significant and the volume of aquatic animal production exceeds that of any single land animal products, taken individually. There are however big geographical differences between countries in the real, quantitative contribution of bluefood for the provision of proteins, fatty acids and micronutrients of interest. Aquaculture products can be tailored to ensure their value in terms of human nutrition and health. As in all food production systems, there is a need for taking full account of nutritional value and food safety issues, keeping in mind long-term sustainability of the sector targeting the sustainable development goals. Such issues bear greater significance in intensive fish farming as practiced in many developed countries. Even in situations prevailing in Asia dealing with species from low-trophic levels and with little supply of additional nutrients into the water bodies, there is an increasing trend towards intensification of aquatic animal production systems, involving variable levels of dietary inputs. In all such feed-based aquaculture, our objective should be to deal with the supply of nutritionally well-balanced diets, ensuring growth, health, welfare and reproduction. The growth of aquaculture also leads to an increasing demand and pressure on feed resources with possible conflicts with other animal production systems or even with human food. Over the years, much progress has been made in developing feeds relying less on capture fishery derived feedstuffs. Irrespective of the farming system and species concerned, optimizing resource utilization is the primary goal to ensure that we supply the humans with products that have high nutritional value and food safety.

Short biography: Sadasivam J. Kaushik earned his bachelor's and master's degrees at the prestigious Presidency College of Madras University in India. He received his Doctor of Science degree from the University of Bretagne Occidentale, France, for his work on the influence of salinity on nitrogen metabolism and arginine requirement of rainbow trout. As an active member of the committee on fish nutrition and production of the International Union of Nutritional Sciences, he was actively involved in fish nutrition research and training. He was the director of the INRA - IFREMER Joint research units on fish nutrition and also headed the hydrobiology research station at Saint Pée sur Nivelle, France. He has more than 300 scientific papers and books on fish nutrition and feeding. He has been active in coordinating European research projects in fish nutrition and metabolism. Further to his retirement from INRA, he held a European research area chair position in the University of Las Palmas, Spain.

Keynote Speakers

Topic 2: Marine Biotechnology, Biomedicine & Biochemistry

Ana Grenha

Centre for Marine Sciences, Facility of Sciences and Technology, University of Algarve

Thursday, 3rd September

01:15-02:00 p.m.

Presentation Title: New therapies growing on the basis of natural (marine) materials

Abstract of the presentation: New therapies are progressively focusing more on advanced technologies involving drug encapsulation, frequently involving natural materials that provide distinct properties. While terrestrial resources have been explored for long, the sea has gathered attention more recently and is now increasingly explored to identify novel drugs and materials that may offer advantages to therapeutics. Marine organisms are strongly contributing to new approaches and are potential sources of bioactive compounds and biomaterials with important pharmaceutical applications. Materials like chitosan, alginate, carrageenan and fucoidan are nowadays widely studied for the production of drug carriers. In parallel, the sea has already provided anticancer drugs and other therapeutic molecules, demonstrating the biomedical potential of ocean resources. This talk will showcase new therapies that are being proposed supported by the use of natural materials, including those of marine origin.

Short biography: Ana Grenha has received her PhD in Pharmacy-Pharmaceutical Technology from the University of Santiago de Compostela in 2007. She joined the University of Algarve in Portugal for a Professorship in Pharmaceutical Technology in 2007 and received her Habilitation in Pharmaceutical Technology at the University of Lisbon in 2022. Since July 2023 she is an Associate Professor in Pharmaceutical Technology. She is the PI of the Drug Delivery Laboratory, part of the CCMAR, which is mainly dedicated to respiratory drug delivery, with a particular focus on inhalation. She has been the PI of several funded projects, and has authored ca. 50 papers in international peer-reviewed journals, 6 book chapters and 2 patents (1 pending) in the field of drug inhalation. She has supervised 4 PhD thesis and approximately 15 Master thesis, all in the field of drug delivery. She was awarded the prize Investigador Universidade do Algarve 2022 and in 2020 received the Robalo Cordeiro Award from the Portuguese Society of Pneumology, attributed to a scientific project on pulmonary immunisation strategies. She is currently the Director of the Integrated Master in Pharmaceutical Sciences at UAlg, is a member of the Board of Directors of CCMAR-UAlg since 2018 and is part of the Drug Delivery to the Lungs (DDL) Conference Committee since 2019.

Keynote Speakers

Topic 3: Global Changes, Ecosystem Services & Human Impacts

Tasman Crowe

University College Dublin, Ireland

Thursday, 4th September

09:00-09:45 a.m.

Presentation Title: Expanding Ireland's network of Marine Protected Areas – an evidence-led participatory approach

Abstract of the presentation: Networks of Marine Protected Areas (MPAs) can be among the most effective tools for conserving marine ecosystems and promoting their restoration and sustainable use. The Global Biodiversity Framework includes a target of 30% global coverage by protected areas by 2030, which is reflected in the EU Biodiversity Strategy. As such EU member states are working towards this target, each proceeding in different ways. In Ireland, approximately 10% of the maritime area is currently protected under the EU Birds and Habitats Directives. A process is underway to establish national MPAs to close the gap to target. A first step was the commissioning of a report from an expert advisory group, which has formed the basis for draft legislation. Interim ecological sensitivity assessments are being used to inform planning for offshore renewable energy and to develop the methodology for selecting features for protection, collating relevant data, engaging with stakeholders and using systematic conservation prioritization modelling to identify networks of sites that could potentially be designated to meet specified network objectives at minimal cost to existing or planned sectoral activities. This talk will present key aspects of this process, preliminary results and some comments on the career trajectory that enabled my involvement as chair of the advisory group.

Short biography: Professor Tasman Crowe is Vice President for Sustainability at University College Dublin. Previously, he was director of the UCD Earth Institute and co-founder and co-director of a new interdisciplinary BSc in Sustainability. He is chair of Ireland's National Biodiversity Forum, a member of the Independent Advisory Committee for Nature Restoration and co-chairs an advisory group to government on the expansion of Ireland's network of MPAs. His research characterises impacts of multiple stressors on marine ecosystems and the services and benefits they provide to society, including their cultural value.

Talk

Marine Natural Park of the Algarve Reef

Pedra do Valado

The Marine Natural Park of the Algarve Reef – Pedra do Valado is Portugal's first Marine Protected Area of Community Importance. Officially designated in 2024, the park covers 156 km² and extends along the Algarve coast between Lagoa and Albufeira, encompassing depths of up to 50 m. Pedra do Valado is one of the country's most biodiverse marine areas, harbouring approximately 70% of the Algarve's marine biodiversity. Nearly 900 species have been recorded within the park, including 12 species new to science.

The protected area was established following a science-based, participatory process involving the Centro de Ciências do Mar (CCMAR), the Oceano Azul Foundation, the municipalities of Albufeira, Lagoa and Silves, and more than 70 additional stakeholders, including fishers' associations, tourism operators, regional authorities, state laboratories and non-governmental organisations. The park was created to conserve marine habitats and threatened species while promoting sustainable fisheries, responsible tourism and scientific research.

Owing to its exceptional biodiversity and well-preserved reef habitats, Pedra do Valado represents one of the most important areas for marine biodiversity conservation along the Portuguese coast.

Patrícia Nogueira

Environmental Policy Manager of AIMM

Patrícia holds a Master's degree in Marine Biodiversity and Conservation and is currently finishing her PhD in Marine, Earth and Environmental Sciences at the University of Algarve. Her research focuses on marine ecology and primary production. In addition to her scientific background, she worked for three years as an aquarist and has experience in environmental education with multiple types of audiences. At AIMM, Patrícia is responsible for the communication and outreach of the Marine Protected Area located in the Parque Natural Marinho do Recife do Algarve – Pedra do Valado.

Round Table

Thursday, 3rd September

04:15 – 05:30 pm

Marine Protected Areas (MPAs): focus on the process of creating the Algarve Reef Marine Natural Park

Moderator

Nuno Sales Henriques

CCMAR - Centre of Marine Sciences

Short biography: Nuno is a researcher at CCMAR since 2011. In 2025, he completed his PhD at the University of Algarve on topics closely aligned with this proposal, graduating with the highest possible distinction (Summa cum laude). Throughout his career, Nuno has developed extensive experience in fisheries and ecological data collection and analysis, field survey design, participatory processes and stakeholder engagement. He has played a central role in participatory and co-management initiatives, working closely with fishing communities, management authorities and other stakeholders to support sustainable fisheries and marine conservation outcomes.

Participant

Jorge Gonçalves

CCMAR - Centre of Marine Sciences

Short biography: Jorge Manuel dos Santos Gonçalves, has a PhD on fisheries ecology by the University of Algarve (UALG) in 2000 and he is a senior researcher of the Centre of Marine Sciences of the Algarve (CCMAR) and an Invited Professor at the University of Cadiz (UCA-FCMA). He is a member of the board of directors of the Centre of Marine Sciences of the Algarve (CCMAR) in which he is also the coordinator of the Habitat and Biodiversity Mapping work package. His drive is to improve Marine Protected Areas efficiency and promote better fisheries management leading to sustainable fisheries and marine biodiversity conservation.

Participant

Alberto Queiruga

Oceano Azul Foundation

Short biography: Alberto Queiruga Maneiro is an environmental scientist and professional diver with over a decade of experience in marine conservation across Latin America, Africa, and Southeast Asia. As a Project Manager, he currently leads international efforts to design and establish Marine Protected Area (MPA) networks, directly contributing to the global 30x30 target. His expertise integrates strategic planning, stakeholder engagement, and scientific monitoring. Alberto has successfully managed multidisciplinary teams and projects focused on coral reefs, seagrass beds, sea turtles, sharks, and seabirds, fostering collaborations with governments, NGOs, and local communities to ensure long-term ocean protection.

Participant

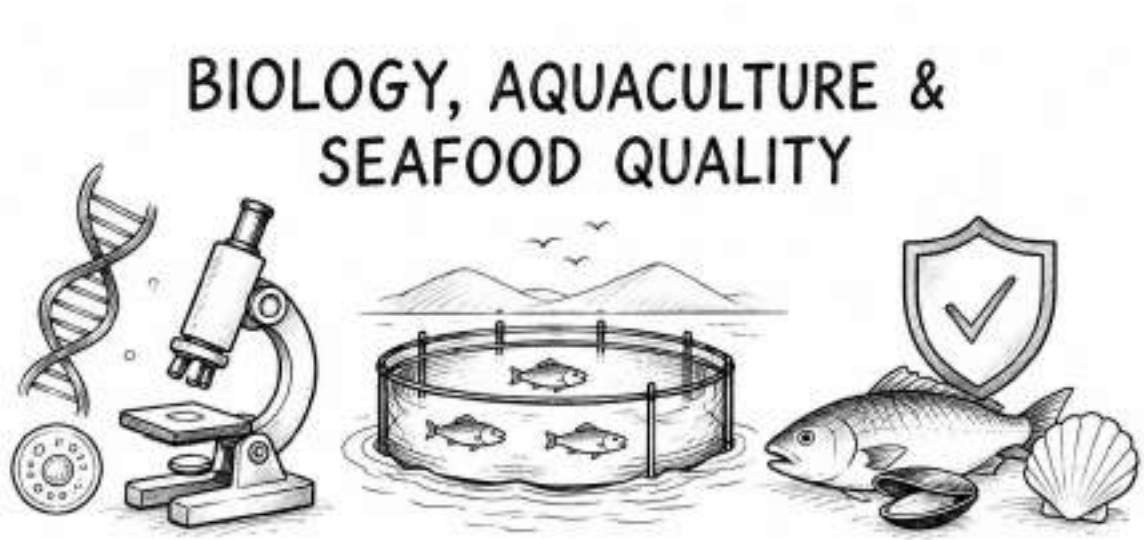
Miguel Rodrigues

BioReef AP and DiveSpot

Short biography: Miguel Rodrigues studied Marine Biology in the University of Algarve and has been managing a dive center in Armação de Pêra, Algarve, since 1997. He is a certified diving instructor and a scientific diving researcher, with thousands of hours of experience on the Algarve Reef. He served as the president of the Armação de Pêra Fishermen's Association and is the founder and current president of BioReef AP, a non-profit organization dedicated to scientific research and environmental literacy. Miguel spearheaded the initiative that led to the classification of the MPA of the Algarve Reef – Pedra do Valado. He was instrumental in highlighting the challenges faced by the reef and collaborated with CCMAR, the University of Algarve, the Oceano Azul Foundation, the Armação de Pêra Parish Council, and local municipalities to develop a participatory co-creation process for the MPA.

Oral Presentations

Topic 1



Transgenerational effects of thermal and nutrient stress priming in the golden kelp *Laminaria ochroleuca*

Benjamim Portugal[1], Júlia Soares[1], Brigitta Varga[1], Luis Barreto[1], Ester A. Serrão[1], Gareth Pearson[1], Neusa Martins[1]

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Ocean warming and the increasing frequency of marine heatwaves pose considerable challenges to kelp forests and their aquaculture potential. *Laminaria ochroleuca*, one of the most thermally tolerant kelp species in the northeastern Atlantic, is a promising candidate for sustainable seaweed aquaculture in southern Europe, however its production remains constrained by sea surface temperatures approaching or exceeding its thermal tolerance limits. Priming, the exposure of organisms to sub-lethal stressors to enhance subsequent stress tolerance, has emerged as a promising strategy to improve resilience in marine primary producers, but its efficacy in kelp gametophytes and its potential for transgenerational carry-over effects remain poorly understood. This study investigated whether thermal and nutrient-deficiency priming of *L. ochroleuca* gametophytes enhances thermal tolerance and reproductive performance, and whether these effects are transferred to the sporophyte generation. A cold-adapted population (Roscoff, France) and a warm-adapted population (El Jadida, Morocco) were compared to assess population-specific differences in priming response. Both priming treatments improved gametophyte survival and growth under elevated temperatures and broadened the reproductive thermal window, with primed gametophytes producing more eggs and sporophytes at the reproductive thermal extremes (11ºC and 18ºC) than unprimed controls. Moreover, sporophytes derived from thermally primed gametophytes maintained photosynthetic performance at 26ºC, while offspring from control and nutrient primed gametophytes showed complete photosynthetic collapse, demonstrating evidence of transgenerational thermal priming in *L. ochroleuca*. The findings support the integration of stress priming protocols into kelp hatchery and aquaculture practices to enhance thermal resilience under future ocean warming scenarios.

Acknowledgments: 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) and project AdaptKelp (DOI: https://doi.org/10.54499/2023.16917.ICDT; MPr-2023-12-16551, ALGARVE-FEDER-00772500).

Exploring the microbiome- thermal resilience relationship in *Laminaria ochroleuca* gametophytes

Júlia Soares Aguiar[1], Benjamim Portugal[1], Joana Filipe[1], Brigitta Varga[1], Luis Barreto[1], Ester A. Serrão[1], Gareth Pearson[1], Aschwin Engelen[1], Neusa Martins[1]

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Kelp forests are among the most productive and valuable coastal ecosystems worldwide yet are increasingly threatened by ocean warming and marine heatwaves. Understanding the mechanisms underlying thermal resilience is therefore important for both conservation and climate-resilient aquaculture. Recent evidence in other organisms suggests that host-associated microbial communities may influence stress tolerance, but their contribution to thermal resilience in kelps remains poorly understood. *Laminaria ochroleuca* (golden kelp) displays substantial variation in thermal tolerance across its Atlantic and Mediterranean distribution, making it an excellent model to investigate links between host performance and microbiome composition. This study examines whether microbial communities differ between thermally resistant and sensitive gametophyte strains originating from Portugal, Morocco, France and Italy, using genetically and geographically distinct but equally acclimated cultures, from the CCMAR Biobank. Gametophytes were exposed for 14 days to control (16°C) and lethal (26°C) thermal treatments. Physiological responses, including growth, density and maximum quantum yield of photosystem II (Fv/Fm), were assessed at the beginning and end of the experiment. Bacterial communities associated with each strain and treatment were characterized using 16S rRNA gene metabarcoding sequencing. We hypothesize that thermally resistant strains harbor more diverse, stable and functionally beneficial microbiomes, particularly under heat stress, whereas thermally sensitive strains will exhibit microbial dysbiosis characterized by a loss of beneficial symbionts and an increase in opportunistic taxa. By linking microbiome composition with host physiological performance across thermal treatments and geographic origins, this study expects to identify bacterial taxa and community characteristics associated with thermal resilience in *L. ochroleuca*. The results will describe kelp holobiont responses to climate change and future testing may ultimately support the development of microbiome-assisted breeding and cultivation strategies for climate-resilient kelp aquaculture.

Acknowledgments: 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) and project AdaptKelp (DOI: https://doi.org/10.54499/2023.16917.ICDT; MPr-2023-12-16551, ALGARVE-FEDER-00772500)

Yeast β-glucans as functional feed additives to improve European seabass resilience against *Tenacibaculum maritimum* infection

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Tenacibaculum maritimum, the etiological agent of tenacibaculosis, is a major health threat in marine fish farming. With limited therapeutic and vaccination options available, dietary immunomodulators such as yeast-derived β-1,3/1,6-glucans (BG) represent a promising preventive approach, yet their capacity to coordinate mucosal and systemic responses during bacterial infection remains incompletely understood. European seabass (*Dicentrarchus labrax*) were fed a Control (commercial-like) diet or diets supplemented with purified BG at 0.06% (BG6) and 0.12% (BG12) for four weeks before a bath challenge with *T. maritimum*. Fish were sampled at 24 h and 7 days post-infection for hematological parameters, plasma humoral immune responses, and gene expression in skin and posterior intestine. Growth performance was unaffected by BG supplementation. At 24 h post-infection, BG12-fed fish maintained circulating leukocyte counts and displayed elevated plasma lysozyme activity. In the skin, BG12 promoted early upregulation of pro-inflammatory (*il8*, *il1b*) and antimicrobial (*hamp*) genes, followed at 7 days by increased regulatory cytokines (*il10*, *tgfb*), reflecting an efficient transition from activation to resolution. Intestinal responses remained largely unaltered, consistent with a compartmentalized modulation focused on the primary infection site. *T. maritimum*-specific IgM was significantly elevated in BG-fed infected fish at 3 weeks post-infection, indicating enhanced adaptive humoral immunity. Overall, dietary BG at 0.12% promotes a balanced, tissue-specific immune response against *T. maritimum,* supporting its use as a functional ingredient to improve disease resilience in aquaculture.

Response of European seabass to a hypoosmotic challenge and potential for modulation with diets containing insect meal

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Northern Mediterranean countries are projected to experience more frequent and severe episodes of heavy rainfall and flooding, which may aggravate salinity drops in European seabass (*Dicentrarchus labrax*) farms. Based on the hypothesis that diet could reduce stress caused by climate-related events, this study analysed: 1) the physiological response of seabass to a hypoosmotic challenge, and 2) the potential of diets with increasing insect meal levels (IM; *Hermetia illucens* and *Tenebrio molitor* mixture) to modulate this response. For 75 days, seabass juveniles were reared at a salinity of 35 ppt and fed with a control diet (15% fishmeal) or one of three diets in which 3-50% of fishmeal protein was replaced with IM (triplicate tanks per diet). After 24 h of fasting, five fish per tank were exposed to water at 5 ppt for four hours. This hypoosmotic challenge induced an acute stress response, marked by increased plasma cortisol, glucose, and lactate. This was accompanied by downregulation of hypothalamic corticosteroid receptors and genes associated with neurogenesis. Additionally, the activity of hepatic antioxidant enzymes (superoxide dismutase and glutathione peroxidase) increased. Although fish fed higher IM levels tended to show a smaller increase in cortisol after the challenge, the IM diets showed little capacity to modulate the stress response. The long-term effects of the hypoosmotic environment ought to be explored in future work.

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Tetrodotoxin trail: how prey composition and gut microbiome predict contamination in *Charonia lampas*

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The trumpet shell (*Charonia lampas*) is a predatory marine gastropod from the Western Mediterranean and the northeast Atlantic. It has recently been linked to food poisoning due to the presence of the potent neurotoxin tetrodotoxin (TTX). This study investigated whether differences in diet and gut microbiome are associated with varying TTX levels, testing the hypothesis that the toxin is transferred through contaminated prey. Using metabarcoding, we analysed diet (COI marker) and microbiome composition (16S rRNA V3–V4 region) in 24 specimens. Additionally, TTX-producing bacterial candidates were isolated from non-edible tissues of *C. lampas,* and the presence of TTX and its analogs was confirmed by LC-HRMS. Our results showed that *C. lampas* has a diverse diet, mainly feeding on specimens from the phyla Echinodermata, Mollusca, and Cnidaria, with a broader diet in TTX-contaminated individuals that ingested taxa such as *Mytilus, Antedonidae, Epizoanthus,* and *Astropecten.* However, no significant effect of TTX contamination on the *C. lampas* microbiome composition was observed. Finally, we were able to isolate TTX and analogues producing candidates and assigned them as belonging to the genus *Vibrio.* These findings improve understanding of the different cues influencing TTX occurrence in *C. lampas* and its implications for food safety. Associations with specific dietary patterns and higher toxicity support an exogenous origin of TTX in trumpet shells. While no significant differences were observed at the genus level, variability in TTX and analogue production appears to occur at the strain level.

Acknowledgements

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Oral Presentations

Topic 2

New broad-host range plasmids to heterologously express cyanobacterial biosynthetic pathways

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The constantly increasing uncultured cyanobacterial diversity demands an expansion of the synthetic biology toolbox available to efficiently explore their metabolic potential. Heterologous expression of cyanobacterial biosynthetic pathways in model bacteria is a promising approach to explore this burgeoning metabolic potential without the need for strain culturing. The fast-growing *Escherichia coli* and the cyanobacterial model host *Anabaena* sp. PCC 7120 are widely used to express cyanobacterial biosynthetic gene clusters (BGCs). However, few plasmid backbones that allow expression in both heterologous hosts are available to date. Hence, swapping heterologous hosts due to compound toxicity or sub-optimal expression often requires assembly of the BGC into a new plasmid. In this work, we present a set of nine new pSEVA plasmids for heterologous expression in *E. coli* and *Anabaena* sp. PCC 7120. The RiPP-encoding *dol*BGC was used to test expression using the pSEVA251 backbone in both *E. coli* and *Anabaena* sp. PCC 7120. In both hosts, the expression of the non-, mono- and bis-phosphorylated dolichospermamides was achieved under the control of the tetracycline-inducible promoter, P_{tetO}. Nonetheless, LC-MS data from the cyanobacterial host indicates constitutive BGC expression evidencing a preference for native BGC regulation over non-native regulation. Afterwards, the pSEVA251 backbone was modified to facilitate heterologous host exchange. The backbone alterations include: 1) excision of the multiple cloning site; 2) assembly of an inducible promoter (P_{tetO}, P_{nirA} or P_{rha}) combined with different antibiotic resistances (neomycin/kanamycin, chloramphenicol or streptomycin/spectinomycin); 3) assembly of a green fluorescent protein downstream of the BGC assembly locus to monitor transcription; 4) insertion of a restriction enzyme recognition sequence to swap the promoter and antibiotic resistance facilitating heterologous host exchange. An environmental BGC (RiPP) was assembled into the newly designed plasmids and is currently being used to evaluate expression in both hosts under the control of the different inducible promoters.

Bioengineering GRP-EVs: A Human Cell Production Platform

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Gla-rich protein (GRP) is a vitamin K-dependent protein with anti-inflammatory and anti-calcification activity, requiring gamma-carboxylation for full functionality. Reduced GRP levels are associated with vascular calcification, inflammation, and cardiovascular disease, highlighting its therapeutic potential. Extracellular Vesicles (EVs) are promising nanocarriers due to their biocompatibility and ability to protect bioactive cargo, potentially overcoming the limited solubility of GRP under physiological conditions. Following our previous demonstration of gamma-carboxylated GRP (cGRP)-loaded EV production in a baculovirus-insect cell system[1], this work aimed to establish an Expi293F cell scalable platform for cGRP-EVs production. Expi293F cells were transiently transfected to overexpress GRP, and EVs were isolated from conditioned media by differential ultracentrifugation (30,000g and 100,000g). EVs were characterized for their physical, morphological, and molecular features, by nanoparticle tracking analysis, transmission electron microscopy, Western blot and ELISA. EVs were successfully isolated, and GRP was detected in all EV fractions. Vitamin K supplementation was required for efficient GRP loading and gamma-carboxylation, resulting in increased cGRP levels in EV. In THP-1 macrophages functional studies, EVs were non-cytotoxic and showed anti-inflammatory activity. Overall, Expi293F cells enable production of cGRP-EVs, supporting a serum-free human platform for scalable EV engineering. Ongoing studies will evaluate anti-calcification effects in relevant human cell models.

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CDKL2-Mediated Functional Rescue in a Zebrafish Model of CDKL5 Deficiency Disorder

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CDKL5 deficiency disorder (CDD) is an early-onset epileptic encephalopathy characterised by drug-resistant seizures and severe motor and neurodevelopmental impairments[1,2]. Recent evidence suggests that CDKL2, a kinase closely related to CDKL5, may partially compensate for CDKL5 loss of function[2]. This project utilises the zebrafish *cdkl5*^{sa21938} mutant model to investigate whether exogenous expression of human *CDKL2* can mitigate the behavioural and molecular abnormalities associated with Cdkl5 deficiency[1,2]. Single-cell embryos were microinjected with a vector encoding *CDKL2*, alongside a GFP-injected control group. Successful microinjection and embryo viability were confirmed by robust GFP fluorescence in control larvae. Molecular analysis further verified exogenous *CDKL2* expression, predominantly in the *cdkl5*^{sa21938} mutant line. To assess phenotypic rescue, locomotor behaviour was evaluated at two developmental stages. At 1-day post-fertilisation (dpf), double tail coiling assays successfully reproduced the previously reported reduction in motor activity in mutant embryos compared with wild-type (WT) controls, confirming the early behavioural phenotype. Preliminary results of swimming behaviour at 5 dpf revealed a prominent rescue trend; whereas *cdkl5*^{sa21938} control larvae exhibited reduced total distance travelled compared to WT controls, mutants expressing exogenous CDKL2 showed increased locomotor activity, approaching WT levels. Although quantitative analysis and statistical validation are ongoing, these initial data validate the experimental pipeline and provide preliminary evidence supporting the potential of CDKL2 as a compensatory therapeutic strategy for CDD.

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Leveraging Composting Microbiomes and Cellulolytic Phenotypes for Sustainable Textile Waste Management

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Cellulosic textiles represent a major fraction of global fibre production and pre and post-consumer textile waste. Although these materials are inherently biodegradable, their large-scale recycling remains challenging due to fibre crystallinity, chemical finishing, and heterogeneous waste streams. This study investigated composting both as a sustainable end-of-life strategy for cellulosic textiles and as a source of cellulolytic microorganisms with biotechnological potential. Cotton and lyocell fabrics were subjected to industrial and community composting conditions, followed by shotgun metagenomic analysis and selective microbial cultivation. Biodegradation efficiency was strongly influenced by composting conditions and textile fabric structure, with community composting achieving near-complete degradation and shredded textiles reaching degradation efficiencies of up to 97%. Metagenomic profiling revealed bacterial-dominated microbiomes enriched in Actinomycetota and Bacillota, together with a diverse repertoire of carbohydrate-active enzymes associated with cellulose degradation. Microbial cultivations recovered 62 cellulolytic bacterial and fungal isolates. Among these, strains affiliated with *Neurospora* sp. and *Aspergillus* sp. displayed the strongest cellulolytic activity and achieved textile degradation efficiencies above 60% under controlled *in vitro* conditions. Overall, this work demonstrates that composting systems constitute both an effective route for the biodegradation of cellulosic textile waste and a valuable reservoir of cellulolytic microorganisms, providing new insights into promising microbial resources for the development of sustainable textile waste treatment strategies.

Modeling ankylosing spondylitis in zebrafish - *CRISPR/Cas9-mediated erap1a/b deficiency*

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Ankylosing spondylitis (AS) is an autoimmune bone disease, with no cure, characterized by inflammation of the vertebral and sacroiliac joints, leading to vertebral fusion, loss of mobility, pain, hyperkyphosis, and loss of lordotic curvature [1]. Mutations in the *ERAP1* gene (Endoplasmic reticulum aminopeptidase 1), which encodes a protein essential for antigen presentation to MHC-I molecules, have been associated with increased AS susceptibility [2]. The zebrafish (*Danio rerio*) represents a suitable model for studying bone pathologies due to conserved skeletal and signaling pathways. In this work, we generated *erap1a: Δ7* and *erap1b: Δ8* loss-of-function mutants using CRISPR/Cas9 and characterized homozygous larvae. AlphaFold2 predictions confirmed that both mutations produce severely truncated proteins, supporting a complete loss-of-function. Skeletal characterization of homozygous larvae revealed vertebral fusions and malformations of bone structures in *erap1a: Δ7*. Furthermore, qPCR gene expression analysis revealed significant dysregulation of inflammatory markers in *erap1a: Δ7* mutants, linking *erap1a* deficiency to molecular pathways observed in AS. These results establish the *erap1a: Δ7* mutant as a promising model for studying the molecular mechanisms underlying AS, with potential for the future identification of novel therapeutic targets.

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Oral Presentations

Topic 3

Spatial and Temporal Patterns of Deep-Sea Elasmobranch Bycatch in Commercial Crustacean Trawls off Southern Portugal

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Bycatch is the incidental capture of non-target species and is a significant concern in fisheries management, particularly for vulnerable species such as deep-sea elasmobranchs. Understanding the spatial and temporal patterns of bycatch is crucial for developing effective mitigation strategies. This study aimed to quantify the bycatch of deep-sea elasmobranchs in a commercial crustacean trawler off the South and Southwest coasts of Portugal using fisheries dependent data collected between June 2020 and May 2022. The analysis focused on bycatch per unit effort (BPUE) across species, region, season and depth, giving special attention to the most predominant species and to total elasmobranch BPUE. Generalized Additive Models (GAMs) were used to recognize the main drivers of variation in capture rates using depth (96–1200 m), season, and area as predictor variables. Overall, the GAM results indicated that depth was the dominant predictor of BPUE, showing a strong and significant non-linear effect that explained most of the deviance in the model. In contrast, the study area (South vs Southwest) and seasonal effects were comparatively weak. Species specific smooth terms further revealed distinct depth-response curves among the most abundant elasmobranch species (e.g. *Deania profundorum* and *Galeus melastomus*). This study highlights the critical role of depth in shaping elasmobranch bycatch patterns. While regional and seasonal factors play a lesser role, their influence should not be entirely disregarded.

Mixed or fixed: genetic composition and connectivity of green sea turtles foraging in West Africa

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Green turtles (*Chelonia mydas*) travel hundreds to thousands of kilometers between nesting beaches and feeding grounds, facing several threats during their migrations. Understanding their population connectivity is critical for their conservation, especially regarding the less studied East Atlantic foraging aggregations. Here we use two mitochondrial DNA markers, the D-loop and mitochondrial short tandem repeats (mtSTR), to characterize the genetic composition of immature turtles across three West African foraging aggregations, in Mauritania, Senegal and Guinea-Bissau, and apply Bayesian mixed stock analyses (MSAs) to estimate their most likely origin. A total of 446 immature turtles (<90 cm) were sampled. All sites were dominated by the haplotype CM-A8.1 (85.4-94.4%) followed by CM-A5.1 (1.6-9.0%) and ten rare haplotypes. We found 20 mtSTR haplotypes, the most common being "7-12-4-4" (73.9-88.7%). Preliminary MSAs using the D-loop dataset estimated that most immature turtles originate from Poilão Island (80.7-90.6%), in Guinea-Bissau. However, for Mauritania and Guinea-Bissau about 7.2-7.9% of turtles may come from the rookery of Suriname, across the Atlantic, while 12.5% of turtles from Senegal were estimated to originate from the São Tomé and Príncipe archipelago, in the Gulf of Guinea. These findings underscore the critical role of West African foraging aggregations in supporting the persistence of the regional green turtle population breeding at Poilão Island and provide the connectivity data needed to guide coordinated conservation across migratory networks.

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Population genomics for the conservation of the red gorgonian, Paramuricea clavata, in the Parc National des Calanques (France)

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Despite recent advances in sequencing technologies and bioinformatics, population genomics studies remain poorly considered in the design of effective conservation measures. This case study demonstrates how population genomics can support management efforts for the Mediterranean red gorgonian, *Paramuricea clavata,* in the Parc National des Calanques (PNC, France). *Paramuricea clavata* is a habitat-forming octocoral with a key structural role in biodiversity-rich benthic communities. However, its shallow populations (until 40m) are highly impacted by recurrent marine heatwave-driven mass mortality events, raising concerns about the species' evolutionary trajectory. Moreover, the potential role of deep populations as climate refugia ("deep refugia hypothesis") and spillover between protected and neighbouring populations remain unclear. Using whole-genome re-sequencing data from 20 shallow to mesophotic populations from the PNC and its neighbouring areas, we aim to i) map the pattern of genetic diversity, ii) assess connectivity between populations, including testing the potential spillover effect, and iii) test the deep refugia hypothesis. This study will provide a genomic baseline to support conservation and restoration strategies for this valuable species, under the current climate change context, in an emblematic protected area.

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Multi-species seagrass transplantation in salt-production lagoons: lessons for design, cultivation and restoration

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Seagrasses are marine flowering plants that form meadows across shallow seabottoms worldwide. These communities provide essential ecosystem services, including coastal protection, support to fisheries, high biodiversity, and carbon sequestration. However, global seagrass decline contrasts with the limited scalability of current restoration efforts, which often rely on harvesting material from natural donor meadows or small-scale cultivation/nursery facilities. This dependency limits restoration potential and places additional pressures on vulnerable ecosystems. This study focuses on establishing seagrass habitats within salt-production lagoons in southern Portugal. The approach aims to create long-term seagrass sanctuaries in underused lagoons while assessing their seagrass cultivation potential. Three native species, *Cymodocea nodosa, Zostera marina,* and *Nanozostera noltei*, were introduced and monitored. Standardized experiments tested different transplantation densities and fragment spacing configurations for *C. nodosa* and *Z. marina* to assess establishment success and cultivation approaches, including handling practices. For *N. noltei*, transplantation followed a chessboard approach, transferring material from intertidal donor meadow into subtidal lagoon conditions. Preliminary results suggest that sheltered lagoons can provide favourable conditions for early-stage establishment by reducing hydrodynamic stress while enabling survival and growth monitoring. At the same time, practical challenges emerged, including plant loss, breakage, herbivory, and maintaining suitable lagoon conditions. This work highlights the potential of sheltered lagoon systems to support long-term seagrass establishment while providing practical insights into seagrass cultivation, transplantation density and fragment handling. The findings contribute to the development of scalable and low-impact approaches that can support European restoration targets and future seagrass conservation and restoration efforts across dynamic coastal systems.

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Cyanobacteria and cyanotoxins in Portugal: Distribution patterns and emerging risks in the Alqueva multipurpose project

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Cyanobacterial blooms in freshwater systems pose increasing environmental and public health concerns, exacerbated by the production of cyanotoxins. In Portugal, these events have intensified in recent decades, driven by eutrophication, climate change, and increasing water demand. This study combines a nationwide review of this topic in Portugal, with data from sampling campaigns in the Alqueva Multipurpose Project (EFMA), located in Alentejo, South of Portugal. The review (up to 2024) shows a widespread occurrence of toxic cyanobacteria, mainly *Microcystis*, *Aphanizomenon*, *Planktothrix*, and the spread of invasive species *Raphidiopsis*, particularly in southern regions. Multiple cyanotoxins were identified, including microcystins (MCs), cylindrospermopsin (CYN), anatoxins (ATXs), and saxitoxins (STXs) indicating increasing diversity. Although CYN has expanded northwards, CYN and ATXs had not yet been detected in surface waters of Alentejo. To address this, 69 water samples were collected from four EFMA reservoirs (March to October 2025) and analysed by LC–MS/MS for dissolved and particulate MC-LR, MC-RR, CYN, ATX-a, nodularin (NOD), and STX. MC-LR was the most abundant toxin, reaching to 1.9 µg L^{-1}, exceeding the regulatory limit for drinking water (1 µg). Maximum concentrations of MC-RR, CYN, and ATX-a reached 0.35, 0.69, and 0.53 µg L^{-1}, respectively. STX was detected but not quantified, due to concentrations below the quantification limit. Toxin levels increased from late spring to early autumn, peaking between August and October. CYN and MCs were consistently present, while STX and ATX showed a more limited distribution. This study reports, for the first time, CYN and ATX occurrence in Alentejo freshwater. Overall, results highlight the diversity and co-occurrence of cyanotoxins in Portugal, emphasizing the need to expand monitoring beyond MC.

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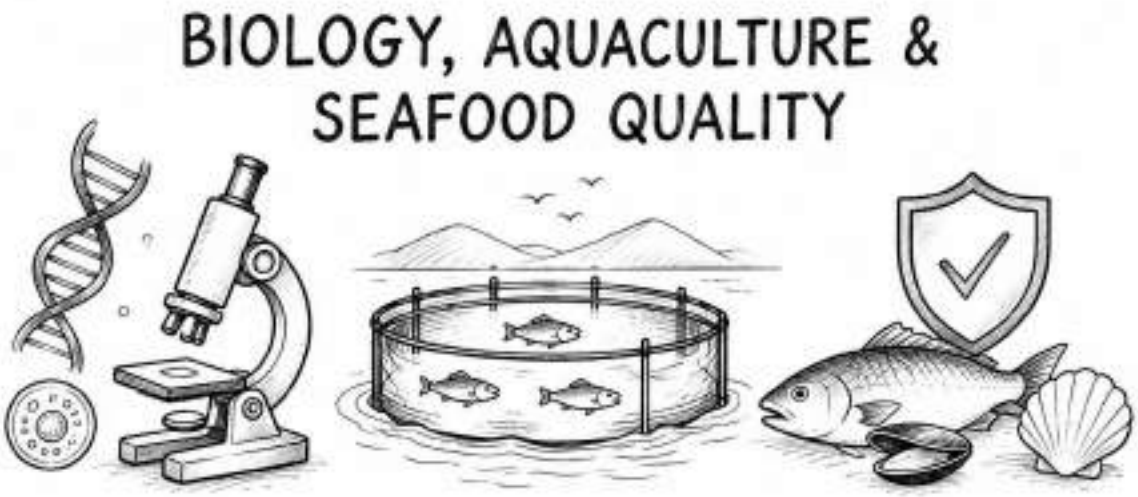
Poster Presentations

Topic 1



Effects of *Salicornia ramosissima* bioactives on European seabass (*Dicentrarchus labrax*) innate immune response: an *in vitro* approach

Aminur Rashid [1,2,3], Marina Machado [1], Sergio Fernández-Boo [1], Henrik Tribler [5], Jonathan L. Stein, [5], Mette H. Thomsen[4], Benjamin Costas [1]

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Salicornia ramosissima is a highly salt tolerant halophyte that has been recognized as a potential source of bioactive compounds with antibacterial, anti-inflammatory, antioxidant, and immunomodulatory properties for several aquaculture species. This study evaluated the effects of three crude extracts from semi-lignified *S. ramosissima* namely, Ext3, XAD, and FERM at three concentrations (0.125, 0.25, and 0.5 mg/mL) on head kidney leucocytes (HKL) from European seabass. Primary HKL cultures were incubated with the extracts for 24h, followed by stimulation with inactivated *Tenacibaculum maritimum* for additional 4h. Cell viability, respiratory burst activity, nitric oxide (NO) production, and transcriptomic responses were assessed at both timepoints. No cytotoxic effects were observed at any concentration following 24h exposure to the extracts or after *T. maritimum* stimulation in this study. Respiratory burst activity showed no significant differences among treatments at either timepoint, although Ext3 and XAD at 0.25 mg/mL showed a trend toward higher activity in both conditions. NO production differed significantly among treatments at 24h (p = 0.006), with XAD and FERM at 0.25 mg/mL showed lower NO production compared to controls. Transcriptomic analysis is currently ongoing, and the resulting data will be critical for elucidating the molecular mechanisms underlying these cellular responses. These findings will offer important insights into the effects of each extract on immune cell function and determine whether the extracts have the potential to activate or exacerbate pathogen-directed responses.

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Protein hydrolysates support gilthead seabream performance and mucosal health under temperature fluctuations

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Gilthead seabream (*Sparus aurata*) production is constrained by thermal fluctuations, particularly low temperatures, impairing growth and epithelial barrier function. Protein hydrolysates from animal by-products are promising functional ingredients due to their high digestibility and presence of bioactive peptides. This study hypothesized that dietary inclusion of poultry or swine protein hydrolysates could mitigate the effects of thermal fluctuations on growth and the mucosal barrier. Juveniles were fed three isoproteic and isoenergetic diets containing either 3% commercial fish hydrolysate (CPSP90; COM), poultry hydrolysate (POULTRY) or swine hydrolysate (SWINE) for 12 weeks under constant (CT; 22 °C) or fluctuating (FT; 22–15–22 °C) temperatures, alternating every 4 weeks. Growth performance and feed utilization were evaluated, and anterior intestine and dorsal skin samples were collected for histological assessment. FT led to lower body weight and feed intake. Mucosal health was also affected, as shown by increased skin epidermal thickness after 4 weeks of exposure to 15 ºC and reduced intestinal goblet cell abundance with FT. The inclusion of dietary animal by-product hydrolysates partially mitigated the negative impact of FT: compared to COM, POULTRY tended to increase feed intake and daily growth index (p=0.06) and significantly enhanced epidermal and intestinal mucous cell abundance despite FT, while SWINE significantly enhanced skin collagen deposition during the low-temperature phase of the FT regime, suggesting improved thermal resilience.

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Dietary Supplementation with *Euglena* Biomass Improves Antioxidant Status in Senegalese Sole Postlarvae

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Algae are promising ingredients to promote fish larval robustness and antioxidant defenses during sub-optimal rearing conditions due to their composition in bioactive compounds. In this study we assessed how algae biomass inclusion could modulate the growth performance and antioxidant system of *Solea senegalensis* postlarvae. Individuals aged 30 days after hatching (DAH) were distributed across 9 tanks, and three experimental diets were randomly assigned to tanks in triplicates until 43 DAH. The dietary treatments consisted of a commercial-like diet without algae supplementation (CTRL), the CTRL diet supplemented with *Euglena gracilis* biomass (EUG), and a third diet supplemented with EUG, *Ulva* sp. and *Tetraselmis striata* (MIX). At 43 DAH, dry weight (DW), total length (TL), condition factor (K), relative growth rate (RGR), and antioxidant-related parameters, including superoxide dismutase (SOD) and catalase (CAT) activities, the reduced to oxidized glutathione ratio (GSH:GSSG), and lipid peroxidation (LPO), were evaluated. Dietary treatments did not affect growth performance, except for TL, nor LPO and CAT activity. Fish fed the EUG diet exhibited a significantly lower TL than those fed the MIX diet, whereas the CTRL group did not differ from either treatment. Both algae-supplemented diets significantly reduced SOD activity, while the GSH:GSSG ratio increased significantly in fish fed the EUG diet. The lower SOD activity in both algal-supplemented treatments may reflect reduced oxidative pressure and a diminished need for endogenous enzyme production, potentially resulting from direct reactive oxygen species scavenging by algal bioactive compounds. These findings support the use of *Euglena* biomass as a functional ingredient capable of improving the antioxidant system of Senegalese sole postlarvae.

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Dietary grape seed extract primes turbot postlarvae for thermal challenge

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Nutritional modulation during early development can influence physiological resilience in fish. Plant-derived polyphenolic extracts, such as grape seed extract (GSE), are rich in bioactive compounds with antioxidant potential, but their effects during fish sensitive developmental stages remain unclear. This study aimed to evaluate how dietary GSE supplementation modulates growth performance, antioxidant status, and gut metabolic responses in turbot (*Scophthalmus maximus*) postlarvae and its ability to cope with a challenging event. Postlarvae (initial dry weight: 22.6 ± 8.0 mg) were reared in triplicate tanks in a recirculating aquaculture system and fed either a commercial-like diet (CTRL) or the same formulation supplemented with grape seed extract (GSE) from 29 to 51 days after hatching (DAH). Growth, antioxidant status, and mRNA expression of genes related to antioxidant defence, cellular stress, mitochondrial regulation, digestive capacity, nitrogen and lipid metabolism, and gut integrity were analyzed at the end of feeding period and after an acute thermal challenge of +5 °C for 24 h. Dietary treatments did not affect turbot growth (357.9 ± 111.3 mg; 47.5 ± 5.7 mm). However, survival was significantly lower in fish fed GSE (69.0%) than in CTRL fish (77.7%). Under standard conditions, larvae fed GSE exhibited "molecular readiness", significantly upregulating genes related to cellular stress (*sirt3*), mitochondrial dynamics (*opa1*) and gut integrity (*tjp1*). When challenged, GSE fed fish showed lower lipid peroxidation, along with proactive response to thermal challenge, with significant upregulation of antioxidant (*cat*, *gpx4*), digestive (*tryp*), and metabolic pathways (*atp5f1a*, *got2*, *gdh*) compared to CTRL. While dietary GSE significantly improved antioxidant and metabolic resilience, survival rates remained unchanged. These findings suggest that, whilst GSE is a potent metabolic primer, future studies should focus on further optimization of inclusion levels to translate these physiological benefits into survival outcomes during early ontogeny.

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Evaluating the potential of halophyte extracts to improve Atlantic salmon (*Salmo salar*) welfare during a simulated heatwave

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A demand for sustainable and innovative strategies to improve animal health and welfare in aquaculture is increasing and functional diets with halophytes extracts display high potential due to their health-promoting properties (antioxidant and anti-inflammatory)[1]. Hence, this study aimed to assess the effects of *Salicornia ramosissima* bioactive compounds in improving Atlantic salmon (*Salmo salar*) health and welfare when submitted to chronic stress by means of a heatwave. Fish were subjected to an experimental design comprising two temperature conditions, 13°C and 19°C, with the latter simulating heatwave scenarios. A total of 18 tanks were used in two separate RAS, and fish were distributed among three dietary treatments (CTRL, EXT3_0.5%, and XAD_0.5%), each conducted in triplicate tanks under both temperature conditions. Fish were hand fed the experimental diets for four weeks, after which sampling was performed. Plasma humoral immune parameters were evaluated through peroxidase activity and nitric oxide analyses, while the haematological profile was assessed by determining total white blood cell (WBC) and red blood cell (RBC) counts. Results showed no statistically significant differences in peroxidase activity, nitric oxide production or total RBC counts between diets and temperature conditions. However, total WBC counts were significantly higher at 19ºC compared to 13ºC. Additionally, gene expression analyses in the head kidney and brain are currently underway to further elucidate the molecular mechanisms underlying immune regulation and stress responses.

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Trait-based screening of *Laminaria ochroleuca* gametophytes reveals intraspecific variation in thermal tolerance

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Global warming is reshaping kelp forest distributions and threatening warm-edge populations. *Laminaria ochroleuca* is a warm-temperate habitat-forming species distributed along the south-western European coast and increasingly exposed to thermal stress at its southern distributional limit[1]. However, intraspecific variation in thermal tolerance across genotypes and populations[2] remains understudied, limiting trait-based strain selection for aquaculture and restoration. Here we present the first large-scale thermal screening of *L. ochroleuca* gametophytes across five geographically distinct populations, using SAMMBA, a high-throughput macroalgal phenotyping pipeline[3]. Over 200 strains were exposed to control (16 °C) and heat stress (26 °C) conditions for two weeks in 384-well plates and monitored through HD imaging and automated ImageJ analysis. Thermal performance was quantified using normalized gametophyte area, enabling high-throughput assessment of survival and growth. Thermal resilience varied among strains and geographic origins, with some populations exhibiting higher survival under heat stress, consistent with local adaptation. These results highlight the importance of population origin in determining thermal resilience in *L. ochroleuca*.

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Carbon fluxes in suspended shellfish aquaculture: developing an integrated metabolic assessment for bivalves and associated fouling communities

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An accurate assessment of the ecological footprint of shellfish aquaculture requires quantifying carbon fluxes not only from the target species but also from the entire biological system associated with production structures. In suspended longline systems, aquaculture lanterns accumulate diverse fouling communities — dominated by mussels, macroalgae and invertebrates — whose metabolic activity may substantially change local carbon dynamics through photosynthesis, respiration, and biogenic calcification. This study puts forward the development and validation of an integrated incubation system to quantify carbon fluxes across two biological components of offshore bivalve aquaculture: the target species *Venerupis corrugata* (grooved carpet shell) and the fouling community colonising the culture structures. Specimens were incubated in temperature-controlled chambers to develop calcification and respiration protocols and wet-to-dry biomass conversion factors. To analyse the biological community as a whole, we designed and tested a sealed incubation system using a 500 L tank, capable of housing an aquaculture lantern (80 × 42 × 42 cm), integrating simultaneous measurements of dissolved oxygen, total alkalinity, dissolved inorganic carbon, and nutrients. This approach allows independent evaluation of organic (net community production) and inorganic carbon fluxes (calcification), with alkalinity measurements corrected for nutrient dynamics. The system was applied across three light regimes — high irradiance, intermediate irradiance, and darkness — using replicate lanterns to characterise community metabolism under contrasting conditions. Together, these tools establish a replicable measurement system capable of showing ecologically meaningful metabolic signals at the practical scale of aquaculture infrastructure. By measuring all biological components in the system, we obtain a comprehensive picture of the carbon footprint of suspended bivalve aquaculture, with implications on the assessment of ecosystem services and carbon accounting in coastal production systems.

Assessing the potential of milk-derived extracellular vesicles in gilthead seabream (*Sparus aurata*) sperm cryopreservation

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Extracellular vesicles (EVs) carry bioactive molecules, including proteins, lipids, antioxidant compounds, and microRNAs, that act as mediators of cellular processes, intercellular communication and cell survival. In mammalians, it has been demonstrated that EVs improve sperm motility, preserve plasma membrane integrity, and protect against oxidative stress. Although no studies have evaluated the use of milk-derived EVs on fish sperm cryopreservation, it is plausible to suggest that it can mitigate sperm damage caused by cryopreservation. To assess that, sperm from ten males was collected and cryopreserved using the protocol described by Cabrita et al., (2005)[1] and adding $1x10^8$ and $4x10^8$ particles/ml of EVs. Sperm quality was evaluated by measuring post-thaw motility and velocity parameters, DNA fragmentation, lipid peroxidation, cell viability and reactive oxygen species. Preliminary results demonstrate that milk-derived EVs can be incorporated in the extender media without deleterious effects, however, no significant improvement was observed regarding the control. The highest concentration showed higher percentage of motile cells as well as VCL and VSL, while lipid peroxidation was higher in cells with the highest concentration. This study represents the first approach to the use of milk-derived EVs in fish cryopreservation extenders. More research needs to be conducted to evaluate different EVs concentrations and sources to explore their potential for fish cryopreservation.

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Immune response of juvenile rainbow trout (*Oncorhynchus mykiss*) under chronic thermal stress: A comparative evaluation of *Salicornia ramosissima* experimental diets

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Climate change stressors can affect fish and induce a chronic stress response, compromising their growth, immune function, and overall survivability. To mitigate these climate-induced organism vulnerabilities, the development of functional, aquafeeds has emerged as a critical frontier in sustainable aquaculture science. Halophytes, such as *Salicornia ramosissima*, are particularly promising candidates to be included in such diets due to their immunomodulatory, anti-inflammatory, and genoprotective capacities. The following study investigated the potential of functional feeds, specifically evaluating the efficacy of *S. ramosissima* enriched experimental diets to bolster resilience and mitigate the deleterious effects of high-temperature stress on juvenile rainbow trout (*Oncorhynchus mykiss*). The fish were randomly distributed in 15 tanks of two recirculated aquaculture systems in either control (16 °C) or elevated temperature conditions (22 °C) to mimic an environmental heatwave. Within each of these conditions, random triplicate groups of 20 fish were fed either a baseline control diet or one of four experimental diets supplemented with varying doses and extracts of *S. ramosissima*, for a period of 4 weeks. Following the 4-week trial, 3 fish from each triplicate group were randomly sampled, blood and plasma were collected for humoral immune parameter analysis, liver for metabolite and oxidative stress analysis, and head-kidney and brain for molecular analysis. Preliminary humoral and oxidative stress results showcased different profiles in the control group (16 ºC) and the high temperature group (22 ºC), while no apparent dietary effect was observed. Further comprehensive results, pending final statistical analysis, will elucidate the efficacy of *Salicornia*-enriched diets in mitigating the thermal stress.

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Dietary *Phaeodactylum tricornutum* promotes better growth and skeletal outcomes in zebrafish larvae

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The increasing global demand for food highlights the relevance of aquaculture as a sustainable source of high-quality protein. However, the sector still depends heavily on fish oil. Microalgae are a promising alternative due to their rich nutritional profile and the presence of bioactive compounds that can support key physiological processes, including skeletal development. In this sense, skeletal deformities are a major concern in aquaculture, leading to substantial economic losses. Because zebrafish share key developmental and genetic features with many aquaculture species, they are a powerful model for exploring nutritional strategies that can later be translated into commercial production. In this work, we investigated the effects of incorporating freeze-dried *Phaeodactylum tricornutum* biomass into zebrafish diets. The microalgae was produced under standard conditions or in a medium supplemented with iron and nitrogen and were added to the feed at 0.5% and 2.5%. Larvae were co-fed with rotifers until 8 dpf and then maintained exclusively on microdiet until 30 dpf. Growth was assessed at 15 and 30 dpf, while cumulative survival and skeletal condition were evaluated at the end of the trial. Although no significant differences in weight were detected, larvae fed with enriched *P. tricornutum* tended to be longer at 15 dpf, and those fed the 2.5% enriched diet were significantly longer at 30 dpf compared with all other groups. The overall incidence of skeletal deformities did not vary between treatments, but deformity severity was higher in fish fed the commercial diet and in those fed with 0.5% *P. tricornutum*. In contrast, both concentrations of supplemented *P. tricornutum* resulted in fewer deformed individuals, despite the absence of statistical significance. Taken together, these findings indicate that *P. tricornutum* cultivated under nutrient-enriched conditions can enhance larval growth and contribute to improved skeletal outcomes, reinforcing the potential of microalgae as functional ingredients for aquaculture feeds.

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Sensing conspecifics: Olfactory detection of chemical cues in the Lusitanian Toadfish

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Teleosts use olfaction to detect natural chemical compounds, odorants that mediate behaviours such as navigation, food location, predator avoidance, and conspecific recognition. The Lusitanian toadfish (*Halobatrachus didactylus*) is a benthic species that uses acoustic communication during the reproductive season; reproductive males vocalize to attract females to their nest. As other teleosts, the toadfish has a well-developed olfactory system. Lusitanian toadfish possess a highly sensitive olfactory system, responding to amino acids, bile acids, and conspecific-derived fluids, as demonstrated by the electro-olfactogram (EOG), with intestinal fluid (IF) being one of the most potent stimuli. Notably, IF olfactory sensitivity differs between reproductive and non-reproductive males. To identify the compounds underlying these differences, IF was fractioned and tested using EOG. Six fractions elicited significantly different olfactory responses between reproductive states. Cross-adaptation experiments revealed partial receptor sharing between active IF fractions and bile acids, suggesting that the biologically active odorants are bile acids or structurally related compounds. Untargeted metabolomic profiling using UHPLC-QTOF-MS in negative and positive ionization modes annotated 166 endogenous metabolites. Most reproductive state differences corresponded to unidentified features, while identified compounds were mainly taurinated bile acids and androgen-related metabolites. These findings indicate that IF composition is modulated by reproductive condition and identify bile acids-related compounds as strong candidates for reproductive chemical cues in Lusitanian toadfish. The study provides new insights into chemical communication in marine fish and suggests that chemical signaling may complement acoustic communication during reproduction.

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Nutritional and functional characterisation of *Sarcocornia perennis* ecotypes cultivated with saline aquaculture effluents

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This study determined the nutritional (proximate composition and minerals), phytochemical (HPLC-DAD) and in vitro antioxidant properties (DPPH and ABTS assays) of two ecotypes of the edible halophyte *Sarcocornia perennis* grown under different salinity gradients using aquaculture effluents. Productivity declined at higher salinity, and ecotypes were rich in moisture, ash, essential minerals, and fibre, supporting their potential as nutritious low-fat vegetables. Thirty compounds were identified, mainly hydroxybenzoic acids (gallic and salicylic acids) hydroxycinnamic acids (chlorogenic and ferulic acids), and flavonoids (epicatechin). The highest total phenolic content was observed in plants grown at lower salinity, while radical scavenging activity on ABTS and DPPH was similar between extracts (EC_{50}< 9 mg/mL). Overall, *S. perennis* shows promise for commercial cultivation in saline environments and could be further explored as a source of nutrients and polyphenol-enriched extracts with antioxidant properties for use in the food industry.

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Early feeding strategies enhance prey capture efficiency in brown trout (*Salmo trutta*) fry

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The degradation of freshwater habitats has contributed to the decline of salmonid populations, with high post-release mortality being a limiting factor in restocking programmes. Early nutritional strategies can influence the feeding behaviour and functional performance of fish, with potential implications for their survival. This study evaluated the effect of live feed inclusion and feeding frequency on the feeding behaviour and swimming performance of *Salmo trutta* fry. In the first experiment, three feeding regimes were tested: a control group fed commercial feed (CTRL), a group fed a diet with continuous inclusion of *Tenebrio molitor* (FR1), and a group fed a diet with late inclusion of the same live feed (FR2). The FR1 diet significantly reduced the latency time to the first catch compared with the control, indicating greater catching efficiency, whilst the FR2 diet showed no consistent effects. The probability of responding to food did not differ between treatments. In the second experiment, a frequent feeding regime (CTRL; three meals daily) was compared with a low-frequency regime (FS; one meal daily). In group trials, the FS treatment resulted in shorter time to capture and higher predatory efficiency, whilst in individual trials no differences were observed between treatments or diet type, although a significant interaction between the two was detected. Critical swimming speed (Ucrit) of the tested fish was not affected by feeding regimes. Taken together, the results indicate that co-feeding with live food improves capture efficiency in fry, whilst reducing feeding frequency increases feeding performance in a group context, but without visible effects on swimming capacity. These results underscore the importance of feeding management in optimising production protocols aiming to enhance the success of restocking strategies.

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Development of a novel purification protocol for myxozoan actinospores using flow cytometry: a model based on *Ellipsomyxa mugilis* (Cnidaria, Myxozoa)

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Myxozoans are microscopic metazoan parasites within the phylum Cnidaria, characterized by a complex life cycle that mainly alternates between fish and annelid hosts. High parasitic loads in fish hosts can trigger major diseases outbreaks, resulting in significant mortality in both wild and cultured fish populations worldwide. Research on host-parasite interactions remains limited, largely due to the difficulty of isolating myxozoans from host tissues, limiting downstream omics, pathology and immunological studies. To address this issue, a standardized and reliable purification protocol was developed using fluorescence-activated cell sorting. The life cycle of *Ellipsomyxa mugilis* was maintained in an indoor laboratory mesocosm through the co-housing of thinlip grey mullet (*Chelon ramada*) and the polychaete *Hediste diversicolor*. This system enabled enduring access to high quantities of actinospores, the infective stage produced by the polychaetes. By combining the wheat-germ agglutinin (WGA) lectin with viability dies (DAPI and propidium iodide) for counterstaining, a significantly pure population of *E. mugilis* actinospores was obtained, with approximately 98,8% viability. Microscopic observations revealed a distinct honeycomb-like binding pattern of WGA on the actinospores surface. These advances provide new opportunities for molecular characterization of myxozoan-host interactions and enable functional studies of actinospores infectivity in fish hosts through subsequent omics and immunologic research.

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Early thermal regime drives species- and tissue-specific microbiome assembly in gilthead sea bream and European seabass

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Aquaculture is an increasingly important pillar of the blue economy and the global strategy to meet growing global demand for high quality protein. Production of predictable high-quality larvae for aquaculture remains a bottleneck in Mediterranean aquaculture, aggravated by rapidly changing climate conditions. Temperature fluctuations during early rearing affect survival, metamorphic success, and long-term health. The microbiome is a critical mediator of host nutrition and immunity, yet how early thermal experience shapes microbial assembly remains largely unknown. Gilthead sea bream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) were reared under species-specific thermal regimes (sea bream: 17, 20, 23°C; seabass: 14, 17, 20°C) during a critical 4 - 8-day window from fertilization to mouth opening. After mouth opening, temperature was standardised (20°C for sea bream, 17°C for seabass) to mimic hatchery practice. At the end of larval rearing (40 days) and mid-metamorphosis (55 days), the intestine, gill, and skin were sampled. The effect of early thermal experience on the microbiome was assessed using 16S rRNA gene sequencing and qPCR. Temperature drove species- and tissue-specific microbiome restructuring. Sea bass showed resilience: no temperature effect in gill or skin at any stage, and core microbiomes were conserved (gill: 58 shared ASVs). In contrast, sea bream microbiota was highly sensitive to early thermal regimes. Metamorphosis triggered a near-complete microbial reset in sea bream; 83-97% of taxa at mid-metamorphosis were new, not present in larvae. A two-way PERMANOVA revealed that the stage-by-temperature interaction was highly significant for all sea bream tissues, with developmental stage alone explaining up to 60% of the variation in gut. These findings offer two practical outcomes. First, for hatchery management: core microbiome size reflects thermal resilience, and the first 4-8 days is a critical window for temperature standardisation, especially for sea bream. Second, temperature-responsive taxa (e.g., Vibrio/Polaribacter ratio) could serve as the basis for a rapid qPCR diagnostic kit, exploiting microbiota status as a tool for non-invasive health monitoring.

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Using passive acoustic monitoring to study marine life and underwater soundscapes

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In recent years, passive acoustic monitoring (PAM) has emerged as an efficient tool for studying underwater ecosystems. Marine organisms rely on acoustic cues to interact with their environment. By recording underwater soundscapes, PAM can be used to assess species presence, behaviour, exposure to anthropogenic noise, and associated responses. Although PAM has become a widely used tool in marine ecology, the selection of recording equipment, deployment strategies, and data analysis methods can be challenging for researchers without an acoustics background. This work provides an overview of the fundamental concepts regarding the design and implementation of passive acoustic monitoring experiments in marine environments. It will introduce the complete acquisition chain, and explain how acoustic quantities can be derived from the recordings. The concepts discussed will be illustrated through a passive acoustic monitoring station deployed off Cape São Vicente, located in south of Portugal, highlighting its applications to biological research.

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Quantifying habitat overlap between an invasive and a native Sciaenidae in the Gulf of Cádiz

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The weakfish *Cynoscion regalis* (Bloch & Schneider, 1801) is a Sciaenidae species native to the northwestern Atlantic coast of North America that was introduced into the Iberian Peninsula (Europe) in the 2000s. In the Guadalquivir estuary (Spain), it was first detected in 2011, and recent studies have confirmed its invasive status and use of the estuary as a nursery area. The meagre *Argyrosomus regius* (Asso, 1801) is a native Sciaenidae inhabiting the same estuary, raising concerns about potential niche overlap and displacement due to shared ecological requirements. This study aims to assess whether weakfish and meagre occupy the same habitats simultaneously and to quantify their temporal habitat use within the estuary. We used coupled Sr:Ca and Ba:Ca otolith signatures and microstructure to retrospectively determine habitat use and quantify habitat overlap between species. Juvenile individuals of both species were collected in spring and summer of 2023 and 2024 in the middle and lower sections of the Guadalquivir estuary using a passive trawl net (1-mm mesh) during new moon periods. Water samples were also collected to link environmental elemental signatures with otolith chemical composition. Additionally, adults of both species were sampled offshore in the Gulf of Cádiz to reconstruct lifetime habitat use. The results of this study will provide important insights for management strategies aimed at protecting native fish populations from potential impacts of the invasive weakfish.

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Evaluating sodium ascorbyl phosphate as a vitamin C biofortification strategy for *Phaeodactylum tricornutum*

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Oxidative stress is a major factor affecting reproductive performance and sperm quality in fish broodstock. Consequently, functional diets are routinely supplemented with antioxidant vitamins, particularly vitamin C, although this increases feed formulation costs and vitamin C is susceptible to degradation during feed processing and storage. The marine diatom *Phaeodactylum tricornutum* is a promising functional ingredient for aquaculture due to its high antioxidant potential and abundance of bioactive compounds, particularly carotenoids. Vitamin C biofortification could further enhance its nutritional value and provide a sustainable strategy for developing functional broodstock diets. This study evaluated sodium ascorbyl phosphate (Stay-C), a stable vitamin C derivative, as a cultivation supplement to assess its potential for vitamin C biofortification of *P. tricornutum*. Two independent 10-day cultivation trials were performed using different supplementation strategies: a single supplementation 24 h before harvest (0.5, 5 and 10 mg L^{-1}) and repeated supplementation throughout cultivation (0.1, 1 and 2 mg L^{-1}). Growth performance was assessed through biomass production, specific growth rate (SGR) and productivity, while biochemical characterisation included chlorophyll a and c, carotenoid content, antioxidant capacity (DPPH and ABTS assays), and vitamin C quantification. Repeated supplementation significantly increased biomass productivity, carotenoid accumulation and antioxidant capacity, with the strongest response at 1 mg L^{-1} Stay-C, whereas chlorophyll content remained unaffected. However, intracellular vitamin C concentration did not increase significantly, indicating that biofortification was not achieved under the tested conditions. Although vitamin C biofortification was not achieved, repeated Stay-C supplementation enhanced biomass productivity, carotenoid accumulation and antioxidant capacity, highlighting the potential of cultivation-based approaches to modulate the functional properties of *P. tricornutum*. These findings provide a basis for optimising future vitamin C biofortification strategies for broodstock nutrition.

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Life history traits and bycatch vulnerability of the common dolphin in southern Atlantic Iberia

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Common dolphins (*Delphinus delphis*) are one of the most frequently bycaught cetacean species in Portuguese waters, yet their life history parameters and bycatch vulnerability remain poorly characterized for the southwestern Iberian region. This study investigates sexual maturity and bycatch vulnerability in common dolphins stranded along the Portuguese coast between 2021 and 2025, using straight body length (SBL) and sexual maturity as the biological metrics. Sexual maturity was assessed using histological analysis of the gonads, and then comparing them with the SBL, using Bayesian cumulative logit models. Females (n=34) were modelled using a binary logistic regression, yielding a length at 50% maturity (x50) of 182.1 cm (95% CrI: 177.3–188.0 cm). Males (n=47) were modelled using a three-stage ordinal approach (immature, pubescent, mature), yielding x50 estimates of 198.2 cm (95% CrI: 189.4–206.3 cm) for the immature-to-pubescent transition and 207.6 cm (95% CrI: 199.8–220.3 cm) for the pubescent-to-mature transition. Bycatch vulnerability was assessed by comparing body size and maturity stage composition between confirmed bycatch and non-bycatch individuals using logistic regression and Fisher's exact tests. Preliminary results show that body length did not significantly predict bycatch probability in either sex. Similarly, no association was found between maturity stage and bycatch status in females while a trend was observed with pubescent males being disproportionately represented in confirmed bycatch events. These results suggest that bycatch in this population is not strongly size- or maturity-selective. Further studies should include larger sample sizes obtained not only from strandings but also from bycaught animals by the fishing vessel and continued monitoring to better characterize bycatch vulnerability in common dolphins along the Portuguese coast.

Of loss and duplication: the genetic evolution of gastric function in cartilaginous fish

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Evolutionary novelty and the mechanisms by which new traits arise remain central, unresolved questions in evolutionary biology[1,2]. The stomach, widely retained in jawed vertebrates, provides a compelling example of independent loss in several lineages, for instance, monotremes and teleost groups (e.g.: Cyprinidae, Syngnathidae)[3,4]. In Chondrichthyes, a similar loss of is found in the Holocephali lineage, whereas Elasmobranchs retain the stomach phenotype. This loss is often attributed to the disappearance, alteration, or inactivation of key genes involved in acid secretion, such as Atp4A and Atp4B, which encode core subunits of the gastric proton pump[3]. This work focuses on investigating the mechanisms of gastric physiology in cartilaginous fish and determine how these adaptations evolved, specifically regarding the role of gene loss in the Holocephali class. To investigate the genomic basics of stomach loss in chondrichthyans, we'll employ a comparative genomic and transcriptomic framework. First, by performing phylogenetic and synteny analyses of gastric-related genes to determine whether gene loss events occurred independently in Holocephali, and to assess the conservation of the surrounding genomic regions. Second, transcriptomic data from multiple tissues and species will be examined to determine whether candidate gastric genes remain active in the agastric Holocephali lineage. As expected, pH sensitive enzymes such as the aspartic proteases (e.g.: pepsin & gastricsin) and specific to the stomach in Elasmobranchs, appear to be absent in the five chimaera species analyzed. Trehalase, an enzyme responsible for hydrolyzing trehalose into two glucose molecules, display the same pattern of loss. Regarding other genes that are almost or totally unique to the stomach of elasmobranchs, exhibit a similar tendency though analysis is ongoing. To conclude, this research fills a void in the understanding of how organ decommission is reflected on a genomic level.

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Molecular characterization of European eel sperm quality during spawning induction

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The European eel (*Anguilla anguilla*) is an endangered species of high market potential in aquaculture. However captive breeding remains a major challenge, due to several reproductive dysfunctions including the inability to undergo spontaneous sexual maturation in captivity. Hormonal inductions of recombinant human chorionic gonadotropin (rhCG) are used to stimulate spermatogenesis and spermiation in male eels, yet these hormonal treatments can impact sperm quality. The antioxidant defence mechanism plays a role in spermatogenesis and in sperm function by protecting cells against damage. Despite the importance in fish reproduction, the relationship between antioxidant capacity and sperm quality in European eel remains unexplored. To understand this relationship two groups of male European eels were induced with two different treatments of rhCG. Sperm samples were classified as high- or low-quality based on motility, osmolarity, and pH. Antioxidant activity was assessed through measurements of total antioxidant status (TAS), superoxide dismutase (SOD), glutathione peroxidase (GPX), and glutathione reductase (GSR). Additionally, the expression of antioxidant-related genes (*gpx4, gsr,* and *sod1*) and the stress-response gene (*hsp70*) were analysed in sperm cells from each treatment and quality group. Understanding the association between physiological and molecular markers and sperm performance, this study aimed to provide insights that can improve the European eel broodstock management and reproductive performance under aquaculture conditions.

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Impacts of dietary antioxidants on the gut integrity of turbot postlarvae

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Turbot (*Scophthalmus maximus*) larval production is a critical stage in aquaculture due to high mortality during the first weeks of development. Feed additives rich in polyphenols, such as grape seed and green tea extracts, may enhance intestinal integrity through antioxidant and immunomodulatory mechanisms. This study aimed to evaluate the effects of dietary supplementation with plant extracts on intestinal morphology and larval performance. Larvae were reared for 21 days (29–50 days after hatching, DAH) in triplicate tanks. Fish were fed either a control microdiet (CTRL) or diets supplemented with grape seed (GSL, low; GSH, high) or green tea (GTL, low; GTH, high) extracts. At 50 DAH, larvae were sampled for standard length and gut histological analysis. Alcian Blue Periodic Acid Schiff staining was applied for histomorphometry and mucin differentiation. *Villus* length and width, lamina propria and submucosa thickness, muscular layer thickness, absorptive area, and goblet cell number and type were quantified. No signs of inflammation were observed in any group. Diets did not affect villus length or intestinal absorption area. The GSL diet increased the number of goblet cells, especially acid goblet cells. Green tea diets had minimal effects on intestinal morphology, and villus width varied without inflammatory association. Compared to both GT diets, both GS diets exhibited more pronounced beneficial effects, characterized by an increased number of goblet cells, which indicate an enhancement of the intestinal mucus barrier and epithelial integrity of Senegalese sole fed diets supplemented with GS.

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Genetic diversity in neutral and non-neutral genes in an Endangered shark species

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Chondrichthyans (~450 My) are the earliest extant jawed vertebrates with fully functional innate and adaptive immune systems[1]. The spiny dogfish (*Squalus acanthias*) is a migratory, circumglobal shark currently classified as Endangered in Europe and Mediterranean Sea according to IUCN due to overfishing combined with K-selected life-history traits[2]. Unpublished neutral marker data (ND2 and nuclear microsatellites) revealed reduced genetic diversity and significant differentiation of the Black Sea population, and to a lesser extent in the Adriatic Sea, relative to populations from the Irish Sea and the west coast of Ireland. To assess the adaptive potential and genetic health of this regionally endangered shark, we targeted immune-related genes, namely Toll-like receptors (TLR1 exon 1; and TLR3 exons 1 and 3) and Major Histocompatibility Complex class I (MHCI, alpha1) genes, across 40 individuals from each population (n = 160). Preliminary results suggest that MHCI and TLR3 exon 1 follow a pattern similar to that observed for neutral markers, with the lowest genetic diversity detected in the Black Sea, followed by the Adriatic Sea. In turn, TLR1 and TLR3 exon 3 show similar levels of genetic diversity across populations. By integrating neutral and adaptive markers, this study aims to clarify whether selective pressures acting on the immune system contribute to population differentiation and to provide insights into the adaptive potential and relative fitness of the studied populations. These findings may contribute to conservation planning by helping identify genetically distinct populations and prioritizing efforts.

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Spatial variation in copepodamides production by *Acartia* spp., *Centropages chierchiae* and *Paracalanus* spp.

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Copepodamides are polar lipids produced and released by copepods. When detected by microalgae, these chemical cues trigger defensive traits, including morphological changes, bioluminescence and the increased toxin production, in harmful algal bloom (HAB) forming species. This study aims to characterize and compare the copepodamides profile in different copepods species between two aquatic environments, Ria Formosa coastal lagoon and Guadiana River estuary. Using Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS), we annotated 47 copepodamides, including 15 new copepodamides structures, and assess their variability between these two environments. Marine water copepods contain both copepodamide subgroups, the copepodamides (CA) and the dihydro-copepodamides (dhCA), whereas brackish copepods were largely dominated by dhCAs. Furthermore, focusing on Ria Formosa, we evaluated spatial variability across sampling points and species-level variability among the three dominant copepod species (i.e. *Acartia* spp., *Centropages chierchiae* and *Paracalanus* spp.). Preliminary results reveal a significant spatial variability across Ria Formosa sampling points and clear species-specific chemical signatures. *Acartia* spp. predominantly produced dhCA, while *Paracalanus* spp. produced predominantly CA. In contrast, *C. chiechiae* exhibited production of both subgroups.

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Gene expression responses of *Mytilus galloprovincialis nr1j1* receptors to natural compounds

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Bivalves are commercially important shellfish that represent a valuable source of food worldwide[1]. They provide nutrient-rich meat, and their production has a low environmental footprint. However, bivalves are filter-feeding species that can accumulate and tolerate compounds that are toxic for human consumption[2]. Bivalves encode a chemical defensome, which comprise a group of molecular mechanisms dedicated to metabolizing and eliminate toxic compounds. As part of the defensome, the PXR receptor, a ligand-dependent nuclear receptor, has been extensively studied for its role as sensor and key regulator of detoxification responses in vertebrates. In marine invertebrates, the nuclear receptor NR1J1 has been described as the invertebrate ortholog of the PXR receptor, and it is suggested to participate in the activation of pathways that regulate the metabolism of toxic substances[3]. *Mytilus galloprovincialis* encode four NR1J1 paralogs; however, their molecular functions are poorly understood[4]. In this study, an *in vivo* approach was used to gather insights into the effect of natural compounds on the expression profiles of *M. galloprovincialis nr1j1* receptors and three candidate downstream genes. Results confirmed that natural compounds modulated the expression of the evaluated genes, suggesting that natural compounds may modulate metabolic pathways involved in the metabolism of toxic compounds, which could positively impact the elimination of toxins.

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Viperin: essential coordinator of antiviral responses in trout cells via CRISPR-Cas9

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Viperin is a key interferon-stimulated gene with broad-spectrum antiviral activity[1], although its role in the antiviral response of rainbow trout (*Oncorhynchus mykiss*) remains poorly understood. This study investigated the role of viperin in innate antiviral responses against viral haemorrhagic septicaemia virus (VHSV) using CRISPR–Cas9-mediated editing of the RTS11 monocyte/macrophage-like cell line. Electroporation of ribonucleoprotein complexes targeting viperin achieved editing efficiencies of up to 50%, after which edited and control cells were stimulated with heat-inactivated VHSV and analysed by RNA sequencing. Partial silencing of viperin disrupted canonical antiviral pathways, particularly the MAVS–TBK1 axis, despite the absence of viral replication, while inflammatory responses mediated by TRAFs and NF-κB remained active. Genes involved in apoptosis, autophagy, and phagocytosis were predominantly downregulated, suggesting a shift toward cellular survival and adaptive responses[2]. Compensatory antiviral mechanisms were also observed, including induction of alternative interferon-stimulated genes such as TRIM45, alongside upregulation of S-adenosylmethionine synthetase and cytidine deaminase, indicating metabolic adaptations linked to nucleotide regulation and epigenetic control of antiviral responses[3]. Overall, these findings identify viperin as a central regulator of antiviral immunity in rainbow trout macrophages and show that even partial loss of viperin significantly alters immune and metabolic responses to viral stimulation.

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Elucidating the roles of intrapopulation genetic diversity and phenotypic plasticity in *Alexandrium minutum*: moving beyond bloom detection to bloom diagnosis

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Harmful algal blooms caused by the dinoflagellate *Alexandrium minutum* pose major threats through the production of paralytic shellfish toxins [1]. Their bloom development and toxicity remain difficult to predict, since natural blooms are composed of multiple coexisting strains that may differ substantially in growth, toxin production, and stress responses [2]. This PhD project investigates how intrapopulation genetic diversity influences the ecological and ecotoxicological dynamics of *A. minutum* populations. The central hypothesis is that genetically diverse populations exhibit greater functional stability under environmental stress because different strains provide complementary adaptive strategies. To test this, the project combines population genetics, experimental ecology, and molecular biology across three levels of ecological complexity. First, a library of *A. minutum* strains will be genotyped using microsatellite markers and functionally characterized with respect to growth and toxin production under different nutrient regimes and biotic stressors. Second, synthetic populations with controlled diversity levels will be examined in long-term microcosm experiments to assess how diversity affects biomass, toxicity, strain dynamics, and transcriptional responses under fluctuating conditions. Finally, these findings will be validated in semi-natural mesocosm experiments. This project aims to provide a mechanistic framework for understanding bloom stability and toxicity. The results are expected to improve HAB forecasting approaches by incorporating genetic diversity as a functional component of bloom risk assessment.

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Exploring the regulation of puberty in Mozambique tilapia using transcriptomic and histological approaches

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Puberty is a key developmental transition in which the hypothalamic-pituitary-gonadal axis becomes fully activated, enabling reproductive competence. Although environmental cues are known to regulate pubertal timing in fish, the underlying neuroendocrine and molecular mechanisms remain poorly understood. In teleosts, photoperiod is an important regulator of reproductive development, but its impact on brain molecular responses during the pubertal window is still unclear. This study examined how photoperiod, used as an environmental signal, affects gonadal development and brain gene expression during early puberty in Mozambique tilapia (*Oreochromis mossambicus*). Fish were reared under a 12L:12D photoperiod until 70 days post-hatching, when histological analysis indicated the onset of puberty. They were then exposed for 30 days to standard (12L:12D), short-day (6L:18D), or long-day (18L:6D) conditions, followed by a return to standard photoperiod. Brain and gonadal samples were collected at 24, 48, and 96 hours post-treatment for histological and transcriptomic analyses. Short-day exposure delayed gonadal development, whereas no differences were observed between standard and long-day groups, indicating inhibitory effects of decreasing day length on pubertal progression. Brain transcriptome analysis revealed dynamic responses to photoperiod manipulation, with 47, 40, and 16 shared responsive genes identified between short- and long-day groups at 24, 48, and 96 hours, respectively. These genes were associated with biological regulation, metabolism, circadian rhythm, development, and reproductive processes. Overall, the results demonstrate that environmental cues influence both gonadal maturation and brain transcriptional activity during a critical pubertal window, providing insights into neuroendocrine mechanisms underlying puberty regulation in teleost fish.

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Dietary supplementation with the microalgae Euglena on the growth and oxidative capacity of Senegalese sole (*Solea senegalensis*) post-larvae

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Optimization of diets for early development stages of marine fish is crucial for aquaculture success. This study evaluated the effects of dietary supplementation with *Euglena viridis* biomass, at two inclusion levels, on the growth performance and oxidative status of Senegalese sole (*Solea senegalensis*) post-larvae. The *in vivo* trial was conducted in 8.8L tanks in RAS under optimal abiotic conditions for the species, for 14 days (30 DAH to 43 DAH). Post-larvae were assigned three dietary treatments, in triplicate: CTRL (commercial-like diet), Low (CTRL supplemented with a low inclusion level of *Euglena*) and High (CTRL supplemented with a high inclusion level of *Euglena*). Key performance indicators (dry weight, total length and relative growth rate) were evaluated at 43 DAH. Oxidative status was also assessed through superoxide dismutase (SOD) and catalase (CAT) activities, reduced to oxidized glutathione ratio (GSH:GSSG) and lipid peroxidation (LPO). Dietary treatments did not affect growth performance. However, both supplemented diets showed lower SOD activity and higher GSH:GSSG ratio compared to CTRL fed fish. These results suggest that *Euglena* supplementation maintains a growth performance equivalent to a commercial diet, while positively influencing redox status. The bioactive compounds present in *Euglena* likely contributed to reactive oxygen species scavenging, reducing the requirement for endogenous SOD, while the higher GSH:GSSG ratio suggests an improved cellular redox balance and antioxidant status in post-larvae fed the supplemented diets.

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Reduced live feed supply promotes metabolic plasticity and compensatory growth in Senegalese sole larvae

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The rearing of Senegalese sole remains constrained by its reliance on live feeds, particularly *Artemia*, which likely leads to suboptimal juvenile quality and may increase production costs. This study evaluated larvae reared under a co-feeding regime from mouth opening, reducing *Artemia* supply by 50%. Larvae were reared in triplicate tanks from 2 to 27 days after hatching (DAH) under two regimes: a control (CTRL) co-feeding protocol and an experimental group (CTRL-50%) with half of the amount of Artemia but identical microdiet levels before transitioning to a common commercial diet from 17 to 27 DAH. Physiological resilience was assessed through a 24-hour thermal challenge (+5°C) at 15 DAH. Performance was evaluated through biometric measurements together with biochemical and molecular markers related to oxidative stress and cellular function. While survival, condition factor, and relative growth rate did not differ significantly, *Artemia* reduction decreased larval weight and length by 17 DAH. During the thermal challenge, CTRL-50% larvae exhibited a higher lap3:ialp ratio, suggesting a shift from digestive activity towards cellular protective mechanisms. Together with the maintained GSH:GSSG balance (1.76), these results indicate an adaptive response to thermal stress that may have contributed to preserving cellular integrity and survival revealing a considerable physiological plasticity in CTRL-50% larvae. By prioritizing cellular integrity over growth during the stress, these larvae maintained normal development and survival rates similar to the CTRL fish. Subsequently, by 27 DAH, larvae exhibited compensatory growth, reaching biometric values similar to CTRL. These results demonstrate that early live-feed restriction, together with high-quality inert microdiets, triggers a physiological response that supports sole metabolic resilience. This strategy represents a promising approach to reduce hatchery dependence on Artemia while maintaining larval robustness and improving production sustainability.

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Impacts of season and effluent water on *Laminaria ochroleuca* farming and its microbiome

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Aquaculture is a rapidly growing sector that requires sustainable solutions to meet increasing food demands while reducing environmental impacts. Integrated Multi-Trophic Aquaculture (IMTA) systems offer such a solution by integrating species from different trophic levels. *Laminaria ochroleuca* is a promising kelp species for IMTA due to its tolerance of warm-temperate waters and its distribution from southern UK to Morocco. Its future more northern distribution may be influenced by changes in temperature and nutrient availability. This project investigates the holobiont of *L. ochroleuca* under farming conditions, focusing on the role of the microbiome in host development, health, environmental adaptation, and productivity. Particular attention will be given to fucoidan, a commercially valuable polysaccharide whose composition varies according to environmental conditions, cultivation season, and tissue type. We hypothesise that fucoidan composition and properties are influenced by tissue-specific microbiota and the cultivation. The project will also evaluate whether manipulating host–microbiome interactions can improve kelp growth and yield while maintaining fucoidan quality. The findings may support the development of new strategies for managing seaweed aquaculture.

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Single vs. multiple hormonal injections in male European eel

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The European eel does not undergo spontaneous sexual maturation in captivity due to neuroendocrine inhibition of the hypothalamic-pituitary-gonadal axis, relying on hormonal induction to stimulate spermatogenesis and spermiation. However, male responses to hormonal treatments remain highly variable, compromising sperm availability and quality. This study compared two recombinant human chorionic gonadotropin (hCGrec) injection protocols in European eel males: a weekly injection of 1.5 IU/g fish (WG) [1] and a boost protocol consisting of 1000 IU per fish at the beginning of treatment, followed by 250 IU per fish after 8 weeks (BG) [2]. Males from both groups (n = 18 fish/group) were sampled at weeks 8, 11, 14, and 17. Protocols were compared through biometrics, sperm volume, sperm motility and kinetic parameters, membrane integrity, malondialdehyde (MDA) levels as a lipid peroxidation marker, and DNA integrity. Eye index increased in both treatments, with BG males showing higher values at the final sampling. At the first sampling, BG males showed significantly higher sperm volume (2.8 ± 1.6 vs. 1.3 ± 0.9 mL/100 g fish) and total motility (62 ± 27 vs. 36 ± 26%) than WG. Sperm viability remained high in both treatments, with BG males showing higher values at the final sampling. BG also showed significantly higher MDA levels at the first sampling (122 ± 75 vs. 59 ± 26 nM MDA/10^6 spermatozoa). DNA fragmentation did not differ between treatments, but decreased over time in BG. Both protocols induced male European eel maturation, with males treated with BG protocol showing a stronger initial seminal response.

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Poster Presentations

Topic 2

Proteotranscriptomic Analysis of stony corals (Scleractinia, Cnidaria): Insights into their biology and putative bioactivity

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Stony corals (Scleractinia) play crucial roles in marine ecosystems by providing habitat for other species and supporting carbon and nutrient cycling. Yet, compared with other cnidarians, such as sea anemones and jellyfish, their bioactive compounds remain poorly explored [1], limiting our understanding of their chemical interactions with natural prey and predators and their potential use as drug leads. Here, we investigate the total proteomic profiles of five underexplored coral species – *Acropora muricata*, *Blastomussa wellsi*, *Catalaphyllia jardinei*, *Micromussa lordhowensis*, and *Montipora foliosa* – through species-specific transcriptomic and genomic datasets. We identified a total of 404 (*C. jardinei*), 1,476 (*M. foliosa*), 1,501 (*A. muricata*), 1,603 (*B. wellsi*) and 2,002 (*M. lordhowensis*) protein clusters, including skeleton-forming proteins, innate-immunity components, lectins, and fluorescent proteins. In addition, antimicrobial peptide candidates and 32 putative toxins were identified in their proteomes, including small cysteine-rich proteins (SCRiPs), actinoporins, and Kunitz-like peptides, revealing a diverse repertoire of bioactive molecules. These toxin families are widely distributed across 37 Scleractinian genomes, with some showing preferential chromosomal associations. Sequence analyses of Kunitz-like peptides showed strong similarity to previously described coral peptides with ion channel and protease activity, particularly in their cysteine arrangements. Together, this study provides the first proteome map for these five species and establish a basis for the study of coral biology and the exploration of novel compounds.

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Electric cell impedance system as a screening tool for nanoplastic exposure across human cell lines

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Nanoplastic pollution is a global environmental challenge, and human health concern. Despite increasing evidence of human exposure, comparative assessment of NP impact across different cell models remain limited, and many studies rely on high NP concentrations that do not reflect realistic environmental exposure[1,2]. In this context, the response of human lung (A549), intestine (Caco-2), dermal fibroblast (HDF) and skin fibroblast (BJ-5ta) cell lines exposed to environmentally relevant levels of 1,000 nm polystyrene nanoplastics (PS-NPs) was evaluated. Upon NP internalization, cell behaviour was monitored using an Electric Cell Impedance System (ECIS), allowing real-time assessment of cell adhesion and wound recovery. PS-NPs were internalized by all cell lines and induced concentration-dependent reductions in cell viability in both lung and intestine cell models. ECIS measurements revealed subtle cell-type-specific responses, including changes in the rate of wound recovery. These findings highlight the value of complementary *in vitro* models to investigate contaminant of the marine environment. The existence of cell-specific responses to NP exposure reveals a hitherto overlooked aspect that suggests toxicity may not be the same in all tissue *in vivo*. Furthermore, we demonstrate the potential of ECIS as a fast, non-invasive screening tool for detecting subtle cellular effects of environmental contaminants.

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Development of site-adapted microbial consortia for hydrocarbon bioremediation in Leixões port environment

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Port environments are recurrently exposed to petroleum-derived pollution, yet most response options remain focused on physical–chemical measures with limited ecological restoration potential. Additionally, ports may also receive river-borne urban/industrial loads from adjacent catchments. A culture-dependent and metabarcoding-guided workflow was used to identify native hydrocarbon-degrading consortia from surface seawater collected at the Port of Leixões (Matosinhos, Portugal). Seawater microcosms were enriched in triplicate with crude oil, diesel oil, or turbine oil for 15 days (dark, agitation), and hydrocarbon-degrader abundance was tracked by the Most Probable Number (MPN) method. Enrichment selected a core set of hydrocarbon-associated taxa and yielded 145 phylogenetically identified bacterial isolates. Across the three oils, common genera included Alcanivorax/Alloalcanivorax, Pseudomonas/Halopseudomonas, and Thalassospira, consistent with the metabarcoding results indicating reduced diversity and community selection under hydrocarbon exposure. Furthermore, a PCR-based screening for hydrocarbon-degrading genes is underway, targeting the genes that encode the following enzymes: alkane monooxygenases (alkB homologs) and flavin-binding long-chain monooxygenases (almA) for alkane degradation, catechol 2,3-dioxygenases (xylE1) for the degradation of BTEX compounds, and naphthalene dioxygenases (nahAc) for PAH degradation. Additionally, for the positive strains, a qPCR protocol will be implemented in order to quantify the absolute abundance of these degradation genes. Overall, these results will lay the foundations for obtaining site-adapted bioaugmentation consortia with high and rapid removal performance in seawater microcosms, supporting the development of autochthonous inocula for port settings.

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Elemental fingerprints of artisanal salts from the Castro Marim Natural Park: Physicochemical characterization and traceability of flor de sal and traditional salt

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Artisanal salt production in traditional saltpans is a centuries-old practice linking coastal wetland conservation and biodiversity protection. The saltpans of the Vila Real de Santo Antonio and Castro Marim Natural Park (VRSACM) serve as a cultural heritage site and critical habitat for halophilic flora, migratory waterbirds, and specialized invertebrate communities., while supplying an essential dietary mineral used globally for seasoning and food preservation. Artisanal salt production sustains the ecological and hydrological balance upon which this biodiversity depends and contributes a unique high value product to the blue economy. The local water mineral composition, seasonal dynamics, and microbial communities impart distinctive physicochemical and elemental fingerprints to artisanal salt that industrial production cannot replicate. A comprehensive physicochemical characterization of two artisanal salt categories, Flor de Sal and Sal Tradicional, was established by determining their elemental fingerprints, traceability profiles, and added-value attributes. Using Microwave Plasma Atomic Emission Spectrometry (MP-AES), concentrations of major (Na, Ca, Fe, Mg, K) and trace elements (Se, Zn, Mn, Fe, As, Cd) were determined across multiple samples. Sodium was the dominant element in all samples. Fe was below the limit of detection in all traditional salt samples but was detected in Flor de Sal. Traditional salt had a higher content of Na and Ca than Flor de Sal, which was enriched in Mg and K. Principal Component Analysis (PC1 = 53.4%, PC2 = 26.8%; cumulative 80.2%) and radar plot visualisation clearly discriminated between categories. Potentially toxic elements (Ni, Co, Cr, Ag, Pb) were below the limit of detection, affirming product safety. These elemental fingerprints establish a scientific basis for origin certification and added-value valorisation, reinforcing the interdependence between Natural Park biodiversity conservation and artisanal salt heritage.

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Valorization of Microalgae extracts in the cosmetic industry

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Microalgae and cyanobacteria are promising, sustainable sources of bioactive compounds with cosmetic relevance, including antioxidant, anti-inflammatory, UV-protective, and antimicrobial properties. These properties are particularly relevant in the context of skin health, where environmental stressors such as smoking and excessive UV exposure elevate oxidative stress, accelerating skin ageing through inflammation and increased activity of matrix-degrading enzymes (e.g., elastase and collagenase). Additionally, UV overexposure promotes hyperpigmentation via tyrosinase-mediated melanogenesis. In collaboration with Necton and Papillon Cosmetic Unipessoal Lda, in this project we aim to evaluate the cosmeceutical value of five industrially produced microalgae species, and test whether extracts from commercially produced biomass can serve as active cosmetic ingredients. The extracts were obtained using three solvents and initially characterized for antioxidant capacity (DPPH and ABTS radical-scavenging) and enzyme inhibition (tyrosinase, elastase, and collagenase). The most active extract achieved 75% and up to 98% oxidation inhibition in DPPH and ABTS assays, respectively, and 62% collagenase inhibition. Building on these results, in vitro anti-inflammatory assays were performed using human cell models relevant to skin inflammation, namely THP-1-derived macrophages and HaCaT keratinocytes. Both preventive and therapeutic approaches were evaluated in THP 1 macrophages, while preventive anti-inflammatory activity was assessed in HaCaT cells. Several extracts showed promising modulation of inflammatory responses, supporting their potential as anti-inflammatory and skin-protective cosmetic ingredients.

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Integrating bisulfite sequencing PCR and CRISPR/Cas-based detection for the analysis of TP53 epigenetic and genetic alterations in circulating cell-free DNA: a proof-of-concept study

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Cancer is one of the leading causes of mortality worldwide. Its early detection represents a major global health challenge requiring innovative biomedical solutions. Genetic and epigenetic alterations in the tumour suppressor gene TP53, the "Guardian of the Genome," are frequent molecular events leading to cancer development, and can be exploited as biomarkers for early diagnosis, prognosis, and disease monitoring. Cell-free DNA (cfDNA) circulating in the bloodstream is a promising and minimally invasive alternative to traditional tissue biopsy for early cancer diagnosis. However, translating this into clinical practice requires assays capable of detecting genetic and epigenetic alterations with high sensitivity and specificity. This proof-of-concept study aims to test two molecular tools, Bisulphite Sequencing PCR (BSP) and CRISPR/Cas12a-based detection system, to screen for TP53 promoter methylation and the R273H hotspot mutation, respectively. Performance of the analytical procedures will be tested using gDNA and cfDNA extracted from A549 (TP53 wild-type) and HT-29 (TP53 R273H-mutant) cell lines, before clinical demonstration with plasma samples from cancer patients and blood donors. This proof-of-concept study will determine the potential and limitations of both molecular approaches for diagnostic purposes.

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Automated membrane-based harvesting of microalgal biomass at industrial scale: performance and energy optimization of a 43 m² membrane system

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Efficient biomass harvesting remains a major bottleneck in large-scale microalgae production, representing a significant share of operational costs. Membrane-based technologies offer high biomass recovery efficiencies and automation potential; however, membrane fouling continues to limit their widespread application.In this study, a 43 m² membrane system was evaluated for continuous harvesting of microalgal biomass from an outdoor raceway at the IFAPA Experimental Station (Almería, Spain). Designed to process 10 m^3 day^{-1} of culture (0.1 day^{-1}), the system successfully completed two independent 40-hour harvesting trials following operational optimization. Stable filtration performance was achieved, with a flow decay rate of 1.5% h^{-1}, preservation of biomass quality, and full membrane permeability recovery after cleaning. Specific energy consumption was 3.7 kWh m^{-3} under the tested conditions. Process analysis revealed that the aeration rate was oversized, and simulations indicated that a 66% reduction in air supply could lower energy consumption to 2.1 kWh m^{-3} without affecting performance. These results demonstrate the feasibility of automated membrane-based harvesting for raceway microalgae production and highlight the importance of process optimization for reducing energy demand and improving economic viability.

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Valorisation of treated flatfish aquaculture effluent for microalgal biomass production in a circular bioeconomy approach

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Aquaculture intensification increases the generation of nutrient-rich effluents, creating environmental pressure and an opportunity for nutrient recovery. Within AQUA4ALL project, this study evaluated the treatment of recirculating aquaculture system (RAS) effluent from flatfish production before its use as a culture medium for marine microalgae. The approach aimed to reduce microbial load while preventing its accumulation in the biomass, supporting microalgal growth, and converting residual nutrients into biomass with potential value for aquaculture, within a circular bioeconomy framework. A preliminary screening of water treatment strategies was performed using: 1) sodium hypochlorite followed by sodium thiosulphate neutralisation, and hydrogen peroxide, and 2) ozonation. Untreated seawater showed 5.63×10^3CFU/mL (TSA), while both effluent treatments resulted in no bacterial growth. This low-dose chemical treatment was selected for the effluent assays due to its microbial control and compatibility with microalgal cultivation. Flatfish RAS effluent was filtered, treated, and tested in ALGEM photobioreactors with *Nannochloropsis oceanica*. Four conditions were compared: seawater supplemented with NutriBloom®, treated effluent without supplementation, treated effluent with nitrates, and treated effluent with NutriBloom®. Non-supplemented effluent showed limited growth, whereas supplemented effluents supported growth patterns comparable to control. NutriBloom-supplemented effluent maintained a positive biomass trend at the end of the assay. Nitrate decreased progressively in all cultures, confirming microalgal assimilation. Thus, treated flatfish RAS effluent can be used as a nutrient base for *N. oceanica* cultivation, provided that microbial treatment and targeted nutritional supplementation are applied. This strategy supports effluent bioremediation while generating microalgal biomass for future aquaculture products.

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Splitting *Spirulina*: a new cyanobacterial genus with anti-inflammatory activity

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The taxonomy of Spirulinales remains unresolved due to morphological convergence and historical misclassification. In this study strain LEGE 11439 is described as a novel *Spirulina*-like cyanobacterial genus, *Mesospirulina marina* gen. et sp. nov., through a polyphasic approach. Strain LEGE 11439, isolated from a marine subtidal environment in Portugal, formed a distinct and well-supported lineage clearly separated from *Spirulina* and *Halospirulina*. Phylogenetic analyses based on the 16S rRNA gene revealed 90.9% sequence similarity to *S.major* PCC 6313 and 90.7% similarity to *H. tapeticola* CCC Baja95Cl2. Phylogenomic reconstruction, a maximum average nucleotide identity (ANI) value of 80.9% relative to *S.major* PCC 6313 and 16S–23S rRNA ITS structures support its taxonomic distinction. Morphologically, *Mesospirulina* gen. nov. differs from *Spirulina* by reproducing exclusively through trichome fragmentation and from *Halospirulina* by possessing thinner coils. In terms of ecophysiology, the new genus distinguished by its more restricted salinity tolerance (2,5–5%) and inability to grow in freshwater. Chemical profiling of acetone fraction revealed a pigment composition dominated by myxoxanthophyll and β-carotene. This fraction exhibited a strong capacity to scavenge nitric oxide (NO) radicals generated *in vitro* and reduced NO production in LPS-stimulated RAW 264. Genome mining identified biosynthetic gene clusters, whereas no cyanotoxin-related genes were detected. These findings underscore the value of integrative taxonomic approaches and identify *Spirulina*-like lineages as promising sources of bioactive compounds.

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Exploring cyanobacterial metabolites for lipid-reducing activity using an OSMAC approach

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Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most prevalent chronic liver disease worldwide and is strongly associated with metabolic disorders such as obesity and type 2 diabetes. Hepatic steatosis, defined as excessive lipid accumulation in hepatocytes, represents the earliest stage of the disease and may progress to steatohepatitis, fibrosis, and cirrhosis. The identification of novel compounds targeting lipid accumulation remains a key research focus. In this context, cyanobacteria have attracted considerable attention as prolific producers of bioactive secondary metabolites. However, their metabolic potential is often underexplored, as many biosynthetic pathways remain inactive under standard culture conditions. The “One Strain Many Compounds” (OSMAC) approach addresses this limitation by modulating cultivation parameters to stimulate the production of otherwise silent metabolites. In this study, the OSMAC approach was applied to five cyanobacterial strains from our in-house culture collection (LEGE-CC), isolated from hypersaline environments. Cultures were grown in Z8 medium at 25 °C under a 14/10 h light/dark cycle, with supplementation of synthetic sea salts at 15, 25, and 60 g/L. Lyophilized biomass was extracted with CH_2Cl_2:MeOH (2:1, v/v) and fractionated by reverse-phase HPLC using a H_2O:MeCN gradient. A total of 120 fractions were screened for lipid-reducing activity in fatty-acid-overloaded human hepatocytes (HepG2 cells). Seven fractions showed a reduction in neutral lipid accumulation, some of which were associated with specific salinity conditions. These results support the potential of the OSMAC strategy as a valuable approach for diversifying and uncovering bioactive compounds from cyanobacteria. LC-HR-ESI-MS/MS-based metabolite profiling of the most active fractions, combined with feature-based molecular networking (FBMN), enabled the identification of candidate compounds potentially responsible for the observed bioactivity.

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Development of resilient and bioactive *Tisochrysis lutea* and *Pavlova gyrans* mutants for sustainable aquaculture

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Microalgae are increasingly recognised as sustainable sources of high-value compounds for aquaculture, providing essential nutrients and bioactive molecules that support fish growth, health, and immune function. Among these, haptophytes such as *Tisochrysis lutea* and *Pavlova gyrans* are particularly promising due to their richness in polyunsaturated fatty acids, including DHA and EPA, as well as fucoxanthin, sterols, and immunomodulatory compounds. However, their large-scale application remains limited by low productivity, sensitivity to environmental fluctuations, and high production costs. This work aimed to enhance the biotechnological potential of *T. lutea* and *P. gyrans* through random mutagenesis, targeting increased accumulation of lipophilic compounds and improved resilience for cultivation in aquaculture effluents. Mutant libraries were generated and screened under selective pressures, including carotenoid biosynthesis inhibitors, aquaculture effluents, and low-temperature conditions. Promising mutants were isolated using fluorescence-activated cell sorting and phenotypically validated under the respective screening conditions. In *T. lutea*, mutant strain M10 exhibited improved growth compared to the wild type when cultivated in aquaculture effluent without vitamin supplementation. In *P. gyrans*, selected mutants displayed markedly altered biochemical profiles and enhanced bioactivities. Mutant strain 26 showed a 53% increase in antioxidant activity and an 80% increase in flavonoid content compared with the wild-type strain ($p < 0.05$), while mutant strain 8 exhibited a 95% increase in flavonoid content ($p < 0.05$). These improvements were associated with promising functional responses in ex-vivo fish gut assays, where all *Pavlova* biomasses induced high gene expression levels. Notably, fish gut exposed to mutant strain 26 showed strong upregulation of genes related to antioxidant responses. Overall, this study demonstrates that random mutagenesis is an effective strategy to improve the resilience, productivity, and functional value of haptophyte microalgae. The enhanced mutant strains obtained in this work represent promising candidates for the development of cost-effective, bioactive, and sustainable ingredients for aquaculture feeds.

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Heterologous expression for the discovery of cyanobacterial natural compounds

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Cyanobacterial natural products (CNPs) represent an extensive group of secondary metabolites with highly diverse structures and bioactivities. At CIIMAR, LEGEcc is a culture collection of over 1,100 cyanobacterial strains, representing a notable biological source of novel chemistry. Although genome mining has enhanced the identification of biosynthetic gene clusters (BGCs) in cyanobacterial genomes, accessing their respective metabolites is still a major challenge since most cyanobacterial strains are not genetically tractable[1]. To circumvent this limitation, heterologous expression is an alternative to discover novel CNPs, aiding in the elucidation of the respective biosynthetic pathways[2]. This work aims to link BGCs from LEGEcc strains to their natural products by expressing them in the heterologous hosts *E. coli* and/or *Anabaena* sp. PCC 7120. For this, BGCs predicted by antiSMASH analysis to encode a RiPP, an indole, a PBDE and an ergothioneine-like compound respectively, were amplified and cloned into replicative vectors. The constructs were transformed into the hosts, and expression was conducted using different culture media, supplementations, temperatures, and induction times. Organic extracts from the expressing cells were analyzed by LC-HRMS and compared to empty vector controls. New molecular features were identified in the extracts from BGC-harboring strains, which now paves the way for optimization of the expression, further upscaling, and isolation and structure elucidation of the potential new metabolites.

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Isolation of halotolerant and halophilic bacteria with biotechnology potential from salt flats in the Algarve region

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The Castro Marim artisanal saltpans, located within the Vila Real de Santo António and Castro Marim Nature Reserve (VRSACM), form a fascinating hypersaline ecosystem at the mouth of the Guadiana River, representing a century-old hypersaline ecosystem with strong seasonal fluctuations. These extreme conditions foster microorganisms with unique adaptations [1]. Culturally dependent methods were used to isolate and purify 20 isolates in Marine Agar (MA), which were characterized by macroscopic and microscopic approaches, Gram staining, and screened for biotechnological traits, including enzyme production (amylase, protease, lipase, cellulase, phosphatase), siderophores, salinity tolerance assessment, and antimicrobial activity against *E. coli*, *P. aeruginosa*, *S. aureus* and *B. subtilis*. Isolates of interest were identified by 16S rRNA gene sequencing and phylogenetic analysis, and the genomes of the most interesting strains were sequenced for further analysis. Promising isolates were identified via 16S rRNA sequencing and phylogenetic analysis, revealing taxa from the *Halomonadaceae* family and the genera *Pseudoalteromonas*, *Bacillus*, and *Yoodia*. Two strains stood out for strong lipase and siderophore production. This study highlights the biotechnological potential of saltpan-adapted halophilic bacteria.

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Expression of melanin-encoding biosynthetic gene clusters in marine Actinomycetota

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The interest in natural pigments has been increasing due to the urgent need to replace petrochemical-based pigments. Actinomycetota is a phylum of Gram-positive bacteria known to produce numerous biotechnologically relevant compounds, including pigments. Among these, melanin displays interesting biotechnological properties, such as dark color, UV-protection and antioxidant properties. In this work we assessed melanin production of macroalgae-associated Actinomycetota isolates and evaluated the feasibility of melanin production in a heterologous system. Genome mining of 15 isolates using antiSMASH identified 8 putative melanin biosynthetic gene clusters (BGCs) from 7 isolates. All BGCs contained the 2 core biosynthetic genes (*melC1* and *melC2*) but varied in cluster size and genomic context. For each BGC, the core genes were amplified by PCR and implemented into a *Streptomyces* sp. heterologous chassis using a phage-based integrative system. The expression of the BGCs and production of melanin in the expression hosts was compared to that of the native host. Microbial melanin can contribute to the replacement of petrochemical pigments although further optimization will be required to achieve industrial production yields.

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Microfluidic isolation of bacterial consortia for targeted pharmaceutical removal in wastewater

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Pharmaceutical compounds are commonly found in conventional wastewater treatment plants (WWTPs) effluents, showing limited removal efficiency. Bioremediation can be a powerful and sustainable nature-based strategy to efficiently remove pharmaceuticals. In this work, two microfluidics-based approaches, Micro-lure in Microfluidics and Entrapment (µLUME) and Interspecies Aggregation Combinatorics, Microfluidics and Entrapment (iACME), were employed to isolate bacterial consortia capable of degrading pollutants from raw wastewater samples. The uLUME approach is based on the application of microsized porous vaterite particles with adsorbed pollutants to attract and attach bacterial consortia, whereas iACME focuses on changes in cell-membrane charges to induce formation of bacterial aggregates. The consortia were then encapsulated in alginate, and the consortia candidates with the best performance were selected based on their respiration in the presence of specific pharmaceuticals. Different pharmaceutical concentrations and presence/absence of a second carbon source were tested for each candidate to improve their growth and potential metabolic abilities. DNA was extracted from cultures under distinct growth conditions for the assessment of the microbial community composition of each consortium and the impact of growth conditions on community structure by 16S Amplicon Sequencing. Overall, this study will provide new insights into novel approaches for the isolation and development of microbial consortia for targeted removal of pharmaceuticals in WWTPs and highlight possible connections between bacterial dynamics and bioremediation processes.

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Deciphering transcriptomic landscapes of microalgal mutants for advanced food and feed applications

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The development of mutant microalgae with improved organoleptic and pigment profiles has revolutionized the food products commercialized by Necton and Allmicroalgae[1]. However, the metabolic pathways driving these enhanced phenotypes remain largely uncharacterized. This project aims to bridge the gap between random mutagenesis and rational strain improvement by integrating molecular characterization with functional validation. The initial phase utilizes transcriptomic profiling to uncover mechanisms underlying enhanced pigmentation in *Chlorella* sp. (white and yellow) mutants and bioactivity in a *Pavlova gyrans* mutant. By comparing mutants to wild-type strains, we aim to identify novel transcripts and metabolic bottlenecks associated with industrially valuable traits. These insights will inform the development of a molecular panel of marker genes for high-throughput screening, providing a robust tool for the rapid selection of stable, optimized mutants of these species, generated through random mutagenesis. Subsequently, functional validation of these optimized mutants will be conducted to assess their potential as human and aquaculture nutritional ingredients. This includes *in vitro* and *ex vivo* models to evaluate antioxidant and anti-inflammatory properties, as well as gut barrier function. This integrated approach strengthens the link between fundamental research and applied biotechnology, supporting the sustainable development of next-generation, commercially relevant algal products.

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Salinity-driven microbial succession in Mediterranean artisanal saltworks: Ecological insights and biotechnological potential

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Artisanal solar saltworks are unique human-managed coastal ecosystems where progressive seawater evaporation generates steep environmental gradients that shape microbial community structure and ecosystem functioning. Despite their ecological importance and potential as reservoirs of extremophilic microorganisms, with high biotechnology potential Mediterranean artisanal saltworks remain poorly characterized. This study investigated microorganisms in artisanal saltworks located in Castro Marim, southern Portugal. Water samples were collected along the evaporation sequence, including inlet ponds, intermediate ponds, evaporation ponds and crystallizers. Culture-dependent microbial isolation and 16S rRNA amplicon sequencing was performed. A pronounced environmental gradient was observed, with salinity increasing from approximately 30 PSU in inlet ponds to over 300 PSU in hypersaline ponds. Microbial communities exhibited clear taxonomic turnover along the salinity gradient, with richness decreasing from 1,267 OTUs in low-salinity ponds to 417 OTUs in hypersaline environments. Lower salinity ponds were enriched in Cyanobacteria-associated taxa, whereas hypersaline ponds showed increased abundance of halophilic microorganisms, including members of the genera Halomonas and Salinibacter. Culture-dependent microbial isolation and functional screening revealed the high biotechnology potential of Portuguese artisanal saltworks that represent reservoirs of extremophile biodiversity. The results provide the basis for understanding ecosystem functioning in Mediterranean hypersaline environments and uncover the biotechnological potential of microorganisms adapted to extreme salinity.

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A culturable bacterial survey of corals from the southern coast of Portugal

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Corals host diverse microbial communities that influence their health, resilience and responses to environmental change. Yet, most microbiome studies have focused on tropical species and rely on culture-independent approaches. As a result, the culturable fraction of microbiomes associated with warm-temperate corals remains poorly characterised, despite its importance for experimental work and microbiome-based interventions. Here, we surveyed culturable bacteria associated with six temperate coral species from the southern coast of Portugal, including five gorgonians - *Paramuricea grayi* (yellow and purple colour morphs), *Spinimuricea atlanticus*, *Eunicella verucosa*, *Leptogorgias sarmentosa* (yellow and purple colour morphs), *Ellisella paraplexauroides*, and one hexacoral, *Dendrophylia ramea*. Bacteria were isolated on Marine Agar and R2A Agar and identified by nearly full-length 16S rRNA gene sequencing. The study yielded 93 distinct bacterial strains, demonstrating substantial diversity among culturable bacteria at both the class (6) and genus levels (34). The genera *Pseudoalteromonas* and *Vibrio* genera emerged as the dominant bacterial groups. Certain coral species, notably *Paramuricea grayi* (yellow colour morph), displayed enhanced diversity of culturable bacteria (25 strains from 15 different genera). Many isolates corresponded to taxa rarely reported from corals or with poorly understood ecological roles. This pioneering study cultivated bacteria associated with this array of temperate coral species, establishing a database and biobank of bacterial isolates, which will provide a foundation for future investigations into host–microbe interactions and support coral restoration initiatives.

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Actinobacteria-mediated solid-state fermentation of macroalgae for biotechnological applications

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Macroalgae are promising resources for aquaculture, although their incorporation into feeds is limited by low digestibility and suboptimal nutritional profiles. Solid-state fermentation (SSF) using marine actinobacteria remains underexplored as a strategy for macroalgal biomass valorization. This work evaluated *Streptomyces* strains isolated from *Chondrus crispus* and *Codium tomentosum* as biocatalysts for macroalgal SSF targeting aquaculture and biotechnological applications.Ten *Streptomyces* strains were screened in 3-week SSF assays using both macroalgae species. Based on phenotypic responses, two strains were selected for extended 6-week fermentations under different conditions. Fermented biomass was lyophilized and extracted with ethanol or methanol:acetone (1:1), followed by antimicrobial assays against human and fish pathogens.*Streptomyces* strains effectively colonized and degraded the macroalgal substrates, showing distinct performances depending on strain and culture conditions. Extraction yields varied according to solvent system and substrate, with methanol:acetone resulting in higher recoveries. Antimicrobial activity assays showed modest inhibition halos (7-11 mm) against *Aeromonas hydrophila*, *Yersinia ruckeri*, *Lactococcus garvieae* and *Enterococcus faecalis*.Antioxidant assessments and nutritional characterization assays will clarify the potential of Actinomycetes-mediated SSF to improve macroalgal biomass for innovative aquafeed applications. Overall, these findings suggest SSF as a promising strategy for marine biomass valorization.

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Functional characterization of matrix Gla protein (MGP) domains using rAAV vectors

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The matrix Gla protein (MGP) gene encodes a member of the Gla protein family, which is secreted primarily by chondrocytes and vascular smooth muscle cells, and functions as a physiological inhibitor of mineralization and regulator of mineralization in the skeletal extracellular matrix 1. Loss of function mutations in the MGP gene can cause Keutel Syndrome in human patients, characterized by abnormal cartilage calcification, peripheral pulmonary stenosis, and facial hypoplasia 1. In addition, previous data have shown that MGP downregulation can lead to ectopic calcifications. However, the contribution of its functional domains, γ-carboxyglutamic acid (Gla) and phosphorylated serine residues, to this inhibitory activity may vary depending on the tissue. Consequently, our goal was to understand how these domains affect mineralization. We transduced cells capable of mineralizing their extracellular matrix, SaOS-2 and MC3T3-E1, using gene delivery mediated by a genetically modified recombinant adeno-associated virus (rAAV) vector encoding cDNAs of wild-type (WT) or mutant MGP. Mineralization was assessed by alizarin S red staining and quantified using the CPC method. The preliminary results obtained in SaOS-2 cells suggest that the Gla residues are important for MGP's ability to inhibit mineralization, while preliminary results from MC3T3-E1 cells suggest that both functional domains are important for MGP's function.

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Exposure to iron overload can inhibit mineralization process in ATCD5 cells and contribute to ferroptosis onset

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Osteoporosis is characterized by low bone mass and bone resorption. Several factors can contribute to bone loss and osteoporosis susceptibility, leading to premature osteoblast death and increase osteoclast activity. The iron metabolism is highly regulated, but there is no active mechanism for iron excretion. This means that through aging iron can progressively accumulate, contributing to onset and progression of bone loss phenotype and eventually induce cell death by ferroptosis. This study aimed to evaluate the impact of iron in the mineralization process using ATCD5 cells treated with 50 and 100µM of iron citrate. Erastin was used as positive control for ferroptosis. In this study we have analyzed cell metabolic activity (XTT assay), mineralization deposition (Alizarin Red staining) and gene expression of markers associated with bone metabolism and ferroptosis (qPCR). Results showed that iron loads tested had an impact on the mineralization process, with evidence for the interference of iron on mineral deposition, and at gene expression with downregulation of osteocalcin, for example. In addition, results showed that iron metabolism was affected and ferroptosis onset was suggested by downregulation of *Gpx4* and *Fth*. Using vitamin D as a possible rescue treatment, it allowed for a recovery in metabolic activity but without recovery of the levels of mineralization. We can conclude that iron concentrations used did not promote a clear ferroptosis phenotype, but affected the mineralization process, probably by interfering with mineral formation.

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Adaptation of microalgae strains for improved resistance to flue gas and waste streams toxicity while achieving high lipid productivity

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CO_2 capture using microalgae has attracted significant interest due to their ability to grow in wastewater, achieve higher carbon uptake rates than terrestrial plants, and produce high-value products and biofuels[1,2]. This study aimed to develop a microalgae-based strategy for CO_2 capture from bioenergy production systems by identifying strains capable of tolerating flue gas toxicity and utilizing wastewater while maintaining high lipid productivity. An initial nutrient assay identified two freshwater strains (T18 and T27) as the most promising, based on their superior growth, nutrient uptake, and lipid contents of approximately 20% and 28%, respectively. To assess wastewater tolerance, the selected strains were cultivated in drainwater supplemented with increasing concentrations of digestate from the anaerobic digestion of urban wastewater sludge, remaining viable at up to 20% and 30%, respectively. Based on these results, both strains are currently undergoing adaptive evolution through repeated digestate supplementation. Future work will focus on adaptation to elevated CO_2 concentrations by progressively lowering the culture pH setpoint. This work paves the way for microalgae-based systems to mitigate industrial CO_2 emissions while supporting sustainable bioenergy production and a circular use of resources.

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Computational discovery of antimicrobial peptide candidates from brown algae using integrated ai and bioinformatics approaches

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The increasing prevalence of drug-resistant infections has created an urgent need for alternative therapeutic solutions. Marine algae are gaining attention as a rich yet underutilized source of bioactive peptides. Their diverse proteomes offer considerable potential for the discovery of compounds with antimicrobial properties and insights into natural disease resistance mechanisms. This study explores brown algae as a novel reservoir of therapeutic peptide candidates, using a computational approach to identify safe and effective candidates. Protein sequences were subjected to simulated trypsin digestion to generate a large set of peptide fragments. These were evaluated through a multi-step screening process combining artificial intelligence (AI) approaches, including deep learning [1] and machine learning [2], as well as sequence-based models to predict antimicrobial activity while filtering out toxic and haemolytic peptides. The pipeline yielded a curated selection of candidates with strong predicted bioactivity and safety profiles. Several identified peptides exhibited favourable physicochemical and safety profiles, suggesting potential for future therapeutic and antimicrobial applications, as well as possible relevance to innate defence mechanisms in brown algae species. These findings highlight the potential of brown algae as a source of novel bioactive peptides and demonstrate the effectiveness of computational tools in accelerating early-stage peptide discovery. This approach supports the development of sustainable, marine-derived resources for future health-related research.

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Enhancing *Nannochloropsis oceanica* productivity by exploring bacteria-microalgae interactions

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Currently, microalgae are being used in a wide variety of industries such as pharmaceutical, cosmetic, food and feed due to their capability of producing high-value bioactive compounds including lipids, vitamins, proteins, and pigments. *Nannochloropsis* is a genus of microalgae known for its high content of polyunsaturated fatty acids as well as its rapid growth and biomass accumulation which led to its extensive use in aquaculture for fish larvae and rotifer rearing. Compelling new research has indicated the important role that bacteria have on the productivity and quality of microalgal biomass, yet the identity and role of specific bacterial strains associated with the industrial cultures remain poorly characterized. This project investigates how microalgae-associated bacteria can influence *Nannochloropsis oceanica* growth and biomass quality. A total of 43 bacterial strains isolated from industrial *N. oceanica* cultures were identified by DNA extractions and 16S rDNA sequencing. Co-cultivation assays were established between selected isolates and *N. oceanica* to assess their effects by monitoring microalgal growth and biochemical composition namely protein and total lipid content and fatty acid profile. The identification of the bacterial isolates at the species or genus level was achieved. Isolates belonging to the genera *Pseudoalteromonas, Marinobacter* and *Corynebacterium* showed positive results on the growth of *N. oceanica* in the co-cultivation trials, while *Oceanobacillus*, *Alcaligenes* and *Brevibacterium* bacteria had no effect on the microalga. Only one of the 43 tested bacterial isolates negatively affected the microalga. These data validate the hypothesis that bacterial interactions alter the performance and quality of *N. oceanica*. Moreover, beneficial effects may depend on specific bacterial combinations highlighting the complexity of microbial consortia in natural and industrial systems.

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A novel device for dry powder dispersion in *in vitro* respiratory applications

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The pulmonary route is raising increased interest for drug delivery, and dry powder formulations are currently the most popular in respiratory therapy. However, one of the major challenges in preclinical evaluation is reproducing realistic lung conditions during *in vitro* testing. Conventional methods often require dry powders to be suspended in liquid media before testing, which does not accurately reflect the actual respiratory environment. To address this limitation, our research group developed a simple and cost-effective aerosolization device capable of dispersing dry powders directly over surfaces, such as cell culture plates or membranes used in release studies.[1] The device operates through a syringe-driven air-blow mechanism to disperse dry powders and was produced using 3D printing technology, being optimized to fit commonly used testing platforms (6-well and 12-well plates). Two model dry powders based on locust bean gum (LBG) and mannitol, obtained through spray-drying and showing different aerodynamic and morphological characteristics, were used to validate the device. The aerosolization efficiency was assessed through quantification of the aerosolization yield (YA) upon testing different powder dosages (1, 5 and 10 mg), syringe air volumes (20 and 50 mL) and air-blow modalities. Results showed consistent and reproducible performance, with YA generally ranging 40% - 50%, independently of powder type and operating conditions. Overall, the developed prototype demonstrated effective dry powder aerosolization, representing a promising tool for improving *in vitro* studies of inhalable formulations, particularly in early-stage development phases.

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Cyotoxicity assessment of LEGE-CC cyanobacterial aqueous extracts intended for textile applications

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Cyanobacteria produce various pigments to harvest light energy efficiently. These pigments, including chlorophylls, carotenoids and phycobiliproteins, exhibit a range of bioactive properties that support their application in several industrial sectors, such as cosmetics, food and textiles. In the textile industry, the increasing demand for natural colourants is largely driven by strict environmental and health regulations. A main issue in the use of these dyes in textiles is the effects on skin. Therefore, the aim of this work was to evaluate the cytotoxicity of phycobiliproteins aqueous extracts at relevant concentrations relevant to the textile industry in skin representative cell lines. Cyanobacteria strains were obtained from LEGE-Culture Collection and cultivated in Z8 medium. Aqueous extracts were prepared through cell rupture by freeze-thaw cycles (-20ºC and 25ºC, respectively). The phycobiliprotein content was measured spectrophotometrically[1]. For cytotoxic assays, cells viability was determined spectrophotometrically by the reduction of the 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide tetrazole salt (MTT). These assays were conducted in keratinocytes (HaCaT), fibroblasts (3T3-L1) and endothelial (HCMEC) cells to represent potential skin exposure to dyed textiles[2]. The tested concentrations of cyanobacterial aqueous extracts were non-toxic, maintaining viability above 82.15% in all cell lines. In some cases (e.g. LEGE 191319), 3T3-L1 and HaCaT viability increased after 48h of exposure, compared to the control group. 3T3-L1 showed the lowest viability, particularly with LEGE 15486 and LEGE 16657 extracts. HCMEC displayed the lowest viability at 48h. Extracts concentration had no significant effect on cell viability. These findings highlight the potential of cyanobacterial aqueous extracts as safe and sustainable textile colourants.

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Seaweeds from the Cape Verde archipelago: Antioxidant potential and biotechnological applications

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Seaweeds represent a diverse group of organisms with different morphologies and colors that live in highly dynamic habitats. They synthesize a diverse array of specialized metabolites, with a large spectrum of biological activities[1]. Although abundant along the coast of Cape Verde, they remain poorly explored[2]. Therefore, this study investigates the biotechnological potential of three brown seaweed species collected in São Vicente, with particular focus on their antioxidant properties. The samples were extracted combining different solvent mixtures and extraction methods, with the optimal solvent-extraction method combination for each species being selected after a statistical analysis. Subsequently, the selected extracts were chemically characterized by HPLC-PDA and LC-MS/MS. A set of bioassays were conducted to evaluate the free radical scavenging capacity of the extracts, namely against superoxide anion, hydroxyl, and nitric oxide radicals. Finally, the cytotoxicity of the extracts was assessed in human keratinocytes, as long as the anti-inflammatory potential using murine macrophages. Statistical analysis revealed that the solvent composition significantly affects the evaluated parameters ($p < 0.05$ in ANOSIM test), while the extraction method did not. All selected extracts exhibited similar pigment profile, good antioxidant activity (specially against nitric oxide radicals), and no evidence of cytotoxicity.

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Small fractions, big impact: When less is more in microalgal cytokine modulation

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Chronic inflammation underlies many non-communicable diseases, including autoimmune and metabolic disorders, and there is a clear need for new, more selective anti-inflammatory approaches. Microalgae represent an emerging yet underexplored source of structurally diverse metabolites with promising anti-inflammatory potential. This PhD project explores microalgae-derived compounds as modulators of inflammation, using a fractionation-guided approach to reveal low-abundance bioactives that are masked in crude extracts. Fifteen crude extracts from five microalgal species were evaluated in LPS-stimulated THP-1 macrophage differentiated cells by quantifying TNF-α and IL-1β. The crude extracts showed little effect on these cytokines. In contrast, preparative HPLC generated 22 fractions with significant cytokine inhibition and low cytotoxicity, and a subsequent microfractionation step produced 74 microfractions, of which 40 structurally simpler fractions maintained strong, non-toxic cytokine-suppressive activity. Ongoing LC-MS/MS and mechanistic studies aim to define the molecular targets, bioavailability and digestibility of these metabolites. Together, the data indicates that microalgae harbour potent anti-inflammatory constituents that remain undetectable at the crude-extract level, and that a fractionation-guided strategy is an effective way to dissect complex mixtures and advance promising candidates towards functional food or complementary therapeutic applications.

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FusionAMP: A hybrid sequence–physicochemical fusion model for antimicrobial peptide prediction

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Antimicrobial peptides (AMPs) are promising candidates for combating antimicrobial resistance, but their experimental identification remains costly and time-consuming. We present FusionAMP, a hybrid deep learning model that combines sequence learning with physicochemical descriptors for AMP prediction. FusionAMP encodes peptide sequences using an embedding layer followed by a packed bidirectional LSTM with attention pooling, enabling efficient handling of variable-length sequences and highlighting informative residues. In parallel, lightweight physicochemical features derived from the amino-acid sequence—including composition, mean hydrophobicity, mean charge, and normalized length—are processed by a multilayer perceptron. The resulting sequence and physicochemical representations are fused and used for binary AMP classification. Model evaluation follows a publication-safe protocol, using a fixed train/validation split, validation-only threshold selection, and final testing on an untouched dataset. FusionAMP is evaluated on benchmarks derived from AMPlify [1] under both balanced and imbalanced regimes, with model selection guided by validation F1-score and PR-AUC, respectively. In practical inference scenarios on large peptide datasets, FusionAMP demonstrated substantially faster execution compared to the original AMPlify implementation, reflecting its lightweight architecture and efficient sequence handling. Results demonstrate stable performance and strong robustness under class imbalance, supporting FusionAMP as an efficient and reproducible approach for computational AMP screening.

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Understanding the enzymatic basis behind the biosynthesis of the unusual chemical features of fischerazoles

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Natural products are an endless reservoir of molecules whose intricate chemical structures imply the activity of unique enzymes. With many powerful enzymes yet to be characterized, biocatalysis proves to be one of the main strategies to reach the goals of the European Green Deal, tackling molecules synthesis while addressing matters of health, economy and environment. Following metabolomic approaches, our group successfully identified a set of C15 lipopeptides (fischerazole A-C,) from feeding perdeuterated hexadecanoic acid (C16) to cyanobacterium *Fischerella* sp. (PCC 9431). After structure elucidation, the isolated compounds showed a peculiar C8 pendant vinyl group. Isotope supplementation revealed that all the original 16 carbon atoms were incorporated into the fischerazoles, with one of them being extruded into the pendant allyl group. This implies an unprecedented biocatalytic contraction of a fatty acid-derived alkyl chain. Bioinformatic analyses revealed the biosynthetic gene cluster (BGC) of the lipopeptide, encoding a SAM-methyltransferase (FshF), that proved to be necessary and sufficient to cause the unique chain contraction[1]. Here, we propose a comprehensive approach to understand the mechanism of FshF. Metagenomics and sequence-based screening is applied to look for homologous bacterial BGCs from MAGs obtained from our lab and elsewhere. Over 10 target FshF homologues have been identified in this way, and analyzing their structural properties and docking, revealed promising hypotheses. Recombinant protein expression for biochemical dissection will be achieved by means of heterologous expression and cell-free systems. Activity of selected enzymes will be tested by in-vivo and in-vitro assays. Finally, one selected enzyme will be engineered with directed evolution and computational design to improve industrial performance.

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Bioprospection of three novel microalgae strains for pharmaceutical and cosmeceutical applications

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Overpopulation, the emergence of new diseases, lack of new bioactive compounds, source scarcity, make a highly challenging scenario with significant health and social impacts. In response to these concerns, new finding of bioactive compounds can help in the development of new drugs. Historically, most drugs come from or were inspired by nature, underscoring its impact on the development of new active ingredients based on natures rich diversity of chemical compounds[1]. In this work, three microalgae strains (T13, T18 and T27) from MarBiotech group (CCMAR) were cultivated at semi-pilot scale in 120L plastic bubble column photobioreactors using tap water supplemented with Nutribloom Plus® (NB+; Necton SA), sodium carbonate and calcium chloride. The biomass was recovered, freeze-dried and biochemically characterized. Additionally, organic extracts were prepared using acetone, ethyl acetate and ethanol 96% and bioactivity assays were performed to assess the presence of antioxidant and chelator[2,3]. Furthermore, the potential to inhibit the enzymes elastase, collagenase, and tyrosinase enzymes was assessed. T13 exhibited the highest lipid content (19%) dominated by polyunsaturated fatty acids (PUFAs). Regarding antioxidant assays, T13 ACE and T18 ACE demonstrated 87.8% and 81.6% ABTS inhibition, respectively. Regarding anti-enzymatic assays, anti-collagenase presented the highest inhibition with T13 ACE and EA with 49.9% and 47.6%. Overall, the selected microalgae strains presented relevant biochemical profiles and bioactivities, highlighting T13 for its high lipid content, potent antioxidant activity, and promising anti-aging potential, providing wide biotechnological applications, particularly to the pharmaceutical and cosmeceutical industries. Additional studies are needed to delve deeper and complement this initial work.

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Cyanobateria and microalgae: a sustainable solution for agriculture

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Over the years, the agricultural sector has relied on chemical products to mitigate losses from biotic and abiotic stresses and to meet the increasing demand for food. However, the negative impacts of the widespread and prolonged use of these inputs prompted the European Union to stop approving new agricultural chemicals, pressuring the need for biobased solutions. Cyanobacteria and microalgae are photosynthetic organisms, rich in bioresources of natural compounds with great potential for various agricultural applications. Samples from crops in Northern Portugal were collected and enriched in different culture media. Cyanobacteria and microalgae were isolated and selected to evaluate their potential. The selected strains were scaled up, centrifuged, freeze-dried, and extracted via sonication and Accelerated Solvent Extraction for biostimulants and biocontrol, respectively. To assess the biostimulants' potential, Solanum lycopersicum seeds were exposed to the extracts applied at 10 µg/mL. After 7 days of incubation, roots were photographed and measured. For biocontrol assays, three pathogenic bacteria, *Xanthomonas euvesicatoria*, *Pseudomonas syringae* pv. *actinidiae*, and *Erwinia amylovora* were exposed to the extracts at 100, 200, and 400 µg/mL in 96-well plates. Around 100 strains of microalgae and cyanobacteria were isolated. Some extracts showed biostimulant potential, and 12 of 81 extracts reduced bacterial viability by over 50%, but only one had this effect on all three bacteria. These organisms show great potential for developing more sustainable agricultural products.

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Sex-Linked Gene Dynamics in Cnidaria: From DMRT Evolution in Anthozoa to Differential Expression in *Hydra vulgaris*

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The Dmrt family genes encode transcription factors with a DM domain, a type of zinc finger motif that binds to the DNA minor groove and regulates genes, especially those involved in sexual determination[1]. However, little is known about the distribution of Dmrt genes in Cnidaria, a basal aquatic phylum of Metazoa comprising the subphyla Anthozoa and Medusozoa[2]. We identified 53 complete orthologous Dmrt genes in Anthozoa genomes – sea anemone *Nematostella vectensis* and 29 stony corals species – revealing 5 distinct phylogenetic clades, highlighting their diversification within Anthozoa. Differential gene expression analysis of *Hydra vulgaris* (Medusozoa) found no sex-biased expression of Dmrt genes in males (n=3) and females (n=3). Still, periculin-1-like and protein boule-like, previously linked to sex determination, were among the top 15 differentially expressed genes. These findings suggest that other regulators of sexual differentiation could be active and advance our understanding of the molecular basis of sex determination in Cnidaria.

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Comparative transcriptomic responses of *Mytilus* sp. exposed to silver and titanium dioxide nanoparticles

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Silver (Ag) and titanium dioxide (TiO_2) nanoparticles (NPs) are increasingly detected in aquatic environments, but their sublethal effects remain understudied. Here, RNA-seq identified differentially expressed genes (DEGs) and affected pathways in *Mytilus* sp. to distinguish shared and treatment-specific responses. Gill transcriptomic responses were obtained from a controlled 14-day exposure experiment conducted at Indigo Rock Marine Research Station (Ireland [1]). Four groups were analyzed: Ag NP control, Ag NP-treated, TiO_2 NP control, and TiO_2 NP-treated. Raw sequencing reads were quality-filtered and quantified prior to differential expression analysis. Functional interpretation included COG, GO, and KEGG enrichment analyses, with protein–protein interaction networks explored using STRING. A total of 492 and 376 DEGs were identified following exposure to Ag and TiO_2 NPs, respectively. Despite a 57% overlap in GO terms, only six DEGs (0.7%) were shared between treatments, confirming mechanistically distinct responses. Distinct cellular response mechanisms were identified for each NPs: Ag NPs appeared to trigger active defense responses, whereas TiO_2 NPs were associated with cytoskeletal suppression and impaired structural integrity. Our findings highlight distinct transcriptomic responses of *Mytilus* sp. to Ag and TiO_2 NPs, underscoring the need for nanoparticle-specific risk assessment frameworks in aquatic ecotoxicology.

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Microbiome and *Vibrio* surveillance across an industrial microalgae production line

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Microalgae production technology has expanded rapidly, yet biological contamination remains a critical challenge, causing significant biomass losses. Bacteria of the genus *Vibrio* pose a persistent threat due to their ubiquity and potential pathogenicity. Mitigating these risks requires a deep understanding of the microalgae-associated microbiome and the development of targeted biocontrol strategies, such as using *Vibrionaceae*-inhibiting probiotic bacteria (VIPB). This work represents the first of four tasks of a project aimed at developing mitigation strategies against *Vibrio* strains. The specific objective of this initial phase was to analyze the microalgae-associated microbiome across an industrial scale-up line and conduct surveillance of targeted bacteria to establish a baseline for future probiotic applications. The microbiome of a production line ranging from laboratory flasks, columns, flat panels, to a large-scale tubular photobioreactor was analyzed through 16S rRNA gene amplicon analysis via QIIME 2 and R/phyloseq. A highly conserved core microbiome, dominated by typical algal symbionts, such as *Marinobacter*, was identified throughout the production line. Significant community shifts were observed through the different reactor types, although *Vibrio* surveillance revealed a low relative abundance across all stages during the sampling period analyzed.

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Poster Presentations

Topic 3

Predicting changes in Minho estuary functioning and ecological status: 40-year comparison and future directions

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Estuaries are among the most productive ecosystems, supporting highly variable food-webs[1]. The Minho River estuary (NW Iberian Peninsula) has been classified as a Natura 2000 site given its ecological diversity[2], although it has seen a marked increase in environmental stress[3]. The current state and evolution of estuaries can be represented by physical, biogeochemical and physiological processes through ecosystem models that integrate and simulate fluctuations of (a)biotic factors[3]. An ecosystem model for the Minho Estuary was calibrated/validated by Martins et al. 2019[3], using data from 1982, to study macroinvertebrates biomass variations and benthic food-web responses to abiotic conditions. Recently, newer biotic and abiotic data were obtained (2021-2022, Gomes et al. unpublished), which allow to re-parametrize the model and perform current conditions simulations with higher accuracy. Therefore, our objective is to compare results from the published model with the recent calibration to understand how the system has changed after 40 years, as well as, to understand how emerging contaminants affect the estuarine ecosystem. To attain these, the published model is being updated using Aquatox v3.2 (U.S. EPA), accounting for current environmental parameters, input biomass values, additional functional groups/species, and an accordingly restructured trophic matrix. Preliminary results suggest an increase in polychaete biomass, which can be understood as a sign of system deterioration[1], accompanied by an increase of community respiration. Increasing and refining the knowledge on the mechanistic processes behind the Minho Estuary benthic communities through this type of models will enhance ecosystem-based management of the river's basin and contribute to informed decision-making.

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Transport of paint flakes in the Douro estuary: A numerical modelling approach

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Although paint particles (PP) are often included in size-based definitions of microplastics (MP), they are increasingly recognized as a distinct category due to their composition, behavior, and potential hazards. Unlike synthetic polymers, PP originates from paint coatings containing complex multilayered binders and inorganic fillers, resulting in higher density, greater brittleness, and elevated toxin concentrations [1]. Consequently, their transport dynamics, settling behavior, and sediment interactions can differ substantially from conventional MP, yet they are often treated similarly in environmental modelling. Within the HEurope INSPIRE project, an offline coupled system based on a hydrodynamic model and a particle-tracking model (PTM) simulates the release, dispersion, and fate of PP accidentally emitted from recreational marinas in the Douro Estuary. The PTM incorporates particle-specific properties, including two density distributions and corresponding settling velocities, to represent the heterogeneous structure of PP. Simulations were conducted under two configurations: baseline conditions (current practices) and post-intervention conditions, following installation of an innovative filtration technology capturing MP and PP at the source in the Douro Estuary. Results estimate each marina's contribution to total PP load, identify accumulation hotspots, and estimate PP residence time. The framework also evaluates filtration technology impacts on PP concentration and load and assesses scalability potential through broader multi-marina implementation to reduce PP pollution in estuarine and adjacent marine environments.

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Microplastics under climate change: Modelling the dynamic case of the Minho estuary

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Coastal zones, especially estuaries, are significantly vulnerable to pollutants and other environmental threats[1,2]. Due to their ubiquity, persistence, and potential for bioaccumulation, microplastics (MPs) pose a major threat to these ecosystems and human health[3,4]. Nevertheless, these emerging contaminants are not the only threat to these environments. Recent studies have shown that MPs' concentrations vary with tidal cycles and seasonal river discharges[1], parameters that are directly influenced by climate change and sea level rise (SLR), potentially aggravating the current threat. The present work has been carried out in the Minho estuary, where a hydrodynamic model was implemented to produce solutions (currents, salinity, and temperature) for present and future conditions (100-year SLR projections), considering the Shared Socioeconomic Pathways (SSPs) scenarios 4.5 and 8.5. The hydrodynamic model results were used to force a particle-tracking model that also accounts for the MPs' specific characteristics. The outputs from the models demonstrate the effect of SLR on MPs' behaviour, helping to understand future dynamics and to predict hotspots and MPs' residence times within this estuary. In this way, the results obtained enable the implementation of targeted future measures for MPs control and monitoring, demonstrating the potential of modelling frameworks to guide estuarine pollution management.

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Assessment of non-enzymatic antioxidant profiles in spinach cultivated with biorefinery-derived cyanobacterial biomass

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Cyanobacteria are widely recognized for their industrial relevance, with applications spanning the pharmaceutical, agricultural, food, cosmetic, textile, and biofuel sectors, highlighting their potential within integrated biorefinery frameworks. Following cyanobacterial biomass processing, residual material is often discarded, despite still containing multiple other high-value compounds. This study evaluated the effects of cultivating spinach with residual cyanobacterial biomass following targeted aqueous pigment extraction on its non-enzymatic antioxidant profile. Spinach plants were cultivated for 40 days under four conditions: control, commercial fertilizer (CF), and two cyanobacterial biomass formulations (4-M and 11-M). Non-enzymatic antioxidants measured included vitamins A, C, and E, β-carotene, lutein, Total Phenolic Compounds (TPC), and Total Antioxidant Capacity (TAC) as 2,2-diphenyl-1-picrylhydrazyl and Ferric Reducing Antioxidant Power. Plant growth parameters were also assessed (fresh weight, bolting, and photosynthetic efficiency). Plants treated with 4-M showed significantly higher (P<.05) β-carotene and lutein, with increases of 99% and 69%, respectively, when compared with the control and 62% and 47%, compared with CF. The 4-M group also had significantly higher (P<.05) TPC when compared to the CF group, with increases of 42%. The remaining non-enzymatic antioxidants did not show statistically significant differences (P>.05). No significant differences (P>.05) in plant morphology or photosynthetic efficiency were observed. Overall, previously extracted residual cyanobacterial biomass showed promising effects comparable to CF by enhancing plant metabolic content without compromising the plant weight, supporting its application as a sustainable agriculture biostimulant and highlighting the potential of cyanobacterial biorefinery strategies within a circular economy framework.

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Addressing current challenges in aquatic nanocontamination research

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Nanotechnology has become increasingly integrated into everyday life, and the growing release of engineered nanoparticles (NPs) into aquatic environments raises concerns about their potential impacts on organisms. However, the characterization and monitoring of NPs remain challenging, often relying on resource-intensive methodologies. In this context, the present work aims to develop and optimize tools for environmental nanocontamination monitoring while improving our understanding of NP interactions in aquatic systems. Specifically, it seeks to identify opportunities and challenges associated with the NanoSpacer platform, a microfluidic confinement technology coupled with dark-field microscopy (DFM) that enables real-time NP imaging using conventional optical microscopy. The study combines (i) the evaluation of the NanoSpacer platform for NP detection, visualization, and characterization, and (ii) exposure assays involving microalgae and bacteria from freshwater, estuarine and marine environments exposed to different types of NPs. Preliminary assessments have highlighted several challenges, including the low environmental concentrations of NPs, the complexity of environmental sample matrices, and NP transformations, which may affect NP detection, and interactions. To address these challenges, image analysis and artificial intelligence-based approaches are currently being developed to support NP tracking and characterization. The integration of established methodologies with emerging analytical and computational approaches is expected to provide a comprehensive understanding of NP behavior and biological interactions.

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Estrogenic activity assessment of recalcitrant pharmaceuticals in Ria Formosa wastewaters using the Dicentrarchus labrax estrogen screen (DLES) test

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Pharmaceuticals (PhCs) are persistent aquatic contaminants entering aquatic systems via wastewater treatment plant (WWTP) effluents. Compounds with endocrine-disrupting activity are of particular concern, yet estrogenic risk assessment tools remain calibrated to human estrogen receptors, overlooking the distinct receptor repertoire of marine vertebrates such as fish, which face lifelong exposure and potential bioaccumulation. The estrogenic activity of treatment-recalcitrant PhCs on fish-specific estrogen receptors remains largely uncharacterised. Here we show that several treatment-recalcitrant PhCs detected in wastewaters at the Ria Formosa coastal lagoon (Portugal), activate nuclear estrogen receptors of European sea bass (*Dicentrarchus labrax*). A 3-year seasonal monitoring campaign across Ria Formosa WWTPs and environmental waters identified five recalcitrant PhCs (carbamazepine, metoprolol, bezafibrate, azithromycin, sulfapyridine) persisting through treatment and detected at low-to-trace environmental levels. Using the DLES reporter gene assay, we evaluated activation of the three nuclear sea bass estrogen receptors (sbEsr1, sbEsr2a, and sbEsr2b) across a concentration range (10^{-11} to 10^{-7} M) of each compound. Azithromycin, metoprolol, bezafibrate and sulfapyridine significantly activated at least one receptor; carbamazepine showed no activation. The most sensitive receptor, sbEsr2b, was activated by all four compounds, with metoprolol and sulfapyridine eliciting responses across multiple concentrations. Bezafibrate additionally activated sbEsr1 and sbEsr2a at 10^{-7} M, the only compound to recruit both. These findings support the integration of marine species-specific bioassays into environmental hazard and risk assessment frameworks for PhCs pollutants in coastal ecosystems.

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Swimming abilities of “Little Fish” post-larvae of São Tomé: Implications for recruitment success

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The “Little Fish” or “Peixinho” is the local name given to endemic amphidromous species of the genus *Sicydium* that occur in São Tomé Island. Amphidromous fishes depend on successful migrations between freshwater and marine environments to complete their life cycle. Shortly after hatching in rivers, larvae drift to the ocean and later return to freshwater habitats as post-larvae, where they grow into adults and reproduce. During the pelagic larval phase, dispersal is strongly influenced by currents, and successful recruitment depends on the ability of individuals to overcome coastal and riverine flows, making swimming capability a critical ecological trait. Therefore, the present work aims to evaluate the swimming abilities of these gobiids by determining the critical swimming speed (Ucrit), a widely used and robust measure that serves as a proxy for *in situ* swimming capacity. For this purpose, 42 wild-caught post-larvae were tested under controlled laboratory conditions using a multi-lane swimming chamber. Critical swimming speed (Ucrit) ranged from 2.08 to 23.0 cm s^{-1}, with a mean value of 18.98 ± 6.05 cm s^{-1}. These results suggest that Peixinho´s post-larvae exhibit considerable swimming abilities, highlighting the potential importance of swimming performance for upstream migration and, consequently, recruitment into freshwater habitats. Understanding the swimming capabilities of amphidromous post-larvae provides valuable insights into recruitment dynamics and population connectivity, particularly in tropical island ecosystems facing increasing pressures from climate change, altered river flows, and habitat degradation.

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Widespread ingestion of anthropogenic particles by post-larval and juvenile amphidromous gobies in São Tomé island

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Small anthropogenic particles (AP), including microplastics and other textile-derived fibres, are now widespread in aquatic ecosystems. Amphidromous gobies support the traditional goby-fry fishery in São Tomé and Príncipe and play an important role in local food security. Despite their ecological and socio-economic importance, AP ingestion by their early life stages has not yet been investigated. This study quantifies the AP ingestion in post-larval and juvenile gobies (*Sicydium bustamantei, S. brevifile, and Awaous* spp.) collected from four rivers during wet and dry seasons. A total of 360 individuals were digested using 10% KOH, after which recovered particles were visually classified according to shape, size, and colour. A subset of particles was further analysed for polymer composition using Fourier-transform infrared spectroscopy (FTIR), while ingestion patterns were analysed using hurdle models to account for zero inflation. Anthropogenic particles were detected in 66.1% of individuals, with a total of 557 particles recovered and a mean ingestion rate of 1.54 ± 2.10 particles per individual. Although species, river, and season did not significantly influence particle counts, *S. brevifile* showed a higher probability of ingestion compared to *Awaous* spp. Fibres accounted for the majority of the ingested particles (85%), with black and blue being the predominant colours. Particle lengths ranged from 40 to 25790 µm, with most exceeding 1000 µm, and *S. bustamantei* and *Awaous* spp. ingested significantly larger particles than *S. brevifile*. FTIR analyses indicated a predominance of cellulosic fibres, suggesting mainly textile-derived sources. These findings demonstrate that early life stages of amphidromous gobies in São Tomé are widely exposed to AP likely originating from imported goods or donated materials. These findings highlight the urgent need for research into the toxicological effects of AP ingestion on fish populations, the implications for local food security, and the development of effective waste management and river catchment protection measures.

Doing science anywhere: investigating fish larvae swimming abilities across disparate resource landscapes

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Investigating the mechanisms that regulate fish larvae recruitment to nursery areas is crucial to accurately modeling larval dispersal and gaining a better understanding of fish ecology, life cycles, and population dynamics. However, studying fish larvae presents significant practical challenges, particularly when working with wild-caught specimens where specialized capture and maintenance are required. This presentation outlines the successful application of identical methodological frameworks to investigate larval swimming abilities in two vastly different contexts: a highly equipped research facility in Portugal and a remote, resource-limited field setting in São Tomé island. Rather than presenting a direct biological comparison, parallel case studies are outlined, to demonstrate how a core experimental design can be tailored to highly different species and working environments. While the resulting datasets represent distinct species and ambient conditions, they collectively validate the robustness of the methodology. The data shows that reliable behavioral results can be gathered effectively without the need for state-of-the-art local facilities. Ultimately, this work highlights the importance of "doing science anywhere" by adapting established frameworks to challenging circumstances. Creating these adaptable approaches in developing nations, rather than waiting for perfect infrastructure, is vital to avoid missing critical windows for biodiversity conservation.

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Reconstructing population dynamics of the European sardine (*Sardina pilchardus*) using palaeogenomics

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Current fishing policies have been consistently leading to a high level of overfishing, which deeply affects the marine species and reduces their populations to a small portion of their historical levels. However, our knowledge of how anthropogenic pressures have affected these species is scarce, mainly due to the lack of reliable catch records. *Sardina pilchardus*, the European sardine, is one of the most affected species, particularly in southern Europe. The European sardine (*Sardina pilchardus*) is a species of considerable economic and social importance, with a distribution extending from the waters off Senegal in West Africa to the North Sea. As one of the principal targets of purse-seine fisheries throughout its range, it represents a valuable marine resource and provides an essential source of income and food security for numerous coastal communities. Current genetic analyses have shown that sardine populations are divided into three main genetic clusters: one comprising individuals from the Azores and Madeira; a second encompassing Iberian populations, which occupy the centre of the sampling range; and a third containing Mediterranean and Canary Islands individuals, with Iberian sardines showing signs of admixture. In our work, we use state-of-the-art ancient DNA approaches to recover DNA from specimens that preceded the periods of most intensive fishing and we compare their genetic profiles with current populations, allowing us to evaluate the genetic continuity of the populations over time, comparing their genetic diversity and helping to create essential baselines for stock delineation, fisheries management, and determining appropriate fishing effort across the various FAO areas.

Conservation and economic potential of the whale shark (*Rhincodon typus*) in Cabo Verde

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Despite its ecological importance, knowledge on the behaviour and distribution of whale sharks (*Rhincodon typus, Endangered - IUCN*) remains limited, highlighting the need for improved research and conservation efforts. The Cabo Verde archipelago supports several threatened shark species. As this study shows, Cabo Verde is an important residency and aggregation area for whale sharks, as approximately 76% of all the positions recorded from the seven tagged individuals were in the study area around Cabo Verde, of which nearly 85% were recorded around Boa Vista Island. Focusing on Boa Vista, where conservation measures are already well established for other animals, shark-focused protection remains comparatively limited. Indeed, only 3% of the existing Marine Protected Areas overlap with the whale shark home range in the area, and less than 1% of the core area is currently protected. Thus, we aim to evaluate the spatiotemporal distribution of whale sharks in Cabo Verde, particularly around Boa Vista, and assess the degree of overlap between shark occurrences and existing Marine Protected Areas and recently identified Important Shark and Ray Areas. In addition, the study explores the potential economic importance of whale shark by reviewing ecotourism case studies from other regions such as the Maldives, Mexico and Indonesia. Some economic approaches aiming to assign monetary value to species with no market price, are relevant for endangered species because they recognise the ecological, cultural, and economic losses associated with their decline. By integrating ecological and economic perspectives, this study provides an assessment of the relevance of whale sharks around Boa Vista and identifies priorities for future research supporting strategy decisions on conservation initiatives.

Behavioural similarity and negotiation strategies between breeding partners in the little auk (Alle alle)

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In the unpredictable environment of the High Arctic, the reproductive success of long-lived seabirds relies on the efficient coordination of biparental care. For the Little Auk (*Alle alle*), while the general patterns of incubation and provisioning are well documented, the role of individual personality in driving these daily negotiations remains unclear. Specifically, neophobia creates a functional conflict between self-preservation and the demands of parental care. When an individual hesitates to approach the nest due to perceived risk, it introduces unpredictable delays into the pair's routine, disrupting their ability to maintain synchrony. Using a novel-object experiment across three breeding seasons (2020, 2021, and 2025) in Hornsund, Svalbard, this study quantifies individual neophobia (via latency to enter the nest), parental effort (provisioning rate), and pair coordination (visit alternation rates). Beyond establishing individual personality profiles, this research examines how neophobic traits shape parental coordination and evaluates whether personality similarity within pairs (matched versus mixed neophobic profiles) facilitates better cooperative strategies. Ultimately, these findings aim to provide key insights into the behavioural mechanisms that support reproductive stability in a rapidly changing Arctic.

Microplastic pollution on the north-west Portuguese coast: Linking coastal dynamics, human pressures, and public awareness

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Microplastics (MP) are now widely recognised as persistent contaminants in coastal systems, yet their spatial variability and accumulation patterns remain difficult to predict because they are shaped by physical and human drivers. Along the north-west Portuguese coast, seasonal variability, hydrodynamic phenomena, the presence of major river systems, tourism, and local land management practices play a critical role in shaping how these particles accumulate along the shoreline. This study examines the factors driving MP contamination by comparing beach sediment samples collected from six selected sites along the North Portuguese coast considering different degrees of exposition to the Ave and Douro rivers influence. These estuaries were selected because their drainage areas are marked by a legacy of industrial activity and dense urban runoff. Sampling was conducted in 2026 considering seasonal variability: late winter, marked by severe storm events, and late spring, characterized by seasonal tourism and increased recreational use of beaches. Field measurements of MP abundance and particle characteristics are combined with site-specific environmental information, available socio-economic data, statistical analysis, and numerical modelling. This integrated approach aims to identify how coastal circulation, river plume dynamics, and anthropogenic pressures drive the establishment of accumulation hotspots, potential retention zones, and transport pathways. A social dimension is also included to explore how local communities and visitors perceive the problem and whether simple educational interventions can encourage more responsible environmental behaviour.

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Meagre (*Argyrosomus regius*) stock trends along its Atlantic distribution: drivers and regional contrasts

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Meagre (*Argyrosomus regius*) is a commercially valuable top-predator fish whose spawning aggregations are targeted by specialized fisheries[1] across its Atlantic range, from France to Mauritania. Despite its ecological and economic importance, and its reported local extinctions[2], coordinated stock assessments remain absent. Here we analyze CPUE time series using complementary approaches to identify regional trends and their drivers. Min/Max Autocorrelation Factor Analysis (MAFA) and Dynamic Factor Analysis (DFA) revealed a dominant declining trend across most regions, associated with the rising Atlantic Multidecadal Oscillation (AMO). Regional Generalized Additive Models identified sub-region-specific drivers including Gironde River discharge, sea-surface temperature and North Atlantic Oscillation (NAO) for France; fishing pressure and primary production for Iberia; and the Tropical North Atlantic Index for Mauritania. A north-to-south gradient from non-lagged to lagged environmental responses suggests progressively impoverished stock status towards lower latitudes, consistent with historical overfishing in Mauritania[3] and ongoing depletion in the Gulf of Cádiz. French stock, driven by year-to-year environmental variability, appear comparatively healthier but require monitoring given the lagged nature of meagre population responses. These results highlight the need for spatially tailored, environmentally-informed management strategies across the species' Atlantic range.

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What the water carries: how coastal hydrodynamics shapes vertebrate eDNA detectability in northern Portugal

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Environmental DNA (eDNA) metabarcoding is increasingly used in aquatic biodiversity monitoring, yet the dispersion of particulate genetic material in river-influenced coastal systems may complicate ecological interpretation. Using an eDNA metabarcoding dataset comprised of 96 surface water samples from northern Portugal, we investigated how river plume influence and water column stratification shape vertebrate eDNA detectability and community composition across five functional groups (pelagic marine fish, demersal fish, river fish, diadromous fish, and cetaceans). Bayesian models using salinity and a stratification index as proxies revealed a generalised negative effect of salinity and a positive effect of reduced stratification on taxa detectability. Detectability of river-associated fish showed the strongest response to low salinity, while demersal fish were most influenced by stratification conditions. Surface-associated taxa detectability showed consistently weak responses to both predictors. PERMANOVA analysis showed that community composition across samples was not significantly structured by river plume and stratification. Spatial predictions revealed areas of consistently elevated richness, particularly near the Douro River's mouth, with clear seasonal variation. These findings demonstrate that hydrodynamic processes shape surface eDNA detectability in coastal systems, with broad implications for marine biodiversity monitoring.

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Fine-scale behaviour and foraging tactics of whale sharks (*Rhincodon typus*) in low oxygen environments

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Climate-driven ocean deoxygenation is altering ocean ecosystems, compressing habitat and shifting species distributions. Within oxygen minimum zones (OMZs), large pelagic fishes such as sharks often avoid hypoxic waters while exploiting enhanced foraging opportunities at shoaling OMZ boundaries, whereas other species appear to tolerate low oxygen conditions. To understand these contrasting patterns, integrating dissolved oxygen (DO) measurements with fine-scale behavioural responses is needed to identify physiological thresholds shaping species' responses to deoxygenation and associated foraging conditions. The Cabo Verde archipelago, within the eastern tropical Atlantic (ETA) OMZ, provides a natural system to investigate how large pelagic species cope with low DO. Among the species inhabiting these waters is the whale shark (*Rhincodon typus*), the world's largest fish, whose vertical habitat use remains poorly understood. Using accelerometers and DO sensors, we studied vertical behaviour and fine-scale movements of five whale sharks tagged in Boa Vista, Cabo Verde. Preliminary results show consecutive dives throughout the day, although only two individuals exceeded 200 m depth. A clear diel pattern was observed, with shallower depths at night and deeper waters during the day. Individuals experienced temperatures from 12 to 29 °C but spent >50% of their time between 23.5 and 24 °C. Sharks occupied mostly well-oxygenated waters (~4.7 mL/L DO), occasionally entering waters as low as 1.6 mL/L. Vectorial dynamic body acceleration (VeDBA), a proxy for activity, indicated higher activity at intermediate depths than at the surface and revealed prolonged U-shaped dives (up to 7 h) to 60–70 m, potentially associated with bottom foraging. Additionally, a surface-feeding event was also observed. Our findings improve understanding of whale shark ecology in low-oxygen pelagic habitats and how large marine species may respond to deoxygenating oceans.

Spatial determinants of biodiversity associated with mussel beds on rocky intertidal shores of the Iberian Peninsula

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Mussel beds are key habitat-forming structures on rocky intertidal shores, increasing substrate complexity and creating microhabitats that can reduce desiccation, buffer thermal stress, and support diverse associated communities. This study evaluated how the richness and diversity of organisms associated with mussel beds vary across spatial and microhabitat scales along the Iberian Peninsula, considering: region, orientation, and position in relation to mussel beds (inside/outside the mussel bed). Photoquadrat surveys were conducted on 3 regions (north, centre, and south), with sampling performed inside and outside mussel beds and in warmer sun-exposed and shaded orientations. Images were analysed using CoralNet, a machine-learning platform for ecological image annotation, applying a 100-point grid per image and a customized label set. The classifier achieved 84% accuracy across 61 categories, with all automated predictions manually reviewed to ensure reliability. Species richness and Shannon diversity were estimated and analysed using Gaussian linear models and ANOVA. Species richness varied significantly among all tested factors, with region showing the strongest effect ($p < 0.001$), followed by orientation ($p = 0.0044$) and position ($p = 0.023$). The Central region showed the highest richness and diversity values, while shaded areas supported richer and more diverse communities than sun-exposed, consistent with the role of fine-scale thermal heterogeneity in shaping intertidal assemblages. Inside mussel beds quadrats also presented higher richness and diversity than outside mussel beds quadrats, reinforcing the role of *Mytilus* as an ecosystem engineer and biodiversity facilitator. Overall, these results show that biodiversity associated with mussel beds is mainly structured by regional-scale, but also responds to fine-scale orientation and position. This highlights the importance of combining long scale and fine-scale approaches in rocky intertidal biodiversity monitoring, particularly under ongoing climate change.

Development of new tools for optimizing gametophyte-spraying techniques in kelp restoration

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Kelp forests are important marine habitats that provide crucial ecosystem services. In the last few years, their abundance has started to decrease, as they are victims of climate change and anthropogenic pressures[1]. Following this global trend, along the Portuguese coast, the perenial *Laminaria ochroleuca,* the golden kelp, has experienced significant range contraction, highlighting the need for new restoration techniques, as conservation alone provided no results. The aim of this study is to improve the techniques for spraying gametophytes during SCUBA diving fieldwork. The main challenge is to find a method to ensure that male and female gametophytes sink close to each other, to allow reproduction. To do so, different substances that would be added to cultures were tested. The experiment quantified how different concentrations of Ficoll, PVP and an increase of salinity would affect the sinking rates and toxicity response of *L. ochroleuca* gametophytes. For the sinking experiment, gametophytes were added to each treatment and poured into cuvettes blocks to measure sinking velocity. For the toxicity experiment, gametophytes were added to each treatment for three hours, and then monitored for two weeks to assess survival and reproductive output. The sinking experiment has shown that all treatments at different concentrations improve the sinking velocity, while the toxicity test has shown that there is no toxicity effect on gametophytes when exposed for only three hours. Since PVP and Ficoll are both polymers, the ideal in the context of environmental restoration is to use an increase in salinity to augment the density of the water and the sinking velocity.

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Prokaryotic contribution to Mercury transformations in Arctic thermokarst lakes sediments: A metagenomic study

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Thawing permafrost is transforming Arctic landscapes, leading to the widespread formation of thermokarst lakes. These dynamic ecosystems function as biogeochemical hotspots, where intensified prokaryotic activity has important implications for the transformation and cycling of mercury (Hg), a globally relevant pollutant. However, the role of prokaryotic communities in these sites remains poorly understood. In this study, we investigated the prokaryotic drivers of Hg transformation in sediments collected from six Arctic thermokarst lakes across distinct permafrost regions. To achieve this, we employed genome-centric metagenomics based on whole-genome sequencing (WGS), enabling the reconstruction of community composition, functional potential, and diversity of prokaryotes associated with Hg methylation and demethylation. Complementary network analyses were performed to infer potential ecological interactions, functional linkages, and cross-pathway connections that shape Hg-related microbial processes. Our findings provide a comprehensive ecological characterization of prokaryotic communities inhabiting thermokarst lake sediments. By uncovering both the taxonomic diversity and the functional pathways underlying Hg transformations, this work advances our understanding of the ecological mechanisms through which thermokarst lake dynamics influence Hg cycling in the rapidly changing Arctic.

Metabarcoding of Mediterranean monk seal (*Monachus monachus*) scats from the isolated Madeira population reveal a diverse cephalopod prey assemblage

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The Mediterranean monk seal (*Monachus monachus*) is one of the world's most endangered pinnipeds, with limited ecological knowledge due to its reliance on secluded coastal caves for reproduction and resting[1]. DNA metabarcoding of scat samples provides a non-invasive approach to dietary analysis, offering higher taxonomic resolution and improved detection of soft-bodied prey than traditional methods[2,3]. We investigated the cephalopod component of the diet of the isolated Madeira monk seal population using scat samples collected from Madeira and the Desertas Islands. Three molecular markers targeting the 18S and 16S rRNA genes were applied to maximise prey detection and taxonomic resolution [2,4,5].Preliminary results reveal a diverse assemblage of octopuses, cuttlefish, and squids, including *Sepia officinalis*, *Illex coindetii*, and *Muusoctopus* sp., representing both benthic and pelagic-associated prey. These findings are consistent with the known cephalopod diversity of Macaronesia[6] and highlight the value of multi-marker metabarcoding for characterising monk seal trophic ecology. Ongoing analyses are expected to further refine prey diversity estimates.

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Non-invasive assessment of stress-related hormones in exhaled breath condensate of free-ranging cetaceans

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Anthropogenic pressures trigger physiological stress responses in cetaceans, with consequences for individual health, reproduction and population viability. However, stress assessment in free-ranging animals is challenging because conventional sampling methods are invasive and logistically difficult. Exhaled breath condensate (EBC) offers a promising non-invasive matrix for endocrine monitoring. Therefore, this study aimed to assess stress levels through the hormonal content of cetacean EBC samples collected along the Spanish and Italian coasts during 2024 and 2025. EBC samples were collected from free-ranging fin whales (*Balaenoptera physalus*) and two dolphin species (bottlenose dolphin - *Tursiops truncatus* and common dolphin - *Delphinus delphis*) using Petri dishes adapted for blow sampling, either mounted on a telescopic pole or on unmanned aerial vehicles. Samples were resuspended and processed for hormone quantification using commercial ELISA kits. Cortisol was quantified as the main stress-related biomarker, while testosterone and progesterone were assessed as complementary endocrine indicators. Urea was measured to normalize hormone concentrations and account for differences in sample dilution and recovery. In fin whales, cortisol and progesterone concentrations differed between samples collected in Spain in 2024 and 2025, suggesting inter-annual variation in endocrine status. Comparisons between Spanish and Italian fin whale samples collected in 2025, and between dolphin species sampled along the Spanish coast, showed no significant differences in cortisol concentrations. Overall, this study supports EBC hormone analysis as a non-invasive approach to assess stress-related physiological responses in wild cetaceans. These findings contribute to the development of stress indicators and may support future cetacean health monitoring and conservation-oriented environmental assessment in European marine regions.

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Navigating change: A comparative analysis of EU and Non-EU Small-scale fisheries management approaches

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Small-scale fisheries (SSF) play a vital role in sustaining livelihoods, local economies, food security, and cultural heritage[1]. However, they face growing environmental, socioeconomic, and governance challenges, including resource depletion, habitat degradation, low profitability, and fragmented regulatory frameworks[2]. In response, diverse management approaches have emerged worldwide, ranging from state-led regulations to participatory and ecosystem-based governance systems[3,4]. Assessing the practical outcomes of these systems is essential to understand which governance mechanisms most effectively promote the resilience and sustainability of SSF[5]. The analysis examined governance arrangements, socio-ecological contexts, and sustainability outcomes, highlighting differences between the across European Union's (EU) Common Fisheries Policy and more locally adapted management systems. Findings reveal a global shift from centralized governance towards more participatory approaches, although sustainable outcomes remain constrained by implementation challenges. These results contribute to a broader understanding of the evolution of SSF governance and the factors shaping sustainable fisheries management.

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Resources or predators? What drives toxin production in harmful algae

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Harmful algal blooms have increased in frequency and intensity in recent decades due to excessive nutrient enrichment and climate change, which influences environmental shifts. These events, driven by the rapid proliferation of toxin-producing microalgae have significant ecological, economic, and health impacts. Among the toxins produced, Phycotoxins produced by harmful algae accumulate in marine food webs and increase ecological and health risks, particularly through their transfer to higher trophic levels and their role in cases of shellfish poisoning. Despite recent advances in elucidating their biochemical pathways, the ecological mechanisms governing phycotoxin synthesis remain poorly understood. Two main hypotheses prevail: (I) bottom-up regulation, in which a nutrient imbalance promotes toxin production, and (II) top-down regulation, in which chemical cues from grazers induce phycotoxin to protect harmful algae from grazing. This research will investigate the effect of nitrogen enrichment and copepod-derived chemical cues (copepodamides) on toxin production and bioluminescence in factorial experiments with two species of harmful dinoflagellates: *Alexandrium catenella* and *Protoceratium reticulatum*. Based on current evidence, both species are expected to exhibit increased toxin production in response to nitrogen enrichment and exposure to copepodamides. P. reticulatum spp. is also expected to exhibit increased bioluminescence under these conditions. The project is ongoing; we expect bottom-up and top-down factors to synergistically regulate dinoflagellate inducible chemical defenses.

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Tetrodotoxin and analogues in bivalvia and associated bycatch from the southern portuguese coast

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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 bivalves from European waters, led the European Food Safety Authority (EFSA) to recommend maximum safe limits for Human consumption of shellfish meat. However, TTX is currently only monitored in the Netherlands and France. On the southern coast (Algarve) of Portugal, TTX concentrations were detected in trumpet shells exceeding the levels considered safe for human consumption throughout the year. Nevertheless, there are no data on the incidence of TTX in other species from this area. This study aims to assess the presence of TTX and its 30 chemical congeners in a broad taxonomic range of marine invertebrates including Bivalvia, Gastropoda, Malacostraca and Polychaeta species. Samples were collected within the framework of the monitoring bivalve dredge fishing survey coordinated by the Portuguese Institute for the Sea and Atmosphere (IPMA). A total of 46 species were analysed, including 21 Bivalvia, 10 Malacostraca, 9 Polychaeta, and 6 Gastropoda. TTX concentrations in the were either not detected or remained below the EFSA-recommended safety limit. However, the systematic screening of both bivalves and associated bycatch revealed a widespread presence of TTX congeners. Since the toxicity of the analogues is estimated, rather than fully characterized, their detection across multiple trophic levels increases the potential risk of intoxication and raises concerns for public health.

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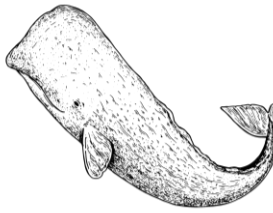

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