

BLUE THINK CONFERENCE

Share Science, Spread Knowledge



BOOK OF ABSTRACTS

Matosinhos, Portugal

23rd and 24th September, 2021



SUPPORT

U. PORTO

Santander
Universidades

BLUE THINK CONFERENCE

Share Science, Spread Knowledge

BLUE THINK CONFERENCE 2021

Share Science, Spread Knowledge

BOOK OF ABSTRACTS

ISBN 978-989-54965-4-9

DISCLAIMER

All texts in this document are the sole responsibility of their authors.

BLUE THINK CONFERENCE

Share Science, Spread Knowledge

CONTENT

THE CONFERENCE	<u>4</u>
ABOUT CIIMAR	<u>5</u>
MESSAGE TO THE PARTICIPANTS	<u>6</u>
ORGANIZING AND SCIENTIFIC COMMITTEES	<u>7</u>
 SESSION 1: ECOLOGY AND EVOLUTION	 <u>8</u>
KEYNOTE SESSION – FELIX FIGUEROA	<u>9</u>
ORAL COMMUNICATIONS	<u>11</u>
FLASH COMMUNICATIONS	<u>17</u>
POSTER PRESENTATIONS	<u>24</u>
 SESSION 2: BIOCHEMISTRY AND BIOTECHNOLOGY	 <u>35</u>
ORAL COMMUNICATIONS	<u>36</u>
FLASH COMMUNICATIONS	<u>44</u>
POSTER PRESENTATIONS	<u>52</u>
 SESSION 3: AQUATIC RESOURCES AND AQUACULTURE	 <u>87</u>
KEYNOTE SESSION – JORGE FERNANDES	<u>88</u>
ORAL COMMUNICATIONS	<u>90</u>
FLASH COMMUNICATIONS	<u>97</u>
POSTER PRESENTATIONS	<u>102</u>

BLUE THINK CONFERENCE

Share Science, Spread Knowledge

CONTENT

SESSION 4: OCEAN DECADE	<u>130</u>
MODERATOR – SANDRA RAMOS	<u>131</u>
MINISTER OF THE SEA – RICARDO SERRÃO SANTOS	<u>132</u>
INVITED SPEAKER – ISABEL SOUSA PINTO	<u>133</u>
INVITED SPEAKER – JOANA LARANJEIRA	<u>134</u>
INVITED SPEAKER – RAQUEL GAIÃO SILVA	<u>135</u>
 FINAL REMARKS	 <u>136</u>
SPONSORS AND CONTACTS	<u>137</u>

THE CONFERENCE

The second edition of the Blue Think Conference (BTC) was organized by the Interdisciplinary Centre of Marine and Environmental Research (CIIMAR) PhD Students' Committee. Our aim was to gather and encourage the students and collaborators affiliated with our institute to share the outputs of their work in a casual and relaxed environment.

The presentations were divided into three sessions entitled **Ecology and Evolution, Biochemistry and Biotechnology** and **Aquatic Resources and Aquaculture**, resembling the three main research lines of CIIMAR. In two of the sessions included a keynote lecture by a renowned scientist who discussed their scientific career, professional experience and most important work achievements.

To commemorate the United Nations Decade of Ocean Science for Sustainable Development, the Blue Think Conference of 2021 concluded with a **roundtable dedicated to the "Ocean Decade"**. During this session, distinguished guests from different fields discussed several topics regarding marine economy and ocean research.

We hope the symposium expands our scientific knowledge and brings together researchers from different investigation groups and faculties. Everyone was welcome to join the discussion of the latest scientific contributions of CIIMAR's young minds.

ABOUT CIIMAR

The Interdisciplinary Centre of Marine and Environmental Research (CIIMAR) is a leading research and advanced training institution of the University of Porto, working at the frontiers of Ocean Knowledge and Innovation.

CIIMAR fosters an integrated approach to ocean and coastal areas promoting the understanding and knowledge on physical, chemical and biological dynamics of these environments and the impact of natural and human disturbances, aiming to unravel links between these processes, grasp ocean and ecosystems functioning and responses to global changes.

CIIMAR uses this knowledge-base to promote the natural capital and the sustained management of marine resources through monitoring of ecosystems health, optimization of aquaculture, and biotechnological exploitation of the resources for environmental and human health applications.

CIIMAR provides innovative solutions and products responding to actual economic and societal challenges. Among them are the demand for high-quality seafood, new drugs and marine products for industrial and medicinal needs, water quality, sustainable fisheries, preparedness for and mitigation of oil and HNS spills, environmental monitoring & risk assessment, preservation of ecosystems services, ocean & coastal management and Ocean Literacy.

GET TO KNOW US AT [CIIMAR.UP.PT](https://ciimar.up.pt)

BLUE THINK CONFERENCE

Share Science, Spread Knowledge

MESSAGE TO PARTICIPANTS

The second Blue Think Conference organized by the PhD students committee of CIIMAR is here. During 23rd and 24th of September 2021, students will have the opportunity to show their works, discuss their ideas, and plan their future. It will be a great serendipity environment, which certainly will make you all grow from scientific and personal points of view.

This is the second conference under this weird period of the covid-19 pandemic, but a positive perspective is on the horizon. This pandemic showed clearly the importance of science and technology in the wellbeing and welfare of our population and the relevance of a sustainable exploitation of our resources. I am sure that in 2022 we will be able to welcome you all and many more in our CIIMAR headquarters at Leixões Cruise Terminal where you can discuss your ideas and future collaborations over a nice drink watching the sunset over the ocean.

I want to thank the PhD students committee of CIIMAR for their enthusiasm in all the activities they organize and wish you all the best for this conference and for all you careers.

Professor Vitor Vasconcelos

Director of CIIMAR



BLUE THINK CONFERENCE

Share Science, Spread Knowledge

ORGANIZING COMMITTEE

Ana Matos

André Santos

Celso Domingos

Diogo Alexandrino

Fernando Pagels

Inês Gregório

Kathleen Abt

Luana Ramos

Mariana Oliveira

Rita Mendes

Sabrina Rodrigues

Sandra Pereira

Tiago Ribeiro

SCIENTIFIC COMMITTEE

Amaranta Kahn

Carolina Castro

Cristina Velasco

David Barros

Dimitri Costa

Joana Fernandes

Joana Martins

Lígia Sousa

Lourenço Ramos-Pinto

Marina Machado

Martina Ilarri

Miguel Semedo

Odete Gonçalves

Sandra Figueiredo

Silvia Chemello

BLUE THINK CONFERENCE

Share Science, Spread Knowledge



SESSION 1: ECOLOGY AND EVOLUTION

MODERATION: INÊS GREGÓRIO AND DAVID BARROS

KEYNOTE SESSION – FELIX FIGUEROA



Dr. Félix López Figueroa has a degree in Biology from the University of La Laguna-University of Malaga, Spain (1983). Doctor in Biology from the University of Malaga (1987). Postdoctoral contract at the University of Munich and Helgoland Biological Station in Germany (1988-1989). Professor of Ecology in the Department of Ecology and Geology of the Faculty of Sciences of the University of Malaga.

He teaches in the Degrees of Environmental Sciences and Biology and in postgraduate (Master and Doctoral Program of Advanced Biotechnology). He is the founder of the research group "Photobiology and Biotechnology of Aquatic Organisms (FYBOA-RNM295)" made up of researchers from the area of Marine Ecology and Microbiology.

Since 2019 he is the Director of the Institute of Biotechnology and Blue Development of the University of Malaga. Researcher in Ecophysiology and Physiology of cyanobacteria, microalgae and macroalgae.



BLUE THINK CONFERENCE

Share Science, Spread Knowledge

Have focused his research on physiological studies on the photocontrol of the synthesis of photosynthetic pigments, photoregulation of carbon and nitrogen metabolism and ecophysiological focus studies on the effect of ultraviolet radiation (UVR) and other variables of climate change on photosynthesis and growth in seaweeds, as well as analysis of photoprotection and antioxidant systems.

Expert in photosynthesis of algae determined by *in vivo* fluorescence of chlorophyll *a* associated with Photosystem II.

Dr. Figueroa has published 4 scientific books, 27 book chapters, 235 scientific articles from the Science Citation Report list, and 29 patents on the use of mycosporin-like amino acid photoprotectors (MAAs) as an antioxidant.

He has supervised 18 Doctoral theses, and 25 Master's and Bachelor's Thesis.

He has promoted the internationalization of the University of Malaga with activities and agreements with Universities of Mexico, Argentina, Chile, Brazil, South Korea, Germany, Peru, Portugal and Israel.

Dr. Figueroa has promoted the transfer of research results to companies in the Aquaculture, Marine Biotechnology and Cosmetics sector.



BLUE THINK CONFERENCE

Share Science, Spread Knowledge



ABSTRACTS

ORAL COMMUNICATIONS



Local ecological knowledge of vulnerable marine species and ecosystems of the Portuguese mainland continental shelf and upper slope

Ana Soares^{1,2}, Sandra Ramos¹, Cristina Pita³, Joana R Xavier^{1,4}

¹CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal

²Department of Biology, University of Aveiro, Portugal

³CESAM – Centre for Environmental and Marine Studies, University of Aveiro, Portugal

⁴Department of Biological Sciences, University of Bergen, Norway

Presenting author email: anasofiasoares.1997@gmail.com

Corresponding author email: anasofiasoares.1997@gmail.com

Abstract

Sponges and corals form highly structured and vulnerable marine ecosystems (VMEs) that serve as refuge areas and feeding grounds for many marine species. Knowledge on the distribution of these VMEs is critical to ensure their sustainable management and conservation but is largely lacking in some areas. This study aims to co-develop a knowledge baseline on the diversity and distribution of these species and habitats by assembling Local Ecological Knowledge (LEK) through participatory approaches with professional fishers operating bottom-contacting gears in mainland Portugal.

In total, 52 fishers and skippers of commercial vessels (trawlers, trammel netters and longliners) were interviewed in fishing harbours from the center and northern Portugal. We assessed fishers' knowledge, by asking them to identify species (from an image catalogue), and provide information on their geographical distribution, and abundance trends over time. In total, 52 out of the 68 species in the catalogue were recognized. The framework-forming scleractinian corals *Desmophyllum pertusum* and *Dendrophyllia cornigera*, alongside a soft coral (likely *Eunicella verrucosa*) were the species most commonly recognized (75–81% on the interviewees) followed by *Dendrophyllia ramea* (40%). Sponges were only occasionally recognized but among these, lithistids and the birds' nest sponge *Pheronema carpeniteri* were the ones that fishers recognized most (21–29% of the interviewees). Fishers were also able to identify several fishing grounds where different species occur, and often referred to the decline of scleractinian coral reef areas to have occurred in the 1980's-90's as a result of intense bottom trawling in the region.

These results show that LEK obtained from participatory approaches can provide valuable information on the spatial occurrence of VMEs, complementing and filling the gaps of conventional scientific knowledge.

Keywords – Fishers' perception; Bottom fisheries; sponges and corals; species distributions; mesophotic; deep-sea



Weekly Fluctuations of Microplankton Community Structure in a Changing Arctic Ocean

Arianna Capparotto^{1,2}, Salvador Macedo^{1,3}, Miguel Semedo¹, Catarina Magalhães^{1,3}

¹CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos, s/n, 4450-208, Matosinhos, Portugal

²Faculty of Molecular Biology, University of Padova, Padova, Italy

³Faculty of Sciences, University of Porto, Porto, Portugal

Presenting author email: arianna.capparotto@studenti.unipd.it

Corresponding author email: arianna.capparotto@studenti.unipd.it

Abstract

The Svalbard Archipelago, in the Arctic Ocean, is among the most sensible regions to climate changes on Earth. The increased amount of glaciers meltwater, especially in Spring and Summer, favors the release of sediment particles, which affect both primary productivity and higher trophic levels. Microbial communities play an important role in this remote oligotrophic ecosystem, by being at the base of the food web and crucially contributing to the biogeochemical cycles. However, the different temporal patterns (weekly, seasonal, annual) that characterize their distribution are still poorly understood. The objective of this study is to provide a starting baseline of the microbial community changes at the weekly timescale, improving the time resolution of our current understanding, and complementing a long-term monitoring program that is being implemented in the region since 2016. Planktonic samples were collected every week during the Spring/Summer transition of 2019 in the Kongsfjorden, a fjord in the Svalbard Archipelago. Water samples were collected using a Rosette sampler at Deep Chlorophyll Maximum (DCM; 15 m) and at 300 m (bottom of the station) and subsequently concentrated in sterivex filters (0.2 µm) for the extraction of DNA. The 16S rRNA genes present in the extracted DNA were further sequenced by a MiSeq Illumina platform and the sequences obtained were processed using DADA2 open-source package in the R environment to characterize the structure and diversity of the prokaryotic communities over the different weeks. From a first analysis of beta and alpha diversity, the species that appear to vary the most are the superficial ones, while the ones sampled in the bottom seem more stable over time. This new knowledge, as well as the subsequent characterization of the driving geochemical factors, will help in the prediction of future trends of microbial communities' structure and function under climate change conditions in this region.

Keywords – Climate change; microbial communities; Svalbard Archipelago; glaciers meltwater; MiSeq Illumina sequencing; Arctic Ocean



New deep-sea glass sponges (Porifera, Hexactinellida) of the tropical central Atlantic seamounts

Celso Domingos¹, Daniel Kersken¹, Kate Hendry², Astrid Schuster³ & Joana R. Xavier^{1,4}

¹CIIMAR – Interdisciplinary Centre of Marine and Environmental Research of the University of Porto, 4450-208 Matosinhos, Portugal.

²University of Bristol, United Kingdom.

³University of Southern Denmark, Denmark.

⁴Department of Biological Sciences, University of Bergen, Bergen, Norway

Presenting author email: bio.celso.domingos@gmail.com

Corresponding author email: bio.celso.domingos@gmail.com

Abstract

Remote and poorly investigated deep seamount environments are increasingly threatened by anthropogenic activities such as bottom fishing, oil and gas exploitation, and climate change. This has resulted in an increased global interest to protect these ecosystems. Glass sponges (Porifera, Hexactinellida) constitute an important and dominant component of the deep-sea megabenthic communities and play key ecological roles, often forming structural habitats in the deep-sea. Currently, there are 143 species of hexactinellids reported for the Atlantic Ocean, the vast majority of which occur in the Northern Atlantic. This study uses an integrative taxonomic approach to identify glass sponges collected on several seamounts (Carter, Knipovich, Vayda) and the Vema fracture zone, located in Areas Beyond National Jurisdiction (ABNJ) across the tropical Atlantic. In total, 28 specimens were analyzed and assigned to 19 species, distributed across 15 genera and 6 families. Of these, 12 species are new to science and are currently being described and illustrated. Phylogenetic reconstructions (mtDNA 16S and rRNA 28S) confirm our taxonomic identifications and support previous results on the monophyly of these hexactinellid families. The discovery of such a high number of new species highlights the low exploration and research efforts in the Tropical and South Atlantic, where much deep-sea diversity is yet to be unravelled. The results of this work are particularly relevant in the context of the United Nation's agreement on the conservation and sustainable use of marine biological diversity in ABNJ, setting the baseline for future protection strategies of these vulnerable deep-sea species.

Keywords – Atlantic Ocean; integrative taxonomy; new species; molecular phylogeny



Molecular evolution of fish olfactory receptors and their role in water-to-land transition

Liliana Silva^{1,2}, Tito Mendes¹, Luana Ramos^{1,2}, Agostinho Antunes^{1,2}

¹CIIMAR/CIMAR, Interdisciplinary Centre of Marine and Environmental Research,

²Department of Biology, Faculty of Sciences, University of Porto

Presenting author email: liliana.silva@ciimar.up.pt

Corresponding author email: liliana.silva@ciimar.up.pt

Abstract

Odor recognition plays an extremely important role in intra/interspecific communication and, ultimately, is fundamental for species' survival. Olfactory receptors (ORs) is a subgroup of G-protein-coupled receptors and due to the highly complex OR repertoires found in vertebrate species, the proposed classification systems are full of ambiguities (for example, the presence of a single intact gene in elephant sharks is comparable to more than 1,000 genes in some mammalian species). In Actinopterygii fish, the OR family remains poorly studied, being characterized solely in some species. Therefore, little is known about the OR repertoire and its molecular evolution in most fish taxa. Aiming to shed some light into the evolution of the OR repertoire in non-model fish species, we performed an exhaustive gene extraction protocol upon 37 fish genomes (comprising Cyclostomata, Chondrichthyes, Sarcopterygii and Actinopterygii species), followed by detailed phylogenetic reconstructions. The performed phylogenetic analyses allowed us to update the old OR classification systems and to define, through an integrative approach, 21 distinct OR families. Subsequent analyses also revealed that the OR repertoire in fishes is highly heterogeneous. Interestingly, we found demarked gene family expansions on ORs sensitive to air-borne molecules, especially in Sarcopterygii and basal lineages of Actinopterygii species, which could be a landmark for water-to-land transition. Finally, the syntenic analysis of OR-encoding chromosomes revealed how whole-genome duplication events impacted the OR repertoire dynamics observed in modern actinopterygians.

Keywords - Chemoreceptors, Vertebrate, Comparative genomics, Gene clusters



Structural and functional changes of macroalgae communities in the north portuguese coast - what has changed in 15 years

Marta Martins¹, Bruna Ruschel Pires¹, Marina Dolbeth¹, Francisco Arenas¹

¹Benthic Ecology Team, CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Matosinhos, Porto, Portugal

Presenting author email: marta__martins@hotmail.com

Corresponding author email: marta__martins@hotmail.com

Abstract

Climate change (CC) is one of the most important scientific research topics worldwide. Understanding its impacts in coastal communities and ecosystems is required to mitigate current climate effects and implement efficient management policy. Although intense debate and research in the CC field of study, much is still not understood, particularly because responses can be highly context-dependent, varying according to local climate and micro-climate changes, diversity patterns and species interactions. Structural differences in communities are becoming apparent, with a decline in species that are more sensitive to local climatic variability, resulting on a decrease on functional variability of the ecosystem. Traditionally, ecosystem functioning measurements were based on taxonomic classification aligned with abundance and diversity estimates. Recently, trait-based approaches (FTBAs) to study marine ecosystem functions and services have been rising. FTBAs use life history traits, such as morphological, biochemical, physiological, structural, phenological or behavioural characteristics of organisms that influence their performance or fitness. In this work, we used an approach that acknowledges the role of local dynamics and species' functional responses towards climate change. Data on macroalgae covering and community structure of the intertidal of five beaches from the north of Portugal was gathered. Two datasets with 15 years apart were compared from a structural and functional perspective. Marked differences between the structure of communities were apparent with a shift from larger canopy species to turf. Such alterations present important functional impacts, like reduction of habitat provided and productivity rates. Our results corroborate the statement that contribution of species or communities for ecosystems functional diversity should be used along with particular characteristics of species to cope with climate. Taken into account that functions and services are directly related, this sort of information will allow to evaluate and foretell ecosystem service provision and use it as a tool for environmental policy and management.

Keywords – Functional traits; Intertidal; Macroalgae; North Portugal; Climate Change



BLUE THINK CONFERENCE

Share Science, Spread Knowledge



ABSTRACTS

FLASH COMMUNICATIONS



The evolutionary history of the family Margaritiferidae using genomic multilocus approaches.

André Gomes-dos-Santos^{1,2}, Elsa Froufe¹, Ivan Bolotov³, Ilya Vikhrev³, Arthur E. Bogan⁴, Chase Smith⁵, Nathan Johnson⁶, John Pfeiffer⁷, Paz Ondina⁸, Prié Vincent^{9,17}, Mohamed Ghamizi¹⁰, Nathan Whelan^{11,12}, Nicole Garrison^{11,13}, Sophie Breton¹⁴, Amílcar Teixeira¹⁵, Yin Peng Lee^{16,17}, Manuel Lopes-Lima^{1,18,19}

¹ CIIMAR — Interdisciplinary Centre of Marine and Environmental Research, Aquatic ecology and evolution (AEE) Lab, University of Porto, Matosinhos, Portugal

² Department of Biology, Faculty of Sciences, University of Porto, Porto, Portugal

³ N. Laverov Federal Center for Integrated Arctic Research, Ural Branch of the Russian Academy of Sciences, Arkhangelsk, Russia

⁴ North Carolina Museum of Natural Sciences, Raleigh, North Carolina, U.S.A.

⁵ Biology Department, Baylor University, Texas, USA

⁶ Wetland and Aquatic Research Center, US Geological Survey, USA

⁷ National Museum of Natural History, Smithsonian Institution, Washington, DC, USA

⁸ Departamento de Zooloxía, Xenética e Antropoloxía Física, Facultade de Veterinaria, Universidade de Santiago de Compostela (USC), Lugo, Spain

⁹ Institute of Systematics, Evolution, Biodiversity (ISYEB), National Museum of Natural History (MNHN), Paris, France

¹⁰ Muséum d'Histoire Naturelle de Marrakech, Université Cadi Ayyad, Faculté des Sciences Semlalia, Marrakech, Morocco

¹¹ School of Fisheries, Aquaculture, and Aquatic Sciences, College of Agriculture, Auburn University, Alabama, USA

¹² Southeast Conservation Genetics Lab, Warm Springs Fish Technology Center, US Fish and Wildlife Service, Alabama, USA

¹³ Department of Biological Sciences, West Liberty University, West Virginia, USA

¹⁴ Department of Biological Sciences, Faculty of Arts and Sciences, University of Montreal, Montreal, Canada

¹⁵ Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Bragança, Portugal

¹⁶ School of Life & Environmental Sciences, Deakin University, Victoria, Australia.

¹⁷ Deakin Genomics Centre, Deakin University, Victoria, Australia.

¹⁸ CIBIO/InBIO - Research Center in Biodiversity and Genetic Resources, Universidade do Porto, Vairão, Portugal

¹⁹ IUCN SSC Mollusc Specialist Group, c/o IUCN, United Kingdom of Great Britain and Northern Ireland

Presenting author email: andrepousa64@gmail.com

Corresponding author email: andrepousa64@gmail.com



Abstract

Freshwater mussels of Order Unionida Gray 1854 are a widely distributed and strictly freshwater group of bivalves that, among other remarkable biological features, have an obligatory parasitic larvae stage and doubly uniparental inheritance (DUI) of mitochondrial DNA. Margaritiferidae is a nearly 200-million-year-old family with 16 extant recognized species distributed throughout the northern hemisphere. This family is the most threatened Unionida family which includes one of the most emblematic freshwater invertebrates, the freshwater pearl mussel *Margaritifera margaritifera* (Linnaeus, 1758), and one of the world's 100 most threatened species, *Pseudunio maroccanus* (Pallary, 1928). Molecular phylogenetics has proven fundamental to clarify the systematics of the Margaritiferidae family. Phylogenetic studies, however, have only relied on a reduced number of markers, either solely based on mtDNA or a combination of a few mtDNA and nuclear markers. In the present study, whole mitogenomes were assembled from low coverage paired-end Illumina reads and 627 nuclear loci were obtained using the probe-based Anchored Hybrid Enrichment strategy, both including representatives from all the genera in the family. Phylogenetic trees were constructed using whole mitogenomes and the nuclear loci individually and combined. By applying these multilocus approaches we aim to recover a comprehensive view of the evolutionary history of the family Margaritiferidae and to evaluate the reliability of the whole mitogenome as a surrogate for phylogenetic studies on the group.

Keywords – Freshwater Mussels; Unionida; Margaritiferidae; Anchored Hybrid Enrichment; Phylogenomics; Phylomitogenomics



Sponges of the genus *Thenea* (Porifera, Demospongiae, Tetractinellida) from Portuguese waters

Daniel Despujols^{1,2}, Celso Domingos² Jorge Lobo-Arteaga^{3,4}, Joana R Xavier^{2,5}

¹ICBAS - Abel Salazar Institute of Biomedical Sciences, University of Porto, Rua Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal

²CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, 4450-208 Matosinhos, Portugal

³IPMA, I.P. – Portuguese Institute for the Sea and Atmosphere, Rua Alfredo Magalhães Ramalho 6, 1495-165 Algés, Portugal

⁴MARE – Marine and Environmental Sciences Centre, NOVA University of Lisbon, 2829-516 Caparica, Portugal

⁵Department of Biological Sciences, University of Bergen, 5007 Bergen, Norway

Presenting author email: danieljundespujols1@gmail.com

Corresponding author email: danieljundespujols1@gmail.com

Abstract

Sponges form a variety of highly structured habitats, known as sponge grounds or aggregations. They play key roles in benthic communities, both in shallow and deep waters. Currently, the genus *Thenea* encompasses 38 species that typically inhabit soft bottom habitats in deep-waters (120–4020 m depth), known to form dense aggregations. *Thenea* is relatively well documented at high latitudes in the Northeast Atlantic, and in the Mediterranean Sea (e.g., *T. muricata* and *T. valdiviae*). There are also some records in the Iberian Peninsula and in the Azores region, however knowledge on the diversity within the genus is relatively scarce. This project aims to investigate the diversity of the genus *Thenea* from the temperate Northeast Atlantic (southern Portuguese continental shelf and the Azores), through the implementation of an integrative taxonomic approach. Sponges were obtained from sampling campaigns on each study area. Morphology based identifications were performed through the analysis of shape, structure, and size of the sponges as well as their skeletal structures (spicules). Molecular based identification was performed through the generation of DNA barcodes of mitochondrial cytochrome c oxidase subunit I and ribosomal 28S gene. Preliminary morphological results show some external variations between the specimens, particularly on color, shape, and surface's texture as well as some slight variations on spicule size. Molecular results reveal two distinct species occurring in the Azores, one of which also occurs in southern Portugal. These results are of great relevance since the study is focused on relatively unexplored locations. Moreover, the species may be new to science, representing a great advancement in the study of this genus.

Keywords – Sponge grounds; Integrative taxonomy; Portuguese continental shelf; Azores; Deep-sea



Insight into the grayling's world: nuclear perspective based on ddRAD-seq

Giulia Secci-Petretto^{1,2}, Steven Weiss³, Gernot K. Englmaier³, Elsa Froufe¹

¹CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal

²Department of Biology, Faculty of Sciences, U. Porto - University of Porto, Portugal

³University of Graz, Institute of Biology, Graz, Austria

Presenting author email: lasecci@hotmail.it

Abstract

Thymallinae is a subfamily of Salmonidae solely represented by the genus *Thymallus* and is among the less well-known genera of salmonids. Graylings (*Thymallus*) are freshwater fishes distributed along the major river basins of Asia, northern Europe, Canada, Montana and Alaska. The greatest diversity is located in the Asian continent, in particular in Russia, Kazakhstan, Mongolia and China. The lack of accessibility to some of the distribution areas has probably contributed to the gaps in the knowledge of these species. Nevertheless, the genus present interesting characteristics from an evolutionary point of view and questions about the number of species, their population genetic and phylogenetic relationships were recently raised. Progress was made with a recent publication based on whole mitogenome sequences, which evaluated the phylogenetic relationships of all *Thymallus* putative species, their evolutionary history and introduces, for the first time in Thymallinae, the use of species delimitation methods. In order to try to fill the gaps left by the study of only maternally inherited markers, we have now proceeded with analyses focused on the nuclear genome. A total of 158 individuals, representing all the known *Thymallus* species in their distribution ranges were sequenced through ddRAD-seq technique. The data obtained allowed inferences on the phylogenetic relationships among the species, comparison with those based on the mitogenomes and a discussion of the incongruences between the two types of markers. Also, particular attention was paid to sympatric zones where two or more species occur and for which, population genetic analyses were performed, including inference of population structure, PCA, and D-statistic calculation for detecting potential ancient introgression. This study will contribute to our increasing knowledge of these species and their phylogenetic relationships as well as support hypotheses concerning the mechanisms of diversification within the genus.

Keywords – *Thymallus*, RAD-seq; phylogeny; sympatry; introgression; hybridization



Aberrant otoliths: characterization of the incidence and type of malformation in different estuarine habitats

Jorge Saraiva¹, Inês Vilar², Ester Dias³, Allan Tainá Souza⁴, Vânia Freitas⁵

¹Estuarine Ecology and Biological Invasions, CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto; Porto, Portugal

²Estuarine Ecology and Biological Invasions, CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto; Porto, Portugal

³Estuarine Ecology and Biological Invasions, CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto; Porto, Portugal

⁴Department of Fish and Zooplankton Ecology, Institute of Hydrobiology, Biology Centre, CAS – Institute of Hydrobiology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic

⁵Estuarine Ecology and Biological Invasions, CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto; Porto, Portugal

Presenting author email: jorgemccsaraiva@gmail.com

Corresponding author email: jorgemccsaraiva@gmail.com

Abstract

The inner ears of teleost fish are composed of otoliths that play an important role in hearing and balance functions. Structural and physical changes in otoliths can affect fish ear function and compromise their survival. Many studies have reported the occurrence of irregularly shaped otoliths with alternate crystalline forms (e.g. vaterite instead of aragonite), attributing this to environmental stress. In Minho River estuary, abnormal otoliths were observed in juveniles of European flounder (*Platichthys flesus*). This study aims to evaluate the incidence of otolith deformity in various habitats of the Minho river estuary and to provide a temporal and spatial description of the prevalence and type of otolith abnormality, using flounder as a model species. To achieve this goal, flounder were sampled in the Minho river estuary, along the salinity gradient, in October 2019, June, and October 2020. The otolith shape, morphometry, and structure were examined, the prevalence of otolith deformity was determined, and an otolith classification based on external morphological criteria was created, with five degrees of abnormality description. Otolith abnormality was found in 77% of the otoliths analysed (N=327), with specimens collected in October 2019 having the highest prevalence (99 %). Along the entire salinity gradient, the normal and first-degree otoliths had the highest incidences. Although vaterite deposition is the most reported type of abnormal development, vateritic otoliths had a low prevalence (4%) where most irregular otoliths were composed of aragonite. This is consistent with studies showing that vateritic otoliths are more observed in farmed fish compared with wild populations. Further studies are needed to understand the extent of this phenomenon and the impacts on fish survival. The high incidence of otolith deformity among juvenile stages found in the present study is relevant as



it suggests a possible impact on the nursery function of the Minho estuary for flounder.

Keywords – Abnormal otoliths; vaterite; flatfish; estuary; nursery



BLUE THINK CONFERENCE

Share Science, Spread Knowledge



ABSTRACTS

POSTER PRESENTATIONS



Green Roofs as biodiversity promoters in urban coastal areas

Ana Francisca Carvalho^{1,2}, Ana Paula Mucha^{1,2}, Maria Paola Tomasino¹, Isabel Aguiar Pinto Mina^{3,4}, Cristina Sousa Coutinho Calheiros¹

¹Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), University of Porto, Matosinhos, Portugal

²Faculty of Sciences, University of Porto, Porto, Portugal

³Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB)

⁴Departamento de Biologia – Escola de Ciências, Universidade do Minho (DB-ECUM), Braga, Portugal

Presenting author email: up201908465@edu.fc.up.pt

Corresponding author email: up201908465@edu.fc.up.pt

Abstract

The rapid expansion of city areas causes alterations in a variety of ecological processes and functions. It also impacts biodiversity by altering the environmental conditions and destroying the natural habitat.

Green roof technology is a nature-based solution that consist on vegetation installed on a constructed structure. Depending on the typology of green roof considered, they may provide a wide range of ecosystem services, such as management of the stormwater runoff, energy savings through internal building's temperature regulation, mitigate the urban heat island effect and increase biodiversity.

Plants in natural habitats benefit from a variety of interactions with soil microorganisms, which are particularly relevant around plant roots. Microbial diversity has a key role in improving plants survival and productivity. However, in green roofs, plants are subjected to different conditions than in natural environment and the knowledge about this associated biodiversity is still limited.

Although this technology is already support by research, the knowledge is still scarce, when considering green roof application to coastal areas and their role as biodiversity promoters.

The overall aim of this work is to study the substrate' biotic community of an extensive green roof, located in Porto city (Portugal), by following its dynamics along time. The microorganisms' biocenosis associated to the rhizosphere of the select plants species (*Helichrysum italicum*, *Festuca scoparia* and *Delosperma cooperi*) and to the substrate, will be carried out by optical microscopy and molecular biology tools to follow the changes over an annual cycle. By characterising the green roof substrate biocenosis, we expect to realize the challenges faced by these microorganisms, and understand their role in the green roofs' performance.

Keywords – Green roofs; Biodiversity; Urbanization; Rhizosphere's Biocenosis

Acknowledgements - This research was supported by national funds through FCT - Foundation for Science and Technology within the scope of UIDB/04423/2020 and UIDP/04423/2020. Authors are also thankful to Eng. Paulo Palha from Neoturf for the collaborative support.



Annotated Checklist of Aquatic Mites from Portugal

Harold Casalís Cantallo^{1,3}, Tiago José Andrade Ribeiro¹, Nuno Miguel Araújo Gomes^{1,2}, Dimítri de Araújo Costa^{1,2}

¹Aquatic Biodiversity and Conservation Laboratory, Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Porto, Portugal

²Aquamuseu do Rio Minho, Vila Nova de Cerveira, Portugal

³University of Aveiro, Aveiro, Portugal

Presenting author email: haroldcantallo.hcc@gmail.com

Corresponding author email: haroldcantallo.hcc@gmail.com, tiago.ribeiro@ciimar.up.pt, nuno@ciimar.up.pt, dimitri.costa@ciimar.up.pt

Abstract

Acari is a Subclass of chelicerates arthropods that contains ticks and mites. This study focuses on aquatic mites from the Portuguese Exclusive Economic Zone, including mainland area, and Madeira and Azores archipelagos, aiming to compile all the existing information and generate a list of species, linking them in each place where they were recorded. For the present work, we considered aquatic species, those that inhabit the marine environment, deep sea, intertidal, freshwater, transition water or brackish and terrestrial species with an associated aquatic lifestyle. Dataset search for this checklist started with the first descriptions of endemic water acarids from Madeira Archipelago performed by Lundblad during the 50's decade. Since then, many authors have given their contribution to cataloguing, recording, and accurately redescribing this vast group.

In search for confirmed occurrences our primary source was The Global Biodiversity Information Facility (GBIF), complemented with an exhaustive and extensive literature review and data biodiversity websites, e.g., European Water Mite Research (Watermite.org), Süßwasserfauna von Mitteleuropa book collection, Interim Register of Marine and Nonmarine Genera (IRMNG), Ocean Biodiversity Information System (OBIS), Integrated Taxonomic Information System (ITIS), and others. At the end, we found 48 valid species fitting these criteria, grouped in 3 Orders, 4 Suborders, 10 Superfamilies, 20 Families, and 24 Genera. In addition, we include a map with the documented occurrences of this species when coordinates were available. Although the list presented is short considering the diversity of aquatic habitats and the number of species contained in the acari subclass, we accomplish the goal of giving a first insight to the diversity of aquatic mites at the Portuguese territory.

Keywords – Acari; Taxonomy; Portuguese Exclusive Economic Zone; Iberian Peninsula; North Atlantic Ocean



Molecular evolution of vertebrate genes: unravelling the origins of birds' endothermy

Hugo Moreno^{1,2}, Tito Mendes², Liliana Silva^{1,2}, Luana Ramos^{1,2}, Agostinho Antunes^{1,2}

¹Department of Biology, Faculty of Sciences, University of Porto, Porto, Portugal

²EGB, CIIMAR/CIMAR, Interdisciplinary Centre of Marine and Environmental Research, Porto, Portugal

Presenting author email: hugocosmoreno@gmail.com

Corresponding author email: hugocosmoreno@gmail.com

Abstract

Homeothermic endothermy, a physiological state characterized by an increased and stable body temperature, evolved independently in mammals and birds, and was a crucial evolutionary step that supported the ecological success and radiation of these two taxa after the Cretaceous-Paleogene mass extinction. Endothermy allowed these taxa to expand their thermal niches and geographical distributions, and to sustain an incremented aerobic metabolism. Comparing with ectotherms, endotherms display more active mitochondria and in higher densities, thus resulting in a significantly higher metabolic rate and increased body temperatures. Although it is assumed that mitochondria played a crucial role in the emergence of homeothermic endothermy in modern vertebrates, the genomic changes that promoted the shift to this physiological state are still unclear.

Hence, through the assessment of avian and reptile genes involved in the mitochondrial regulation and functioning in a comparative approach, we aim to provide valuable clues regarding the ectothermy-endothermy transition in the sauropsid lineage.

Keywords - Natural selection, Bioenergetics, Thermogenesis, Basal metabolic rate; Neofunctionalization



Changing Seas: Unravelling the genomic diversity, connectivity and climate resilience of two deep-sea habitat forming sponges

Inês Gregório¹, Rute da Fonseca², Joana Xavier^{1,3}

¹CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal

²CMEC – Center for Macroecology, Evolution and Climate, University of Copenhagen, Copenhagen, Denmark

³Department of Biological Sciences, University of Bergen, Bergen, Norway

Presenting author email: ines.sousa.gregorio@gmail.com

Corresponding author email: ines.sousa.gregorio@gmail.com

Abstract

The deep-sea is the vastest and most unexplored biome on Earth, hosting a remarkable diversity of species and habitats which provide essential ecosystem goods and services. The impacts that climate change can have on these systems are still largely uncharacterized, leading to knowledge gaps that can compromise the development and implementation of sustainable development and conservation strategies for the oceans, an objective that is at the forefront of the global political agenda.

Sponges play key ecological roles in the deep-sea, serving as ecosystem engineers and ensuring the integrity and functioning of its communities. However, we are still lacking studies predicting range shifts under climate change scenarios and on genetic diversity and connectivity of populations of deep-sea sponges.

This work will combine for the first time advanced ecological modelling and state-of-the-art genomic methods to address key ecological questions on the distribution, population structure, and connectivity of two deep-sea sponges, *Aphrocallistes beatrix* and *Pheronema carpenteri*, that form structural habitats throughout the North Atlantic. Each species' habitat suitability and their adaptive potential and genomic resilience will be inferred for present-day conditions and projected under future climate change scenarios. The outcomes of this work will be a valuable resource to inform policy towards the management and conservation of these vulnerable deep-sea species and the habitats they form..

Keywords – Porifera; deep-sea habitats; genomics; ecological modelling; climate change; VMEs



Evolutionary history of SRPX2 in mammals: a gene implicated in language-related disorders

José Ferreira^{1,2}, Luana Ramos^{1,2}, Daniela Almeida^{1,3}, Agostinho Antunes^{1,2}

¹Evolutionary Genomics and Bioinformatics, CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Porto, Portugal

²Department of Biology, FCUP – Faculty of Sciences, University of Porto, Porto, Portugal

³Department of Biological Sciences, Clemson University, Clemson, SC, United States

Presenting author email: jfseabra07@gmail.com

Corresponding author email: aantunes@ciimar.up.pt

Abstract

Vocal learning, a key component of human language, is a rare and complex behavior defined as the ability to learn and modify novel vocalizations through auditory experience. This trait has evolved independently in a few avian and mammalian lineages. Within mammals, only some eutherian species present the ability for complex vocal learning. This trait is primarily used for sexual communication and mate choice, but it also plays a significant role in species in which social interactions and communication are the foundations of social organization. The increasing number of genomic data and publicly available resources allows the use of comparative evolutionary genomic tools to elucidate the evolutionary history of genes involved in vocal learning across multiple species. Sex chromosomes have been particularly difficult to sequence and assemble due to their repetitive nature, but new long-read sequencing technologies are increasing the number of high-quality sequences available. This opens the door to exciting new opportunities to understand the role of sex chromosomes in vocal learning. In this work, the molecular evolution of the SRPX2 gene, located in the therian X chromosome and implicated in language disorders, will be assessed across 141 mammalian species, including vocal learners and non-vocal learners, by performing phylogenetic and selection analyses. The dataset was built using annotated nucleotide sequences and non-annotated genomes retrieved from the NCBI database. The obtained SRPX2 phylogeny will allow assessing its congruency with the species tree, understanding the evolutionary relationships among species, and detect potential signatures of positive selection in functional regions of the protein-coding gene. Despite being a behavioral trait encoded by a large and complex gene network, the study of individual genes can contribute to better decipher the role of molecular evolution in vocal learning and human language, providing valuable background to medical genetics.

Keywords – Vocal Learning; Language Disorders; Mammals; SRPX2; Phylogenetic Analyses; Positive Selection



Freshwater mussels' behavior to dewatering

Paulo Castro^{a,b,*}, Francisco Carvalho^a, Amílcar Teixeira^c, Fernando Miranda^c, Manuel Lopes-Lima^d, Vanessa Modesto^{a,b}, Elsa Froufe^b & Ronaldo Sousa^a

^aCBMA – Centro de Biologia Molecular e Ambiental, Departamento de Biologia, Universidade do Minho, Campus Gualtar, 4710-057 Braga, Portugal

^bCIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal

^cCIMO – Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253, Bragança, Portugal

^dCIBIO/InBIO - Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, Vairão, Portugal

Presenting author email: castro.pcx@gmail.com

Corresponding author email: castro.pcx@gmail.com

Abstract

Freshwater mussels (FM) are one of the most globally endangered faunal groups on the planet and play crucial ecological roles. Several threats are contributing to their decline, including climate change. In particular, extreme climatic events such as droughts may reduce water levels, and these organisms are limited in their options to resist or adapt to survive these conditions, due to their sedentary nature.

In this context, the horizontal and vertical movement, and mortality of native FM *Unio delphinus*, *Anodonta anatina* and *Potomida litorallis* were assessed in different dewatering scenarios. For this, ramps were built and placed in three semi-artificial river channels. Three scenarios were tested: control (no dewatering), slow rate (4 cm/day) and fast rate (8 cm/day) dewatering. In general, species responded differently to dewatering. Both *A. anatina* and *U. delphinus* were able to respond along with water reduction in both slow and fast rate dewatering. On the contrary, *P. litorallis* failed to adapt to declining water levels in both scenarios and moved the least horizontally. Over 50% of the individuals of *P. litorallis* were dead in both scenarios and only 1 individual of *U. delphinus* died when exposed to fast rate dewatering. This experiment highlights significant interspecific differences in behavior and suggest the adaptation potential between species, where *A. anatina* was the quicker to avoid emersion by moving to deeper zones instead of burrowing, *P. litorallis* favored burrowing to stay immersed and *U. delphinus* showed the most versatile response. Additionally, these results suggest how the structure of FM assemblages and hydrogeography of rivers can play a fundamental role under a climate change scenario and this information should be included in future conservation strategies.

Keywords – Dewatering; Freshwater mussel; Climate change; Behavior responses



Agro-ornamental green walls as an ecological solution for food production in urban and peri-urban areas

Patrícia Alves^{1,2}, Cristina Calheiros², Paulo Palha³, Ruth Pereira⁴

¹FCUP - Faculty of Sciences, University of Porto, Porto, Portugal

²CIIMAR/CIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Porto, Portugal

³Neoturf, Porto, Portugal

⁴Biology Department, GreenUPorto – Sustainable Agrifood Production Research Centre, Faculty of Sciences, University of Porto, Porto, Portugal

Presenting author email: patri.al.alves@gmail.com

Corresponding author email: patri.al.alves@gmail.com

Abstract

Given the increase of human population in urban and peri-urban areas and the need to reduce the carbon footprint of food, it is crucial to produce closer to the urban agglomerations. Edible green walls can represent an opportunity for local food production towards family consumption. These structures also provide several ecosystem services such as biodiversity, human well-being, and air quality improvement. However, there is still a lack of knowledge concerning operational aspects such as irrigation needs, adequate plant species and resilience to abiotic stresses.

This work intends to study the installation, maintenance, viability and yield of an ornamental and edible polyculture green wall. For that, one has been implemented at Campus Agrário de Vairão with the collaboration of the company Neoturf. The species were chosen based on their adaptation to the wall, climate conditions and seasonality. The structure has an area of approx. 8 m², is made of recycled plastic (some from the sea), easy to maintain and disassemble and has minimal water consumption (irrigation is individual and automatic). Various physiological parameters of the plants will be measured, and their development will be monitored. Water consumption will also be evaluated.

The main objective is to understand if this can be a sustainable solution for urban agriculture, supplying family needs, when there is no availability of soil or other infrastructure. The expected results are related to the costs and productivity of the wall, as well as the selection of the most suitable species. Finally, is important to establish bridges like this one where universities work together with companies, so that in the future, more sustainable solutions can be found, and environmental impacts can be reduced.

Keywords – Biodiversity; ecosystem services; urban agriculture; urban environments; water consumption; vertical garden



Microplastics and plankton: Are laboratory experimental responses similar to those found in the field?

Sabrina M. Rodrigues^{1,2}, Michael Elliott^{3,4}, C. Marisa R. Almeida¹, Sandra Ramos¹

¹Ecobiotec, CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, Porto University, Matosinhos, Portugal

²ICBAS - Institute of Biomedical Sciences Abel Salazar, Porto University, Porto, Portugal

³Department of Biological & Marine Sciences, University of Hull, Hull, UKPortugal

⁴International Estuarine & Coastal Specialists Ltd., Leven, Beverley, UK

Presenting author email: smagalhaes@ciimar.up.pt

Corresponding author email: smagalhaes@ciimar.up.pt

Abstract

Microplastics, plastic particles < 5 mm, are considered a ubiquitous pollutant that can impact the aquatic environment and its biota. Due to their characteristics, especially the size, microplastics are hypothesized as causing a major impact on small organisms such as plankton, since microplastics size coincides with their prey size. The interaction between microplastics and plankton is of increasing concern given the position of such organisms at the base of marine food webs with a dominant role in ecosystem functioning. With an increasing scientific interest in the impacts caused by microplastics on these organisms, there is a need to investigate if laboratory experimental responses are similar to those found in field studies. Therefore, we systematically reviewed the scientific literature regarding plankton and microplastics interactions in order to compare field and laboratory experimental findings. This showed that 70% of scientific publications were from laboratory conditions and reported effects on processes such as growth, survival and reproduction. Microplastics interaction with plankton was recorded for 88 taxa, with a dominance of *Daphnia magna*, a freshwater species. Field studies were relatively scarce, representing less than 1/3 of the total 147 publications analyzed, with most focusing on contamination. In addition, microplastics types and concentrations collected in the field differed from those used in laboratory experiments, thereby limiting the comparison between studies. This review indicated that there are several knowledge gaps: in the non-comparability between laboratory and field conditions; in the low diversity of plankton species studied, namely phytoplankton and ichthyoplankton, and in microplastic effects extending from the individual organisms to populations, communities, and ecosystems.

Keywords – Microplastics; Zooplankton; Phytoplankton; Ichthyoplankton; Laboratory/field studies; Microplastics effects

Acknowledgements - This study was partially supported by FCT - Foundation for Science and



BLUE THINK CONFERENCE

Share Science, Spread Knowledge

Technology within the scope of UIDB/04423/2020 and UIDP/04423/2020 and a PhD fellowship to SM Rodrigues (SFRH/BD/145736/2019) and the project Ocean3R (NORTE-01-0145-FEDER-000064).



Medusozoans from the Iberian Peninsula and Islands: current knowledge and bioprospecting potential

Tomás Rodrigues^{1,2,3}, Daniela Almeida^{1,4}, Francisco Guardiola³, Agostinho Antunes^{1,2}

¹Evolutionary Genomics and Bioinformatics, CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Porto, Portugal

²Department of Biology, FCUP – Faculty of Sciences, University of Porto, Porto, Portugal

³Department of Cell Biology and Histology, University of Murcia, Murcia, Spain

⁴Department of Biological Sciences, Clemson University, Clemson, United States

Presenting author email: tomasfcr.portogmail.com

Corresponding author email: aantunes@ciimar.up.pt

Abstract

Bioprospection of natural products from marine invertebrates has focused on less than 1% of its biodiversity. The Cnidaria is the second most bioprospected phylum, but largely focused in the Anthozoa clade (anemones, corals). Such bias raise potential on other clades as sources of natural products, namely the underexplored Medusozoa (jellyfish). The growing interest in medusae, mainly due to the global increase of jellyfish blooms favored by global warming and imposing often high industrial economic losses (e.g., fisheries, aquaculture, tourism), represents an excellent opportunity of study. Here, we aim to evaluate the medusozoans from the Iberian Peninsula and Islands (Continental Portugal and Spain, Azores, Madeira, and Canaries), and their transcriptomics data availability, to guide the selection of interesting understudied species for future bioprospection of biocompounds through integrative omics. A bibliographic assessment of medusozoans (pelagic and benthic) reported in the Iberian Peninsula and Islands was accomplished considering more than 250 articles and books published between 1876 and 2021. Furthermore, the concept of jellyfish bloom was revisited considering the abundance data for each species, and the transcriptomic data of the reported species available at the NCBI database was compiled. Our results revealed a total of 559 species reported for the region, of which 516 are hydrozoans (92.3%), 37 scyphozoans (6.6%), four staurozoans (0.7%), and two cubozoans (0.4%). Overall, 226 were exclusively benthic (40%) and 333 presented a pelagic phase (60%). Continental Spain was the most diverse area with 368 species. The transcriptomic data compiled revealed just 7% of the species with information available: 30 hydrozoans, five scyphozoans, two staurozoans, and two cubozoans. The Iberian Peninsula and Islands showed rich biodiversity of medusozoans. However, there is a considerable lack of transcriptomic data. These first results provide an initial screening to select the most promising species to be object of future bioprospection of natural biocompounds.

Keywords - Cnidaria; Medusozoa; jellyfish; blooms; Iberian Peninsula; Biocompounds



BLUE THINK CONFERENCE

Share Science, Spread Knowledge



SESSION 2: BIOCHEMISTRY AND BIOTECHNOLOGY

MODERATION: SANDRA PEREIRA AND AMARANTA KAHN

BLUE THINK CONFERENCE

Share Science, Spread Knowledge



ABSTRACTS

ORAL COMMUNICATIONS



Biosynthesis of chlorinated lactylates from cyanobacteria

Kathleen Abt^{1,2}, Raquel Castelo-Branco², Pedro Leão²

¹PhD program Biomedical Sciences, ICBAS - Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, Portugal

²Cyanobacterial Natural Products, CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Porto, Portugal

Presenting author email: kabt@ciimar.up.pt

Corresponding author email: pleao@ciimar.up.pt

Abstract

Cyanobacteria are a rich source of bioactive natural products with potential for pharmacological and biotechnological applications. Most of these secondary metabolites are produced by sets of enzymes encoded together in so-called biosynthetic gene clusters. Despite the enormous progress in genome sequencing technologies and computational cluster detection tools, many compounds do not yet have a gene cluster associated and vice versa. Bridging this gap can provide valuable new insights into the unique enzymatic machinery of cyanobacteria, promote compound discovery or enable heterologous production of industrially relevant natural products. Lactylates, compounds consisting of a fatty acyl moiety and an α -keto acid moiety, are an important group of emulsifiers in the food and cosmetic industries. Recently, chlorinated lactylates, the chlorosphaerolactylates, have been reported from cyanobacteria. Here, we used stable isotope labelling and bioinformatics to identify a putative biosynthetic pathway for the chlorosphaerolactylates. A homology search with a known fatty acyl-chlorinating enzyme lead us to a single candidate gene cluster in the genome of the producing cyanobacterial strain. Feeding studies with stable-isotope labelled fatty acids and α -keto acids confirmed label incorporation of both putative building blocks into the compounds. By *in silico* analysis, we showed that the sequence of the predicted non-ribosomal peptide synthetase from the candidate gene cluster confers specificity for α -keto acids instead of amino acids, supporting the connection between this cluster and the lactylates. The discovery of this putative biosynthetic pathway paves the way to engineering the microbial production of industrially relevant lactylates.

Keywords - Cyanobacteria; natural products; biosynthesis



Combination of metabolomic tools and bioactivity screening as a strategy to find hit anticancer compounds from a library of cyanobacterial fractions

Leonor Ferreira¹, Maria Lígia Sousa¹, Marco Preto¹, João Morais^{1,2}, Raquel Silva¹, Ralph Urbatzka¹, Vitor Vasconcelos^{1,2}, Mariana Reis¹

¹CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, Matosinhos, Portugal

²FCUP – Faculty of Sciences of the University of Porto, Porto, Portugal

Presenting author email: lferreira@ciimar.up.pt

Corresponding author email: mreis@ciimar.up.pt

Abstract

Cyanobacteria are photosynthetic prokaryote microorganisms, that exist in diverse ecosystems (from fresh to marine waters, terrestrial land, and even in extreme environments).

To come up with strategies to survive and succeed in different habitats, they produce secondary metabolites that have interest for pharmacological applications.

The Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC - CIIMAR) holds 800 strains of cyanobacteria collected from a wide range of habitats and geographic locations, comprising a rich biodiversity. However, only a limited number of bioactive metabolites were isolated from by LEGE CC strains. Portoamides A-B, hierridin B, and nocuolin A are examples of compounds with anticancer potential, isolated in previous studies.

With such a vast biological collection, it is challenging to explore its full potential. The classic bioactivity-guided process for the isolation of compounds was often ineffective and very time-consuming. It was then crucial to implement a swifter strategy for fractionation, bioactivity testing and selection of promising strains. Therefore, the work developed on project CYANCAN established a process for a semi-automatic natural products library (LEGE-NPL) of cyanobacterial fractions, that can be easily applied to any bioactivity testing, and that can be combined with dereplication by LC-MS/MS.

In this work, 512 fractions from LEGE-NPL were tested for their anticancer potential against two different cell models of HCT 116, colon carcinoma cancer cells - traditional 2D monolayer, and biologically relevant 3D spheroids. The results yielded 11 bioactive fractions, with differences observed between the two cell models. Fractions of interest were then analysed by LC-MS/MS, processed with MZmine 2 and the metabolic networks were constituted using GNPS platform for data organization, dereplication and annotation. MetaboAnalyst was used to performed statistical analysis between active/inactive groups. The combination of these methodologies enhanced the selection of the most promising strains proving as a useful tool for future anticancer compound isolation.

Keywords - Cyanobacteria; anticancer; 3D spheroids; LC-MS/MS; metabolomics



BLUE THINK CONFERENCE

Share Science, Spread Knowledge

Acknowledgements - This research was developed under CYANCAN project PTDC/MED-QUI/30944/2017, co-financed by NORTE 2020, Portugal 2020 and the European Union through the ERDF, and by FCT through national funds and was additionally supported by the FCT strategic fund UID/Multi/04423/2019



Diversity of the Functional Machinery of Native Microbial Communities with the Ability to Degrade Petroleum Hydrocarbons

Maria Luis Bôto^{1,2}, Julie LaRoche³, Catarina Magalhães^{1,4,5}, Ana P. Mucha^{1,4}

¹EcoBioTec, CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal

²ICBAS – Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, Portugal

³Department of Biology, Dalhousie University, Halifax, NS, Canada

⁴FCUP – Faculty of Sciences of University of Porto, Porto, Portugal

⁵Ocean Frontier Institute, Dalhousie University, Halifax, NS, Canada

Presenting author email: mboto@ciimar.up.pt

Corresponding author email: mboto@ciimar.up.pt

Abstract

Bioremediation is the most effective, economic and eco-friendly approach to mitigate oil spills. This approach uses the natural capability of microorganisms to degrade petroleum compounds ensuring the mineralization of the oil and avoiding the risk of recontamination with secondary contaminants. With the development of culture-independent approaches it became possible to assess the whole composition and functionality of these microbial communities. A previous study [1] conducted along the NW coast of the Iberian Peninsula, in the scope of the SpilLess (<https://spilless.ciimar.up.pt>) and BIOREM (<https://biorem.ciimar.up.pt/project.html>) projects, unraveled that microbial communities with potential to degrade some petroleum aromatic hydrocarbons are widely distributed in this geographic area. Based on this information, the five most promising sites were selected for metagenomic analysis.

The aim of this work is to uncover the diversity and distribution of microorganisms capable of degrading petroleum compounds and understand its functional machinery involved on the degradation of those compounds.

Sample collection and process were previously described [1]. The natural and respective oil-enriched microbial communities were characterized at a taxonomic level using DADA2 pipeline and predict key functions with PICRUST2 pipeline. The metagenomic analysis is currently being processed using the Mgnify pipeline (<http://www.ebi.ac.uk/metagenomics>).

Oil-enriched communities were mainly composed by genera known to degrade petroleum hydrocarbons, such as *Acinetobacter*, *Alcanivorax*, *Flavobacterium*, *Pseudomonas*, *Salegentibacter* and *Thalassospira*. The analysis of the respective metagenomes will provide an important understanding of possible habitat-associated patterns and on the understanding on the genes involved in the degradation of hydrocarbons in natural and oil-enriched microbial communities.



This in deep analysis will allow to develop an efficient workflow to isolate the most promising strains with the capability to degrade petroleum compounds and to test their biodegradative potential for different petroleum hydrocarbons, based on their genetic potential, to help implement more effective bioremediation approaches.

Keywords - Bioremediation; Oil spills; Marine microbiome; Next-generation sequencing; Metagenomics

Acknowledgements - Maria Bôto acknowledges the Foundation for Science and Technology (FCT) for the PhD grant SFRH/BD/146523/2019. The authors also acknowledge the project BIOREM - Bioremediation of hydrocarbon pollutants by autochthonous microorganisms in aquatic environment, PTDC/BTA-GES/32186/ 2017.

References

[1] Bôto, M.L. *et al.* (2021). *Front. microbiol.* 12(879). doi: 10.3389/fmicb.2021.633659.



Direct pathway cloning of RiPP gene clusters and its production in *E. coli*

Raquel Castelo Branco^{1,3}, Vitor Vasconcelos^{2,3}, David Fewer⁴, Pedro Leão¹

¹Cyanobacterial Natural Products Team, CIIMAR - CIIMAR/CIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal.

²Blue Biotechnology and Ecotoxicology Team, CIIMAR - CIIMAR/CIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal.

³Faculty of Sciences, University of Porto, Porto, Portugal.

⁴Microbial Natural Products Group, Department of Microbiology, University of Helsinki, Helsinki, Finland.

Presenting author email: rcastelobranco@ciimar.up.pt

Abstract

Cyanobacteria are well-known producers of natural products (NPs), exhibiting remarkably diverse structures and biological properties. One abundant class of NPs produced by Cyanobacteria are the Ribosomally synthesized and Post-translationally modified Peptides (RiPPs) as revealed by recent genome sequencing efforts. However, under laboratory conditions many of the biosynthetic gene clusters (BGCs) encoding RiPPs are silent or expressed at very low levels. As an alternative, heterologous expression of these BGCs in genetically tractable hosts becomes an attractive route to obtain the encoded RiPPs and optimize their yields. In this work, we applied the direct pathway cloning (DiPaC), an emerging synthetic biology strategy that uses long amplification PCR and HiFi DNA assembly to capture several RiPP BGCs and express them in *E. coli*. By employing this newly established expression system and changing culture medium, we increased the production yields when compared to the native host. Thus, this approach proves to be a useful tool for cyanobacterial RiPPs production in *E. coli*. The next steps of this work will focus on the isolation of new cyanobacterial RiPPs and evaluate their bioactivity.

Keywords - Cyanobacteria; natural products; ribosomally synthesized and post-translationally modified peptides (RiPPs); synthetic biology; heterologous expression



Nocuolin A biosynthesis – origin of the carbon skeleton

Teresa Martins^{1,2}, Nate Glasser⁴, Nathaniel R. Braffman⁴, Duncan Kountz⁴, Kathleen Abt^{1,2}, Paulo Oliveira³, Emily Balskus^{4*}, Pedro Leão^{1*}

¹Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal

²Biomedical Sciences Institute of Abel Salazar, University of Porto, Porto, Portugal

³Institute of Health Innovation and Research /Institute for Molecular and Cell Biology, Porto, Portugal

⁴Department of Chemistry & Chemical Biology, Harvard University, Cambridge, MA, USA

Presenting author email: teresamartins_26@hotmail.com

Corresponding author email: pleao@ciimar.up.pt, balskus@chemistry.harvard.edu

Abstract

Natural products are useful for their biological activities, but their intricate structures also intrigue biochemists and inspire them to discover new biosynthetic reactions. Nocuolin A is a cyanobacterial compound featuring a unique 1,2,3-oxadiazine moiety. This structural feature is unprecedented in natural or man-made chemistry and suggests that novel enzymatic chemistry might be present in its biosynthesis. We report here our findings on the origin of the carbon skeleton of the nocuolin A, namely its C₁₃ linear chain and its 4-hydroxybutanone fragment.

Feeding experiments with deuterated and ¹³C-labeled fatty-acids with different chain lengths revealed that the C₁₃ chain is formed by the condensation of two short-chain fatty acids, hexanoic and octanoic acids. The observed deuterium-labeling patterns in following the feedings studies suggests that a new C-C bond is formed between carbons 5 and 6 and is compatible with an alpha-beta unsaturated C₈-derived alkene intermediate and a decarboxylation after the C-C bond forming reaction. In addition, enzymatic assays carried out with the purified fatty-acyl AMP ligase (NocH) from *noc* cluster proved that the enzyme activates hexanoic and octanoic acids, which also represents the first biochemical evidence connecting this putative cluster to nocuolin A. Additional feeding experiments showed that the 4-hydroxybutanone fragment is derived from methionine, likely through S-adenosyl methionine, as suggested by the presence of methionine salvage enzymes encoded by *noc*.

Keywords - Nocuolin A; cyanobacteria; biosynthesis; biochemistry; ketosynthase; methionine



BLUE THINK CONFERENCE

Share Science, Spread Knowledge



ABSTRACTS

FLASH COMMUNICATIONS



Development of two optimized bacterial consortia for the biodegradation of fluorinated pesticides

Diogo A.M. Alexandrino^{1,2}, Ana P. Mucha^{1,3}, Maria Paola Tomasino¹, C. Marisa R. Almeida¹, Maria F. Carvalho^{1,2}

¹CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Porto, Portugal

²ICBAS – Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, Portugal

³FCUP – Faculty of Sciences, University of Porto, Porto, Portugal

Presenting author email: dalexandrino@ciimar.up.pt

Corresponding author email: dalexandrino@ciimar.up.pt

Abstract

The use of microbial consortia for pesticide biodegradation is a promising strategy, often showing higher potential for their productive biotransformation than single strains. Nonetheless, substantial optimization of these high-performing communities is often required to enable their downstream application.

This work aimed to optimize bacterial consortia capable of biodegrading two highly persistent fungicides - epoxiconazole and fludioxonil. For this, bacterial strains isolated from consortia previously enriched with each pesticide were assembled in different combinations, until loss of degradation performance was observed (indicating the absence of a key microorganism). The potential of each strain to biodegrade the pesticides in axenic cultures was also investigated. A metabarcoding approach targeting the 16S rRNA biomarker was also implemented to unravel the taxonomic structure of the enriched consortia from which the strains were isolated.

For epoxiconazole, the combination of *Hydrogenophaga eletricum* 5AE and *Methylobacillus* sp. 8AE led to the degradation of 80% of this pesticide in 28 days, outperforming the efficiency of the consortium containing all the isolated strains. These strains were also able to metabolise the pesticide as axenic cultures, with strain 5AE showing the best performance (52% after 28 days). The abundance of strains 5AE and 8AE represented less than 10% of the enriched consortium, showing that epoxiconazole catabolism is likely driven by minor phylogenetic groups in the community. For fludioxonil, none of the tested bacterial combinations showed biodegradation efficiencies exceeding 20%. Furthermore, the isolated strains represented a small subset of the diversity of the enriched consortium they originated, which combined with their poor biodegradation performances, indicates that key bacterial degraders were not successfully isolated.

This work offers an outlook on the complex microbial dynamics of two enriched consortia exhibiting unusual catabolic proficiency towards recalcitrant pesticides, while also emphasizing the



importance of adequate culture-dependent approaches in the engineering of microbial consortia for bioremediation purposes.

Keywords - Illumina MiSeq amplicon sequencing; bioremediation; microbial dynamics; persistent pesticides; epoxiconazole; fludioxonil

Acknowledgements - Diogo A. M. Alexandrino acknowledges Fundação para a Ciência e Tecnologia (FCT) for the PhD fellowship ref. SFRH/BD/116702/2016. Maria F. Carvalho wishes to acknowledge the CEEC program supported by FCT (CEECIND/02968/2017), Fundo Social Europeu and Programa Operacional Potencial Humano. This work was financed by the Strategic Funding UIDB/04423/2020 and UIDP/04423/2020 through national funds provided by FCT and European Regional Development Fund (ERDF).



Marine inspired fumiquinazoline related alkaloids: synthesis and assessment of antibacterial potential

Mariana C. Almeida^{1,2}, Diana I. S. P. Resende^{1,2*}, Paulo M. da Costa^{2,3}, Madalena Pinto^{1,2}, Emília Sousa^{1,2}

¹Laboratório de Química Orgânica e Farmacêutica, Departamento de Ciências Químicas, FFUP – Faculdade de Farmácia, Universidade do Porto, Porto, Portugal.

²CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental, Matosinhos, Portugal.

³ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal.

Presenting author email: mariana_c_a@hotmail.com

Corresponding author email: * dresende@ff.up.pt

Abstract

As drug resistance continues to rise to worrisome levels, the development of effective antimicrobial agents is essential to the global health [1]. Marine derived indole alkaloids, namely those related to the fumiquinazolines, have shown promising bioactivities, including relevant antimicrobial properties [2]. The major aims of this work are to synthesize new fumiquinazoline related alkaloids inspired by marine molecules, introducing chemical modifications in these molecular scaffolds, and to evaluate their antibacterial activities.

To accomplish these goals a multi-step synthetic pathway was followed [3,4] through conjugation of three amino acids. In the antibacterial screening, the minimum inhibitory concentration (MIC) of each compound towards four bacteria species was determined by the broth microdilution method and a synergism study using the agar disk diffusion methodology was also performed.

Herein, we present the synthesis of natural fumiquinazoline F and glyantrypine as well as of new alkaloids and discuss their structural elucidation by 1H-NMR spectroscopy. In addition, we will also show the results of the assessment of antibacterial activity of the obtained compounds and their intermediates. Current work involves the synthesis of new alkaloids with different substitution patterns to study the structure-activity relationship (SAR) and further studies to assess the antibacterial activity of the compounds.

Keywords – Antimicrobial; fumiquinazolines; marine alkaloids; medicinal chemistry

References

- [1] Cheng, G. et al., *Frontiers in Microbiology*, 7 (2016) 470.
- [2] Resende, D. I. S. P. et al., *Natural Product Reports* 36 (2019) 7.
- [3] Wang, H., Ganesan, A., *The Journal of Organic Chemistry*, 65 (2000) 1022.
- [4] He, F.; Snider, B. B., *The Journal of Organic Chemistry*, 64 (1999) 1397-1399.



BLUE THINK CONFERENCE

Share Science, Spread Knowledge

Acknowledgements - This research was supported by national funds through FCT - Foundation for Science and Technology within the scope of UIDB/04423/2020, UIDP/04423/2020 (Group of Natural Products and Medicinal Chemistry CIIMAR), and under the project PTDC/SAU-PUB/28736/2017 (reference POCI-01-0145-FEDER-028736), co-financed by COMPETE 2020, Portugal 2020 and the European Union through the ERDF and by FCT through national funds, as well as CHIRALBIOACTIVE-PI-3RL-IINFACTS-2019 and R&D&I ATLANTIDA (reference NORTE-01-0145-FEDER-000040), supported by NORTE2020, through ERDF. Mariana C. Almeida acknowledges the BYT+ scholarship to CIIMAR.



Marine microbial diversity of a fuel oil leakage: bioremediation of hydrocarbons in seawater sampled after a spill accident in a port area

Rafaela Perdigão^{1,2}, Maria Paola Tomasino², Cristina Marisa Almeida^{2,3}, Maria de Fátima Carvalho^{1,2} and Ana Paula Mucha^{2,3}

¹ICBAS – School of Medicine and Biomedical Sciences, University of Porto, Porto, Portugal

²Ecobiotec/CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal

³FCUP – Faculty of Sciences, University of Porto, Porto, Portugal

Presenting author email: rafaelaperdigao94@gmail.com

Corresponding author email: rafaelaperdigao94@gmail.com

Abstract

Bioremediation with hydrocarbon-degrading bacteria can be an ecological and efficient technique to tackle oil spill incidents. Maritime ports are highly susceptible to these events. In 2019, a marine leakage of fuel oil occurred during bunkering operations inside a harbor in the SW of Portugal. Contaminated seawater samples and spilled oil were collected 5 days later. In this study, we aimed to (i) survey the native microbial community implicated in the oil spill degradation and (ii) evaluate the efficiency of a hydrocarbon-degrading lyophilized bacterial consortium to degrade the leaked fuel oil. Two parallel experiments were assembled in a seawater/oil ratio of 20:0.5: (i) an enrichment experiment, with addition of nutrients and (ii) a microcosm experiment, with three treatments: natural attenuation (NA) (seawater+oil); biostimulation (BS) (seawater+oil+nutrients); and bioaugmentation (BA) (seawater+oil+nutrients+lyophilized bacteria). Both experiments were incubated for 30 days, in the dark and under constant agitation. Prokaryotic communities of the contaminated seawater and composed samples collected after 15 and 30 days (T15/T30) of enrichment with oil were characterized by next generation sequencing. The abundance of oil-degraders was evaluated along time, by the most probable number (MPN) method. Degrading bacterial strains were recovered and identified after cultivation in solid culture medium. Results indicate a selection of the microbial community after 15 days of enrichment, where microbial diversity decreased, and dominant taxa associated to hydrocarbon degradation increased. Despite diversity increase after 30 days, T15 and T30 communities presented similar taxonomic profiles, with a predominance of the genera *Alcanivorax* and *Thalassospira*. A total of 95 bacterial strains were isolated, including 5 species of obligate hydrocarbon-degraders. The addition of the lyophilized strains to the BA treatment improved the biodegradation process, by increasing the abundance of oil-degraders along time. These bacteria were recovered in the first 3 weeks of experiment, highlighting its role in biodegrading the spilled oil.

Keywords – Bioremediation; oilspill; marine ports; bioremediation agent; lyophilized bacteria; hydrocarbons



BLUE THINK CONFERENCE

Share Science, Spread Knowledge

Acknowledgements - Rafaela Perdigão acknowledges the Foundation for Science and Technology (FCT) for the PhD grant 2020.04689.BD. The authors also acknowledge the project BIOREM - Bioremediation of hydrocarbon pollutants by autochthonous microorganisms in aquatic environment, PTDC/BTA-GES/32186/ 2017.



Bioactivity screening of cyanobacteria with anti-diabetic potential using zebrafish as model organism

Tiago Ribeiro^{1,2}, Mariana Reis¹, Vitor Vasconcelos^{1,2}, Ralph Urbatzka¹

¹BBE (Blue Biotechnology and Ecotoxicology), CIIMAR (Centro Interdisciplinar de Investigação Marinha e Ambiental), Universidade do Porto, Porto, Portugal

²Faculdade de Ciências da Universidade do Porto, Porto, Portugal

Presenting author email: tiago.amribeiro8@gmail.com

Corresponding author email: tiago.amribeiro8@gmail.com

Abstract

Diabetes is one of the most endemic diseases worldwide affecting different ages and social classes. The mechanism involved in the development of the disease is complex but one central process is the uptake of the glucose of the blood stream to the cells, being mainly controlled by the glucose transporters (GLUT's) and insulin signalling. *Danio rerio*, also known as zebrafish, is a well-used model organism in the study of many human diseases and the molecular mechanisms involved on them, with several published works on metabolic disorders like diabetes. In this work, we try to search for compounds that copy the insulin action and can have an important effect on the control of the glucose levels, using the zebrafish as screening model.

A total of 182 organic fractions, obtained from 22 cyanobacterial strains using chromatographic techniques, were screened using the 2-NBDG assay, a fluorescent analogue of glucose, to find fractions that increase the peripheral uptake of glucose. Two fractions had promising activity and were selected for the study of the molecular mechanism and chemical analysis of its constitution. For the mechanism was analysed: 1) GLUT expression using western blot, specially GLUT's 1-4, transporters with known function and related to the glucose homeostasis; 2) expression of genes involved in the glucose metabolism (like PEPCK, for example); 3) analysis of glucose levels using different time points of exposure.

The metabolite profiles of the fractions were analysed by LC-MS/MS and bioinformatic tools, as creation of molecular networks by GNPS data analysis, and correlations of mass peaks to bioactivity.

Overall, two fractions from cyanobacteria revealed promising activity for the treatment of diabetes and future work may identify responsible compounds.

Keywords – Diabetes; zebrafish; cyanobacteria; insulin; GLUT



BLUE THINK CONFERENCE

Share Science, Spread Knowledge



ABSTRACTS

POSTER PRESENTATIONS



Detection and separation of cyanobacteria bioactive compounds with therapeutical applications

Alessia di Flora^{1,2}, Simão Silva^{2,3}, Rita Biltes², Vitor Vasconcelos^{2,4}, Inês Páscoa², Marco Preto², Isabel Cunha²

¹Dipartimento di Ecologia e Biologia (DEB), BEM - Facoltà di Biologia ed Ecologia Marina, Università degli Studi della Tuscia, Italia. Programma Erasmus+ traineeship post lauream

²Blue Biotechnology and Ecotoxicology (BBE) group, CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal

³Departamento de Engenharia Química, ISEP - Instituto Superior de Engenharia do Porto, Porto, Portugal.

⁴Biology Department, FCUP - Faculty of Sciences, University of Porto, Porto, Portugal.

Presenting author email: alessiadiflora@gmail.com, simaobaptistasilva99@gmail.com

Corresponding author email: alessiadiflora@gmail.com, simaobaptistasilva99@gmail.com

Abstract

Cyanobacteria found in marine, estuarine and freshwater systems, produce toxic blooms that are becoming increasingly frequent. The high degree of chemical diversity of their secondary metabolites makes them an exceptionally good source of bioactive compounds. Also, the good growth rate of various strains increases the potential to produce innumerable natural products for a wide range of applications in the fields of pharmaceuticals, nutrition, cosmetics etc. Peroxisome Proliferator-Activated Receptors (PPARs) are nuclear receptors that have been shown to influence the expression of many genes in a variety of signaling networks. Recognized as ligand-activated transcription factors, they are involved in lipid, glucose, and amino acid metabolism, taking part in different cellular processes, including differentiation and apoptosis, inflammatory modulation, and attenuation of acute and chronic neurological damage and cancer. PPARs have been defined as critical sensors and master regulators of cellular metabolism. As a result of a previous work where 106 strains were pre-screened, the present work is focused on 8 of those strains that gave positive hit using a cellular sensor bioassay, also formerly developed. Our work involved several procedures such as large-scale cultivation and harvesting of cyanobacteria cultures, biomass extraction (solvents), fractionation of the crude extract by vacuum column liquid chromatography – VLC, and sub fractionation of fractions by different chromatographic techniques, including low pressure preparative and high-performance liquid chromatographies – HPLC. Subfractions will be analyzed again with the cell-sensor in triplex mode, to guide the identification and isolation of bioactive compounds with therapeutic applications, acting through transactivation of PPARs. The triplex mode allows for the detection of PPAR α , - β and - γ ligands, simultaneously. At the end of this drug discovery pipeline, we expect to have various hit compounds identified, however the process is still in progress.

Keywords - Cyanobacteria; biosensors; PPARs; bioactive compound; therapeutical applications; natural products



Selection and optimization of autochthonous bacterial strains isolated from Port of Leixões for bioremediation of crude oil and maritime fuels

Alice Melzi^{1,2}, Rafaela Perdigão^{2,3}, Maria Paola Tomasino², Maria Bôto^{2,3}, Ana Paula Mucha^{2,4}

¹SAA – Agroenvironmental Sciences / Statale University of Milan, Milan, Italy

²Ecobiotec/CIIMAR - Interdisciplinary Centre of Marine and Environmental Research

³ICBAS – Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, Portugal

⁴FCUP – Faculty of Sciences, University of Porto, Porto, Portugal

Presenting author email: alice.melzi@studenti.unimi.it

Corresponding author email: alice.melzi@studenti.unimi.it

Abstract

Seawater pollution, by petroleum hydrocarbons, can be caused by intensive activities in exploration, production, refining and waste management in oil industries. These events may represent a great threat to public health and environmental safety. Bioremediation of petroleum hydrocarbons, using consortia of autochthonous microorganisms, has been considered an efficient, eco-friendly and cost-effective way to degrade pollutants in contaminated sites. In this line, the aim of this work is to select and optimize, with different carbon sources, the growth of bacteria with potential to degrade different maritime fuels. In order to fulfil this aim, a total of 145 bacterial strains were previously isolated from the seawater of the Port of Leixões (Portugal) after an enrichment with petroleum, turbine oil and diesel. These bacteria are currently being identified using full length 16S rRNA gene amplification followed by sanger sequencing and will then be tested with the most probable number (MPN) method to assess their potential for hydrocarbon degradation. The growth of the best performing bacterial strains will be tested with different carbon sources such as glycerol, peptone, glucose and sodium acetate, in flasks with minimum medium after an initial screening in 96-well plates. This ongoing work will allow us to select bacteria with high potential to degrade different maritime fuels and optimize their growth, for a future application as a bacterial product in an oil spill scenario.

Keywords - Bioremediation; Maritime fuels; Seawater; Hydrocarbon's degradation; Autochthonous microorganism

Acknowledgments - This research was partially supported by the project BIOREM—Bioremediation of hydrocarbon pollutants by autochthonous microorganisms in aquatic environment, PTDC/BTA-GES/32186/ 2017 and POCI-01-0145-FEDER-032186, supported by Fundo Europeu de Desenvolvimento Regional through COMPETE2020—Programa Operacional de Competitividade e Internacionalização (POCI) and FCT/MCTES; by national funds through FCT—Foundation for Science and Technology within the scope of UIDB/04423/2020 and UIDP/04423/2020.



Targeted discovery of fatty acid-incorporating natural products from genome data

Anne Liong^{1,2}, Pedro Leão¹

¹Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIIMAR), University of Porto, Matosinhos, Portugal

²Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Porto, Portugal

Presenting author email: aliong@ciimar.up.pt

Corresponding author email: pleao@ciimar.up.pt

Abstract

Due to the advent of new sequencing technologies, the number of sequenced genomes has increased dramatically over the last years. For bacteria, this wealth of genomic data has revealed a large number of secondary metabolite biosynthetic gene clusters (BGCs), representing a potential source of unexplored compounds with industrial and pharmaceutical interest. Cyanobacteria, a diverse group of gram-negative bacteria, incorporate fatty acids (FAs) into a large fraction of small molecules with medicinal interest, so called natural products (NPs). On average, those photosynthetic bacteria devote around 5-6% of their genomes to secondary metabolism with around five BGCs per genome. The percentage of orphan BGCs (i.e. those without a corresponding NP) is estimated to be 80-90%. However, identifying the NP associated with an orphan BGC is not trivial and a current challenge in NPs research.

In this project, we aim to establish a strategy for the targeted deorphanization of FA-incorporating BGCs. By developing experimental and bioinformatics tools to predict the incorporated FA by a BGC of interest, our method will enable direct access to the encoded NPs. To do so, we focus on a group of FA-incorporating enzymes especially abundant in cyanobacteria: Fatty acyl-AMP ligases (FAALs), as they have the ability to incorporate FAs resulting in a high variety of lipidic NPs with different functionalities.

After selection of target BGCs, FAALs originating from LEGE strains were cloned for heterologous expression in *E. coli*, purified and their specificity determined *in vitro*. The tested FAALs showed FA length specificity *in vitro*. Armed with this knowledge, we performed isotope-labeled FA feeding experiments using a specific FA length for a particular target FAAL-containing BGC. Comparative metabolomics analysis (LC-HRMS) will allow us to connect such BGCs to the corresponding NPs. Hence, this technique should open a new door to unexplored NPs which may possess important biological activities.

Keywords - Natural products; Cyanobacteria; Fatty acyl-AMP ligase; Biosynthetic enzymes; Comparative metabolomics; Mass spectrometry



Assessing spatial green roofs implementation in a historic village

Cristina Silva¹, Joaquim Alonso^{2,3}, Cristina S. C. Calheiros⁴

¹Faculdade de Ciências, University of Porto, Porto, Portugal

²ProMetheus – Research Unit in Materials, Energy and Environment for Sustainability, Instituto Politécnico de Viana do Castelo, Viana do Castelo, Portugal

³CIBIO.InBIO – Research Centre in Biodiversity and Genetic Resources, Campus de Vairão, University of Porto, Vairão, Portugal

⁴Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), University of Porto, Matosinhos, Portugal

Presenting author email: up202000475@up.pt

Corresponding author email: cristina@calheiros.org

Abstract

With the increase of impermeabilization in urban areas is of outmost importance that these territories include vegetation as a building material when planning a resilient development to strike climate change challenges. The inclusion of nature-based solutions, in the contemporary urban environment, has been increasingly recognized as a good practice, used to improve life quality and sustainability. One of these solutions are green roofs, which offer countless ecosystem services by increasing the green area in urban context and acting as an ecological corridor for biodiversity. These green systems can be placed at ground level or on top of buildings, improving not only their thermal comfort and energy savings but also their longevity. Besides that, they reduce the impact of precipitation peaks through rainwater retention and controlled drainage and mitigate urban heat islands. As landscape interventions are becoming more prominent within major city buildings, the aim of this study is to promote green infrastructure, such as green roofs, especially those that can be installed in new or pre-existing buildings. To materialize this new paradigm, strategic planning is required to achieve technical successful and socio-economic viable solutions. In this way, this study intends to assess the potential of green roof implementation in one of the oldest towns of Portugal, Ponte de Lima. Land use evolution, impermeabilized areas, surface temperatures, normalized difference vegetation index (NDVI) and humidity index of the region were considered to define priority areas towards green roofs inclusion. Thus, in this research spatially explicit models included biophysical, ecological and economic variables and local land use planning options, to support local analysis and sustainable proposals in order to transform conventional to green roofs.

Keywords - Sustainable development; green infrastructures; spatial analysis; energy saving; nature-based solutions



Potential of chalcone-triazole hybrids to circumvent bacterial resistance

Daniela Pereira^{1,2,#}, Fernando Durães^{1,2,#}, Joana Freitas-da-Silva^{2,3}, Nikoletta Szemerédi⁴, Eugénia Pinto^{2,5}, Paulo Costa^{2,3}, Madalena Pinto^{1,2}, Marta Correia-da-Silva^{1,2}, Gabriella Spengler⁴, Emília Sousa^{1,2,*}, Honorina Cidade^{1,2,*}

¹Laboratory of Organic and Pharmaceutical Chemistry, Department of Chemical Sciences, FFUP – Faculty of Pharmacy, University of Porto, Porto, Portugal

²CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal

³ICBAS – Institute of Biomedical Sciences Abel Salazar, University of Porto, Portugal

⁴Department of Medical Microbiology, Albert Szent-Györgyi Health Center and Faculty of Medicine, University of Szeged, Hungary

⁵Laboratory of Microbiology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Portugal

Presenting author email: dmpereira@ff.up.pt

Corresponding authors email: hcidade@ff.up.pt, esousa@ff.up.pt

#Authors contributed equally to this work.

Abstract

Antimicrobial resistance is a serious worldwide threat to public health, leading to an increase of mortality rates, prolonged illness, and higher medical cost, among others [1]. There are several mechanisms associated with antimicrobial resistance, being the presence of efflux pumps one of them [1-3]. Several chalcones have shown to display antimicrobial activities [4], namely 5'-lavandulyl-2'-methoxy-2,4,4',6'-tetrahydroxychalcone isolated from marine-derived actinomycete *Streptomyces* sp. C011, which exhibited promising antibacterial activities in both gram-positive and gram-negative bacteria, as well as antiyeast effect against *Candida albicans* [5]. Moreover, some chalcones displayed efflux pump inhibitory activity [3]. Considering the potential of the chalcone scaffold, a series of chalcone derivatives was synthesized and screened for its antibacterial, antifungal, and potential to establish synergy with antibiotics in resistant bacterial strains. Furthermore, they were tested for the inhibition of bacterial efflux pumps, and adaptability and virulence mechanisms, such as biofilm formation and quorum sensing. Two propargylated chalcone intermediates were obtained by Claisen-Schmidt condensation from appropriate substituted acetophenone and benzaldehydes. Afterwards, and considering the metabolic stability and antimicrobial potential of the 1,2,3-triazole ring [6], eight triazolyl-chalcone hybrids were obtained through copper catalysed alkyne-azide cycloaddition (CuAAC). Although none of the synthesized compounds displayed antimicrobial activity alone, it was found a synergic effect of some compounds in association with antibiotics in resistant strains of *Escherichia coli* and



Enterococcus faecalis. Further studies showed that some chalcone hybrids were able to inhibit efflux pumps of the resistant bacterial strain *Salmonella enterica* serovar Typhimurium SL1344 and to inhibit the biofilm formation of *Staphylococcus aureus* MRSA 272123, suggesting their potential to circumvent bacterial resistance and adaptability.

Keywords - Bacterial resistance; Efflux pumps; Chalcones; Click chemistry; Antimicrobial activity

Acknowledgements - This research was supported by national funds through FCT - Foundation for Science and Technology within the scope of UIDB/04423/2020 and UIDP/04423/2020 (Group of Natural Products and Medicinal Chemistry, CIIMAR) and under the project PTDC/SAU-PUB/28736/2017 (reference POCI-01-0145-FEDER-028736), co-financed by COMPETE 2020, Portugal 2020 and the European Union through the ERDF, and by FCT through national funds, and by the structured program of R&D&I ATLANTIDA (reference NORTE-01-0145-FEDER-000040), supported by the North Portugal Regional Operational Programme (NORTE2020), through the ERDF. This research was also supported by IINFACETS, grant number CHIRALBIOACTIVE-PI-3RL-IINFACETS-2019 and CHIRALSINTESE_APSFCT_IINFACETS_2021. Daniela Pereira and Fernando Durães acknowledges FCT for their grants (SFRH/BD/147207/2019 and SFRH/BD/144681/2019, respectively).

References

- [1] J. Tanwar, S. Das, Z. Fatima, S. Hameed, Multidrug Resistance: An Emerging Crisis, Interdisciplinary Perspectives on Infectious Diseases, 2014 (2014) 541340.
- [2] F. Barbosa, E. Pinto, A. Kijjoo, M. Pinto, E. Sousa, Targeting antimicrobial drug resistance with marine natural products, International Journal of Antimicrobial Agents, 56 (2020) 106005.
- [3] F. Durães, M. Pinto, E. Sousa, Medicinal Chemistry Updates on Bacterial Efflux Pump Modulators, Current medicinal chemistry, 25 (2018) 6030-6069.
- [4] W. Dan, J. Dai, Recent developments of chalcones as potential antibacterial agents in medicinal chemistry, European Journal of Medicinal Chemistry, 187 (2020) 111980.
- [5] T.T.T. Hoa, V. Van Nam, D.T.M. Huong, P.T. Dao, N.N. Phuong, L.T.H. Minh, V.T. Quyen, N.M. Anh, B.T. Murphy, H.T.M. Ly, P. Van Cuong, Flavonoids and Alkaloids from Marine-Derived Actinomycete Streptomyces sp. C011, Vietnam Journal of Chemistry, 58 (2020) 610-614.
- [6] A. Rani, G. Singh, A. Singh, U. Maqbool, G. Kaur, J. Singh, CuAAC-ensembled 1,2,3-triazole-linked isosteres as pharmacophores in drug discovery: review, RSC Advances, 10 (2020) 5610-5635.



Anti-inflammatory potential of Cyanobacteria

Fernando Pagels ^{1,2*}, A. Catarina Guedes ¹, Vítor Vasconcelos, ^{1,2} and Graciliana Lopes ¹

¹CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal

²FCUP – Faculty of Science, University of Porto, Porto, Portugal

Presenting author email: fernandopagels@gmail.com

Corresponding author email: fernandopagels@gmail.com

Abstract

Cyanobacteria are a group of photosynthetic prokaryote microorganisms of considerable economic importance, mostly due to their ability to synthesize several high-value compounds. The secondary metabolites of cyanobacteria are associated with a range of biological activities, including anti-inflammatory capacity. Inflammation pathways are typical pathophysiological characteristics of many deleterious diseases, including coronary diseases, type 2 diabetes mellitus, asthma, neurodegenerative disorders, and many types of cancer. The need for new therapies has contributed to the search for new sources of compounds, mainly from natural and renewable sources, such as cyanobacteria.

This study explored the available literature that underlies the anti-inflammatory capacity of cyanobacterial compounds, highlighting the mechanism of action related to the inflammation pathway.

The anti-inflammatory effect of cyanobacteria is specific to the type of compound:

- i) Mycosporine-like amino acids (e.g. mycosporine-2-glycine and shinorine) can act directly in enzymatic activity and expression;
- ii) Peptides (e.g. microcolins and aeruginosins) can downregulate cytokines (in special IL-8), inhibit NF- κ B and reduce iNOS expression;
- iii) Polysaccharides (e.g. Sacran and polysaccharides-rich extracts) can be applied to symptoms relieve, but also in the reduction of cytokines, NF- κ B and enzymatic activity;
- iv) Lipids (e.g. mono- and digalactosyldiacylglycerol fractions) can downregulate NF- κ B, but be also applied in terms of reduction of edema;
- v) Pigments (e.g. carotenoids and phycobiliproteins) can act at most of inflammation pathways, including the reduction of cytokines, NF- κ B and enzymatic activity, and also in terms of reducing leukocyte recruitment.

Finally, other compounds from cyanobacteria were pointed out as potential anti-inflammatory agents, including tolypodiol, coibacin and scytonemin.

Major findings include that the main anti-inflammatory effects of cyanobacterial compounds are related to reduction of cytokines mRNA levels (IL-4, IL-5, IL-6, TNF- α and IL-1 β), reduction of



BLUE THINK CONFERENCE

Share Science, Spread Knowledge

transcription factor (NF- κ B) and enzymatic activity (iNOS, COX-2). Overall, cyanobacteria were found to be a promising source for anti-inflammatory compounds.

Keywords - Natural compounds; bioactive potential; mycosporine-like amino acids; peptides; polysaccharides; pigments; scytonemins; polyphenols



Bioactivity screening of bacteria isolated from extreme environments

Imene Hakem^{1,2}, Mariana Girao², Tiago Ribeiro², Maria Ligia Sousa Silva², Natália Silva², Fátima Carvalho², Vitor Vasconcelos^{2,3}, Bensalah Farid¹ and Ralph Urbatzka^{2*}

¹Department of Biology/ Laboratory of Microbial Genetic, University of Ahmed Ben Bella Oran1, Oran 31000, Algeria.

²Interdisciplinary Centre of Marine and Environment Research (CIIMAR/CIMAR), University of Porto, Avenida General Norton de Matos, s/n, 4450-208 Matosinhos, Portugal.

³Department of Biology, FCUP, Faculty of Science, University of Porto, Rua do Campo, Alegre, 4169-007 Porto, Portugal.

Presenting author email: Hakem1991@outlook.fr

Abstract

Nature is a major reservoir of biologically active molecules. The urgent need of finding novel molecules for pharmaceutical applications is prompting the research to underexplored environments, such as marine ecosystems. In this study, we investigated the microbial extracts by marine bacteria of the genera *Bacillus* sp., *Pseudomonas* sp., *Pseudoalteromonas* sp., *Cobetia* sp., *Streptomyces* sp., *Micrococcus* sp., isolated from different site in Algeria.

The bioactivity screening comprehended the cytotoxicity activity against cancer cells lines grown as a monolayer culture or as multicellular spheroids and against non-carcinogenic endothelial cell line (hCMEC/D3). Antimicrobial activity was determined against pathogenic bacteria using the agar-based disk diffusion method, followed by determination of inhibitory concentration (MIC) values. Finally, glucose uptake (antidiabetic), and lipid-reducing activity (obesity) was analyzed in zebrafish larvae. Bioactivity data were complemented by the characterization of the metabolite profile of different extracts that were associated with observed bioactivities. Three extracts inhibited the growth of *Bacillus subtilis* and/or *Staphylococcus aureus* with MIC value ranging from <0,5 to 100 µg mL⁻¹. One microbial extract affected the viability of colon adenocarcinoma cells (HCT116) in monolayer culture and multicellular spheroids.

Twenty-nine extracts increased the neutral lipid concentration and one extract showed toxicity, in zebrafish. *Pseudoalteromonas* sp. extract demonstrated the most promising results and revealed to be a rich source of metabolites with bioactivities, and consequently, of new molecules with biotechnological importance.

Keywords - Extract, Antimicrobial; Anticancer; antidiabetic; marine bacteria; bioactivities



Exploring the diversity and metabolic potential of deep-sea Actinobacteria from Macaronesia archipelagos

Inês Ribeiro^{1,2*}, Jorge Antunes¹, Mariana Girão^{1,2}, Cíntia Ribeiro^{1,3}, Francisca Branco^{1,3}, Andreia Braga-Henriques^{4,5}, Ana P. Mucha^{1,3}, Ralph Urbatzka¹, Pedro Leão¹, Maria de Fátima Carvalho^{1,2}

¹CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Porto, Portugal

²ICBAS - Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, Portugal

³FCUP - Faculty of Sciences of the University of Porto, Porto, Portugal

⁴MARE - Marine and Environmental Sciences Centre, Madeira, Portugal;

⁵The Regional Secretariat for the Sea and Fisheries, Government of the Azores, Horta, Portugal

Presenting author email: inesfcribeiro@gmail.com

Corresponding author email: inesfcribeiro@gmail.com

Abstract

Natural marine products have been increasingly targeted due to their wide range of biotechnologically relevant bioactivities and under exploration. Marine actinobacteria are known to be a highly prolific source of bioactive secondary metabolites and their bioprospection in extreme environments, such as the deep-sea, may be a key to the discovery of new drugs. In this study, 63 deep-sea samples (sediments, corals and sponges) were collected from the archipelagos of the Azores and Madeira, at depths between 150 and 3199 m, with the aim of isolating actinobacteria capable of producing bioactive metabolites. Two pre-treatments and five selective culture media supplemented with different antibiotics were employed for their isolation. A total of 230 actinobacterial strains, belonging to 19 genera and including potential novel taxa, were isolated. These strains are being screened for diverse bioactivities, with 155 isolates tested up to the moment. Antimicrobial assays revealed 15 extracts with antimicrobial activity against one or more of the test microorganisms - *Candida albicans*, *Staphylococcus aureus* and *Bacillus subtilis*, showing minimum inhibitory concentration (MIC) values in the range of 7.81–1000 µg mL⁻¹. Cytotoxicity assays revealed nineteen extracts capable of reducing the cellular viability of the cancer cell lines HepG2 (liver cancer) and T-47D (breast ductal carcinoma), in at least 30% after 48 h of exposure. The extract of one *Streptomyces* strain is currently being analyzed by mass spectrometry to investigate if it may contain a new natural product. Our results show that deep-sea samples are an important source of actinobacteria, including novel taxa, and may be promising for the discovery of novel molecules.

Keywords - Marine actinobacteria; Deep-sea; Antimicrobial; Anticancer; Azores, Madeira

Acknowledgements - Inês Ribeiro would like to acknowledge the Ph.D. grant SFRH/BD/136357/2018 attributed by Foundation for Science and Technology (FCT). This work



BLUE THINK CONFERENCE

Share Science, Spread Knowledge

was financially supported by the project ACTINODEEPSEA (POCI-01-0145-FEDER-031045) co-financed by COMPETE 2020, Portugal 2020 and the European Union through the European Regional Development Fund (ERDF) and by FCT, Portugal, through national funds.



Ethanol extracts of filamentous and picoplanktonic cyanobacteria improve skin structure and preserve dermal matrix components

Janaína Morone^{1,2}, Graciliana Lopes^{1,2}, Marco Preto², Vítor Vasconcelos^{1,2}, Rosário Martins^{2,3}

¹Faculty of Sciences, University of Porto, Porto, Portugal

²Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), University of Porto, Matosinhos, Portugal

³Health and Environment Research Centre, School of Health, Polytechnic Institute of Porto, Porto, Portugal

Presenting author email: janabavini@ciimar.up.pt

Corresponding author email: mrm@ess.ipp.pt

Abstract

The use of natural products in skin care formulations has become a major concern for modern societies. The undesirable side effects of synthetic compounds and the associated environmental hazards have driven investigation on photosynthetic organisms as sustainable sources of effective and environmentally friendly ingredients. In this way, the use of natural extracts in cosmetics has been highlighted. Cyanobacteria have come into focus as a source of compounds for cosmetic applications. Due to their low culture demands, high grow rates, and ability to produce a wide variability of bioactive compounds, cyanobacteria emerged as an economically and sustainable base for the cosmetic industry. In this study, we evaluated the potential of ethanolic extracts of picocyanobacteria strains of the genera *Cyanobium* and *Synechocystis* and filamentous strains of the genera *Nodosilinea*, *Phormidium*, and *Tychonema* for skin care applications. The cyanobacteria extracts were analyzed for their cytotoxicity against keratinocytes (HaCat), fibroblasts (3T3L1) and endothelial cells (hCMEC/D3), pigments profile, phenolic content, antioxidant potential, and capacity to inhibit hyaluronidase. The total carotenoid content ranged from 118.69 to 383.89 $\mu\text{g g}^{-1}$ of dry biomass, and the total phenolic content from 1.07 to 2.45 mg GAE g^{-1} . Identified carotenoids consisted of zeaxanthin, lutein, canthaxanthin, echinenone and β -carotene, with zeaxanthin and lutein being the most representative (49.82 and 79.08 $\mu\text{g g}^{-1}$ of dry biomass, respectively). The highest antioxidant potential was found for *Phormidium* sp. LEGE05292 and *Tychonema* sp. LEGE07196 for superoxide anion radical ($\text{O}_2^{\cdot-}$) scavenging assay (IC_{50} of 822.70 and 924 $\mu\text{g mL}^{-1}$, respectively). Regarding Hyaluronidase inhibition, *Tychonema* sp. LEGE07196 and *Cyanobium* sp. LEGE07175 showed the best IC_{50} (186.85 and 214.17 $\mu\text{g mL}^{-1}$, respectively). Moreover, an increase in fibroblasts proliferation was registered upon treatment, suggesting their potential for skin regeneration. Thus, the species *Tychonema* sp. and *Cyanobium* sp. seem particularly interesting, for studies directed to application in anti-aging formulations.

Keywords - Cyanobacteria; antioxidant; anti-aging; regeneration



Appetite Reduction by Microalgae in Zebrafish Larvae for Obesity Treatment.

Javier Sanz Moxó^{1,2}, Gabriela Matos³, Jorge Saraiva³, Ana Margarida Costa⁴, Joana Laranjeira da Silva⁴, Ricardo Pereira⁵, Cristina Rocha⁵, Vítor Vasconcelos^{1,6}, Ralph Urbatzka¹

¹Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Porto, Portugal.

²Universidad Católica de Valencia (UCV), Valencia, Spain.

³University of Aveiro, Aveiro, Portugal

⁴Allmicroalgae, Pataias, Portugal

⁵Department of Biological Engineering, University of Minho, Portugal

⁶FCUP - Faculty of Science, University of Porto, Porto, Portugal

Presenting author email: jsanzmoxo@gmail.com

Corresponding author email: jsanzmoxo@gmail.com

Abstract

The increasing number of people suffering from obesity is becoming of great concern. Over 4 million people die per year because obesity leads to diseases such as high cholesterol, diabetes and both respiratory and cardiovascular complications. To solve this problem, an urgent search of innovative therapeutic drugs must be triggered to combat for a suitable human health. Microalgae are important biological resources that have a wide range of biotechnological applications. Known as protein resource, but also as producers of valuable compounds for human health benefits (e.g. polyunsaturated fatty acids, pigments, vitamins), these microorganisms are a powerful source for nutraceutical and pharmaceutical uses, as well as for industrial applications. Moreover, microalgae are known producers of natural compounds with biological activities towards human diseases. The aim of this research focuses on the screening of microalgae extracts in zebrafish larvae for the discovery of appetite reducing compounds. Ninety-two extracts belonging to three strains of microalgae (*Chlorella vulgaris autotrophic*; *Chlorella vulgaris heterotrophic/smooth*; *Nannochloropsis oceanica* and *Chlorococcum*) were obtained by different techniques (Ohmic treatment and High Pressure processing) and solvents. Extracts were screened in two different feeding assays with zebrafish larvae as model organism using fluorescent labelled liposomes and paramecia (*Paramecium bursaria*). In the liposome assay, five extracts reduced the appetite in zebrafish larvae for more than 30%, while seven extracts showed appetite induction > 30%. Active extracts are then submitted to a metabolite profiling with LC-MS/MS to receive insights into responsible compounds for appetite signaling. Molecular techniques will be performed to perceive the molecular mechanisms of action of the metabolites. Since some of the used microalgae species are approved for human consumption, the development of new nutraceuticals for the treatment of obesity could be promising.

Keywords - Microalgae; Marine Natural products; appetite; obesity; zebrafish larvae



***Synechocystis salina* as source of antioxidant compounds: application of Box–Behnken design toward optimization of productivity**

Joana Assunção^{*1,2}, Inês Meirinho^{1,3}, Helena M. Amaro², F. Xavier Malcata^{1,4}, A. Catarina Guedes²

¹LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Department of Chemical Engineering, University of Porto, Porto, Portugal

²CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, Matosinhos, Portugal

³Faculty of Sciences and Technology, University of Algarve, Faro, Portugal

⁴FEUP - Faculty of Engineering of University of Porto, Porto, Portugal

Presenting author email: joanaleonardoassuncao@gmail.com

Corresponding author email: acatarinaguedes@gmail.com

Abstract

Cyanobacteria have been gaining attention over the latest decades owing to their remarkable ability to produce a wide range of compounds with different biological capacities – and thus, susceptible of eventual exploitation within the biotechnological field. Such compounds include molecules bearing antioxidant capacity – known to be important players in prevention and sequestering free radicals that affect human health – and thus likely to be used as ingredients in nutraceutical or cosmetical applications. With the increasing market demand for functional products, an opportunity exists to explore those compounds – as long as the preliminary optimization of the biomass production is performed. Culture conditions (i.e. light, medium concentration, temperature, salinity, and pH) strongly influence accumulation of antioxidants in cyanobacteria – which are often triggered by extreme environmental conditions. Very few studies have focused on optimization of the antioxidant capacity of *Synechocystis salina* – a species offering a potential wastewater bioremediation and production of pigments, phenolic compounds and other bioactive compounds; this study, accordingly, aimed to improve antioxidant capacity of *Synechocystis salina* LEGE 06155, following a Box-Behnken design approach. Temperature (15-25°C), pH (6.5-9.5), and NaCl concentration (10-40 g.L⁻¹) were chosen parameters to be optimized. The antioxidant capacity was analyzed by ABTS, every two days along a 22-day period of growth, for each condition tested. The antioxidant capacity served as objective function, and the best conditions for production of antioxidant compounds were determined.

Keywords - Cyanobacteria; temperature; pH; NaCl concentration; antioxidant properties; optimization



Endocrine disrupting chemicals in gammarids: The Ecdysone Receptor as molecular sentinel

João Pedro Sousa^{1,2,3}, Teresa Neuparth³, Arnaud Chaumot⁴, Luís Filipe Castro^{2,3}, Olivier Geffard⁴, Davide Degli-Esposti⁴, Miguel Machado Santos^{2,3}, Raquel Ruivo³

¹Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal

²Faculdade de Ciências da Universidade do Porto, Porto, Portugal

³CIIMAR- Centro Interdisciplinar de Investigação Marinha e Ambiental, Matosinhos, Portugal

⁴IRSTEA – Institut de Recherche en Sciences et Technologies pour l'Environnement et l'Agriculture, Lyon, France

Presenting author email: jsousa@ciimar.up.pt

Corresponding author email: rruivo@ciimar.up.pt

Abstract

Ecotoxicological risk assessment relies on the representativeness of physiological responses across the taxa. This is still problematic in metazoans, which limits the accurate estimation of adverse outcome mechanisms in a taxonomic perspective. Within the Nuclear Receptor (NR) superfamily, the ligand-induced Ecdysone Receptor (EcR) provides a notorious example. This receptor, considered arthropod specific, has been extensively targeted for the development of insect-specific pesticides and anti-parasitic drugs, with an apparent low toxicity towards off-target species. Yet, once bioavailable such compounds can act as endocrine disrupting chemicals, mimicking or interfering with the signaling of endogenous hormones, and producing atypical or subchronic effects, with emphasis on timing and duration of exposure, and even latent or transgenerational effect. Additionally, the easy dissemination of these compounds makes the aquatic systems the main target. To assess the ecotoxicological status of water bodies, amphipods are extensively used as model organisms, yet amphipod EcR activity is still unknown. With this in mind, we aimed to functionally characterize EcRs from two different species of amphipods (the marine *Gammarus locusta* and the freshwater *G. fossarum*). Using a luciferase-based transactivation assay, the ability of selected compounds to induce the transcriptional activity of the amphipod EcRs was measured. Namely, diacylhydrazine and neonicotinoid insecticides, other compounds with similar structures (aminopyrine, vatalanib), and ecdysteroids as positive control (20-OH ecdysteroid and phytoecdysteroid Ponasterone A). Current observations demonstrate variable transcriptional inductions upon exposure to Ponasterone A, in *G. locusta* and *G. fossarum*. Additionally, co-transfection with the endogenous heterodimeric partner Retinoid X Receptor (RXR) increased the sensitivity towards two diacylhydrazine insecticides, as well as the 20-OH ecdysteroid. This further highlights the ability of RXR to modulate EcR transcriptional profiles. This project was supported by the Programme Nationale de



BLUE THINK CONFERENCE

Share Science, Spread Knowledge

Recherche en Environnement-Santé Travail, France, under a collaboration with INREA.

Keywords - Ecotoxicology; Nuclear Receptors; Neonicotinoids; Ecdysteroids; Insecticides; Transactivation assays



In silico analysis, expression and purification of BrtB, a new C-O bond forming enzyme

João Reis¹, Pedro Leão¹

¹Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), University of Porto, Matosinhos, Portugal

Presenting author email: jreis@ciimar.up.pt

Corresponding author email: pleao@ciimar.up.pt

Abstract

The genomes of bacteria encode a large number of uncharacterized protein functions. This diversity fuels enzyme discovery efforts and will lead to the development of future biocatalysts. We have recently reported a novel enzyme – BrtB - responsible for the O-alkylation of free fatty acids with a series of cyanobacterial chlorinated dialkylresorcinols, the bartolosides. This reaction has little precedent in enzymatic catalysis, where carboxyester formation involving the nucleophilic attack of carboxylates onto alkyl halide electrophiles had only been transiently observed.

BrtB has potential for biocatalysis applications as it shows high promiscuity towards fatty acid length and saturation degree. As such, there is considerable interest in understanding more about this enzyme and its homologs. Here, we provide an initial *in silico* analysis of BrtB and detail our efforts to obtain an efficient protocol for recombinant BrtB expression and purification. Large-scale BrtB purification will enable future structural analysis and lead to a mechanistic understanding of this new biocatalyst.

Keywords - Natural product; biosynthesis; protein purification; enzymatic catalysis



Tricyclic compounds as potential dual BACE-1/GSK-3 β inhibitors: synthesis and neuroprotection studies

Márcia Martins^{1,2}, Miguel Maia^{1,2}, Eva Gil-Martins³, Luís Gales^{4,5}, Fernando Remião³, Madalena Pinto^{1,2}, Renata Silva³, Emília Sousa^{1,2}

¹CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, Porto, Portugal

²Department of Chemical Sciences, Laboratory of Organic and Pharmaceutical Chemistry, Faculty of Pharmacy of University of Porto, Porto, Portugal

³UCIBIO-REQUIMTE, Department of Biological Sciences, Laboratory of Toxicology, Faculty of Pharmacy of University of Porto, Porto, Portugal

⁴IS – Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal

⁵Department of Molecular Biology, ICBAS - Instituto de Ciências Biomédicas Abel Salazar, University of Porto, Porto, Portugal

Presenting author email: msolfasm.1312@gmail.com

Corresponding author email: rsilva@ff.up.pt, esousa@ff.up.pt

Abstract

β -Secretase (BACE-1) and glycogen synthase kinase 3 (GSK-3 β) play an essential role in the formation of β -amyloid (A β) peptides and in the hyperphosphorylation of tau protein, consequently impacting the development of Alzheimer's disease. Therefore, the design of a molecule capable of inhibiting both enzymes is an ascending strategy to find an efficacious treatment [1,2]. Since P-glycoprotein (P-gp) is associated with A β clearance, the activation of this efflux pump is another thinkable strategy [3]. Herein, we report the synthesis of potential dual BACE-1/ GSK-3 β inhibitors and the *in vitro* evaluation of their putative neuroprotective properties, as well as their direct effect on P-gp efflux activity.

A library of ten compounds was obtained by synthesis from the coupling reaction of tricyclic compounds with aliphatic amines with yields between 18 % and 95 %, following the reduction to respective amines. To evaluate their neuroprotective properties, differentiated SH-SY5Y cells were firstly incubated with the synthesized compounds at the non-cytotoxic concentrations of 10 μ M and 25 μ M and, after one hour, submitted to the chemical-induced neurotoxicity using MPP⁺ and iron (II) as neurotoxic agents. Neuroprotection studies revealed two compounds with a slight neuroprotective effect against MPP⁺-induced cytotoxicity, with compounds with halogens in their structures demonstrating potential to protect against iron (II)-induced cytotoxicity. Regarding the effect on P-gp activity, several compounds demonstrated potential for P-gp activation, while none of the tested compounds was identified as a P-gp inhibitor. Hereupon, A β peptide clearance through P-gp is not compromised and can be possibly enhanced using the P-gp activators.

In the future, the neuroprotective properties of the compounds against A β peptide-induced cytotoxic and their effects in the target enzymes will also be evaluated, expecting to discover new



multitarget molecules able to impact the progression of Alzheimer's disease.

Keywords - Alzheimer's disease; dual BACE-1/GSK-3 β inhibitors; tricyclic compounds; differentiated SH-SY5Y cells; neuroprotection studies; P-glycoprotein

Acknowledgments - This research was supported by national funds through FCT—Foundation for Science and Technology within the scope of UIDB/04423/2020, UIDP/04423/2020 (Group of Natural Products and Medicinal Chemistry-CIIMAR), and under the project PTDC/SAU-PUB/28736/2017 (reference POCI-01-0145-FEDER-028736), co-financed by COMPETE 2020, Portugal 2020, and the European Union through the ERDF and by FCT through national funds. Miguel Maia acknowledges his FCT grant (SFRH/BD/146211/2019). Márcia Martins acknowledges her BYT $plus$ scholarship to CIIMAR.

References

- [1] M. Martins, R. Silva, M. M. M. Pinto, E. Sousa, *Pharmaceuticals (Basel)* **2020**, 13 (9).
- [2] M. Maia, E. Sousa, *Pharmaceuticals (Basel)* **2019**, 12 (1), 41.
- [3] E. Gil-Martins, D. J. Barbosa, V. Silva, F. Remião, R. Silva, *Pharmacology & Therapeutics* **2020**, 107554.



Actinobacteria from seaweeds: unveiling their diversity and bioactive potential

Mariana Girão^{1,2}; Isabel C. Azevedo¹; Ralph Urbatzka¹; Pedro N. Leão¹; Maria F. Carvalho¹;

¹Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Porto, Portugal

²Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Portugal

Presenting author email: mariana.martins@ciimar.up.pt

Corresponding author email: mariana.martins@ciimar.up.pt

Abstract

Mining microbial secondary metabolism from untapped marine sources may represent a valuable route to the discovery of chemical novelty. Actinobacteria, superb producers of bioactive molecules, often live in association with marine organisms, as seaweeds, and such promising – yet poorly explored – ecological niche can uncover valuable clinically-relevant compounds, useful to help facing urgent problems as infections caused by antibiotic multi-resistant bacteria or cancer pathologies. In this work, actinobacteria isolated from Chlorophyta, Rhodophyta and Phaeophyceae seaweeds, collected in rocky shores in northern Portugal, are being screened for the production of compounds with antimicrobial and/or anticancer properties. Using selective pre-treatments and culture media, more than 450 seaweed-associated actinobacterial strains were isolated up to the moment. According to 16S rRNA analysis, isolates recovered so far are mainly affiliated with the genus *Streptomyces*, but 26 other genera – including some rare ones - were also retrieved. Antifungal activity against *Candida albicans* and antibacterial activity against *Bacillus subtilis* and/or *Staphylococcus aureus* were detected for some isolates, with minimum inhibitory concentration (MIC) values ranging from 0.487 to 1000 µg mL⁻¹. The cytotoxic activity of the actinobacterial crude extracts is being tested on three human cancer cell lines (breast carcinoma T-47D, colorectal carcinoma HCT116 and neuroblastoma SH-SY5Y) and in a non-carcinogenic cell line (brain microvascular endothelial hCMEC/D3), with some strains compromising only the viability of cancer cells, translating into a possible anticancer asset. Although dereplication data revealed the presence of several well-established antibiotics in most of the active extracts, it also unveiled the likely presence of unknown bioactive compounds that are being isolated following a bioactivity-guided pipeline. Results obtained so far uncovered not only the rich seaweed-associated actinobacteria diversity, but also their potential as a promising source of novel bioactive metabolites, highlighting their importance and biotechnological value.

Keywords - Marine actinobacteria; antimicrobial; anticancer; seaweed bioactive metabolites

Acknowledgements - M. Girão would like to thank a Ph.D. grant from Foundation for Science and Technology (FCT) - SFRH/BD/145646/2019.



Isolation and identification of Actinobacteria from marine sponges from Southern Mozambique and Northern Portugal

Mariana Oliveira^{1,2}, Ilário Lucas Timba³, Isidro Tamele^{1,2,4}, Celso Domingos¹, Joana Xavier^{1,5}, Ralph Urbatzka¹, Maria de Fátima Carvalho^{1,2}

¹CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Porto, Portugal.

²ICBAS - Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, Portugal.

³EBMI-UEM-Estação de Biologia Marítima da Inhaca, Eduardo Mondlane University, Maputo. Mozambique

⁴UEM – Faculty of Sciences, Eduardo Mondlane University, Maputo. Mozambique.

⁵UiB - University of Bergen, Bergen, Norway.

Presenting author email: mariana.oliveira@ciimar.up.pt

Corresponding author email: mariana.oliveira@ciimar.up.pt

Abstract

Marine sponges are generally characterized by a dense and diverse microbiome, which is frequently rich in actinobacteria. Different species of these microorganisms have been shown to produce a diverse range of promising bioactive secondary metabolites that can be valuable for drug development and for reducing the overall disease burden. Most of the clinically relevant antibiotics currently approved had their origin in actinobacteria metabolites, but these bacteria can also produce compounds exhibiting anti-malarial, antitumor, cytotoxic, anticancer, anti-inflammatory, anti-parasitic and immune suppressive activities.

The aim of this work is to bioprospect sponge-associated actinobacteria from unexplored regions for the discovery of novel natural compounds with pharmaceutical applications.

Nine different marine sponges collected on the coast of Southern Mozambique (n=6) and Northern Portugal (n=3) were used in this study. A workflow for the selective isolation of actinobacteria from these sponges was applied, consisting in the use of five different culture media (M2, SCN, NPS, EEA and ISP2) and two plating techniques (direct plating and dilution to extinction). A sample of each sponge was homogenized and, in most cases, pretreated for 20 min at 60 °C. After dilution, samples were either directly plated on different selective agar media or inoculated in 96-well plates containing selective liquid media and were incubated at room temperature for up to six months. Several bacterial strains have been recovered from these samples and isolation and phylogenetic identification are undergoing. The actinobacteria isolated from the analyzed sponges will be in the future bioprospected for the production of novel bioactive compounds.

Keywords - Secondary metabolites; natural products; bioactive compounds; bioprospecting; microbiomes



Phytoremediation of soils amended with metal contaminated digestate – plants and microorganisms roles

Neus Bonet^{1,2}, Verónica Baldasso^{1,2}, Sofia Dias², Carlos R. Gomes^{1,2}, Ana Paula Mucha^{2,3}, C. Marisa R. Almeida^{1,2}

¹Chemistry and Biochemistry Department, FCUP – Faculty of Sciences, University of Porto, Porto, Portugal

²EcoBioTec-- Bioremediation and Ecosystems Functioning – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal

³Biology Department, FCUP – Faculty of Sciences, University of Porto, Porto, Portugal

Presenting author email: neusbonetgarcia@fc.up.pt

Corresponding author email: neusbonetgarcia@fc.up.pt

Abstract

Currently, Anaerobic Digestion (AD) is emerging as the preferred treatment for the organic fraction of municipal solid waste (MSW). The number of anaerobic digesters is increasing worldwide and a huge amount of digestate is being produced which, if inappropriately managed, can lead to a serious environmental impact. The current management practice involves utilization of digestate for land application as soil improver. However, digestates are not entirely harmless. They can contain trace chemicals/ elements (TCs/ TEs), namely trace metals (TMs), which can be transferred to the soil ecosystem. Phytoremediation can be used not only to remove these TMs from contaminated soil but also for the recovery of metals through plant biomass that can be reintroduced into the biodigester, helping close the material loop. The use of non-food energy crops avoids direct competition with food agriculture, further increasing the sustainability of the system.

The aim of the current work is to evaluate the role of plants and microorganisms for metal-phytoremediation of soil amended with metal contaminated digestate.

Two non-food bioenergy crops (*Panicum virgatum* and *Pennisetum alopecuroides*) were evaluated. For that, 60 x 20 cm vertical soil column pot experiments were performed to (i) assessing alterations in soil properties treated with MSW digestate; (ii) determining metals bioavailability (through fractionation) and microbial community changes at different soil depths; (iii) studying plants growth performance and metals uptake capacity. The results of this ongoing work will be presented.

Overall, the results will help develop strategies for digestate safe disposal and valorisation while creating opportunities for metals recovery and reuse in the context of circular bioeconomy. Specifically, it will provide insights into TMs migration within soil profiles after digestate application, which is useful for predicting the potential risk of metal leaching, and it will help unravelling plant-microbe-metal interactions questions.



BLUE THINK CONFERENCE

Share Science, Spread Knowledge

Keywords - Anaerobic digestion; digestate; trace metals; phytoextraction; microbial communities; bioenergy crop plants

Acknowledgments - This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 861088.



Constructed wetlands for metal, antibiotic and AMR removal from anaerobic digestion digestate.

Pau Porras Socias^{1,2}, Carlos R. Gomes^{1,2}, C. Marisa R. Almeida^{1,2}, Ana Paula Mucha^{1,3}

¹EcoBioTec - Bioremediation and Ecosystems Functioning, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal.

²Chemistry and Biochemistry Department, Faculty of Sciences, University of Porto, Porto, Portugal.

³Biology Department, Faculty of Sciences, University of Porto, Porto, Portugal.

Presenting author email: pauporras@fc.up.pt

Corresponding author email: pauporras@fc.up.pt

Abstract

Antimicrobial resistance (AMR) is a global phenomenon that is causing a public health crisis. Anaerobic digestates can be a reservoir of antibiotic resistance genes that need effective treatment strategies to avoid AMR dissemination to the environment.

The objective of this study is to evaluate the potential of constructed wetlands (CWs), at microcosm scale, for the removal of metals and other pollutants, namely antibiotics and AMR, from the digestate liquid fraction, while studying also the effects of metals and antibiotics on the development of AMR within CWs systems.

Twelve laboratory scale vertical subsurface flow CWs systems were assembled in 0.4 m x 0.3 m x 0.3 m containers with gravel, light expanded clay aggregate and sand and with *Typha latifolia* testing four different experimental conditions (liquid digestate doped with sulfonamides, copper, both pollutants or without doping). After recirculating the digestate in the systems during a whole week, the effluent will be removed and replaced with new digestate with the same conditions.

Samples of CWs influent and effluent will be collected, removal rates of organic matter, nutrients, metals and antibiotics determined, and microbial communities characterized. The results of this ongoing work will be presented. It is expected that taxonomic analysis will reveal how the wetland rhizosphere responds to the digestate, to metals and to antibiotics. Moreover, a metagenomic approach will give further understanding about the influence of metals and antibiotics (alone or in a mixture) on pollutants removal and AMR dynamics.

Overall, CWs are a sustainable alternative to treat the liquid effluent of anaerobic digestors and its valuable products can be reused in agriculture or bioenergy production, closing the loop of circular metal bioeconomy.

Keywords - Constructed wetlands; liquid digestate; metals; sulfonamides; antimicrobial resistance; microbial community

Acknowledgments - This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 861088.



The anti-obesity potential of microalgae *Chlorella vulgaris* and its ability to fight associated comorbidities.

Rita Simões^{1,2}, Ana Regueiras^{1,2}, Gabriela Matos³, Jorge Saraiva³, Ana Margarida Costa⁴, Joana Laranjeira da Silva⁴, Vitor Vasconcelos^{1,2}, Ralph Urbatzka¹

¹BBE, CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal

²FCUP - Faculty of Science, University of Porto, Porto, Portugal

³UA - University of Aveiro, Aveiro, Portugal

⁴Allmicroalgae, Pataias, Portugal

Presenting author email: rita.loewe@gmail.com

Abstract

The prevalence of obesity in society has been dramatically rising and established as a global epidemic since the end of the 20th century. Patients who suffer from this metabolic disease have an increased risk of developing associated comorbidities, which are among the leading causes of death worldwide. Drug therapies approved for obesity have low weight loss efficacy, many side effects and the frequently obesity relapse. Thus, it is imperative to find new compounds to fight it. The aim of the work was to evaluate the potential of the microalgae *Chlorella vulgaris* regarding their beneficial effects on human metabolic diseases. Extracts were obtained by High Pressure Processing (HPP), under various combinations of time and pressure using different solvents, and by ohmic treatment, under distinct combination of frequency and time. The different extraction conditions lead to a unique set of metabolites, which in turn lead to distinctive bioactivities. Therefore, bioactivities of each extracts were tested in different models: the zebrafish Nile red fluorescence fat metabolism *in vivo* assay, anti-steatosis *in vitro* assay with HepG2 human hepatocyte carcinoma cell line, anti-inflammation *in vitro* assay with RAW264.7 mouse monocyte macrophage cell line, and anti-diabetes *in vivo* assay in zebrafish monitoring uptake of a fluorescent glucose analogue. Toxicity was evaluated by cytotoxicity in cells and lethality or malformations in zebrafish larvae. CSC11, CSC20 and CSC23, acetone and ethanol 96% extracts obtained by HPP without applied pressure, had the most promising combination of bioactivities. Mass spectrometry (LC-MS/MS) was applied to characterize the metabolic profiles of active and non-active extracts, aiming to identify the responsible molecules for bioactivity. Complementary studies were also carried out on target genes and proteins to determine the molecular mechanisms of action. The results suggested that some of the extracts could be used as potential therapeutic agent against obesity and its comorbidities.



BLUE THINK CONFERENCE

Share Science, Spread Knowledge

Keywords - Anti-obesity; *Chlorella vulgaris*; anti-diabetes; High pressure processing; anti-inflammatory; zebrafish

Acknowledgements - This work was supported by the AlgaValor project (POCI-01-0247-FEDER-035234), financed by the European Regional Development Fund (ERDF) through COMPETE2020-Operational Program for Competitiveness and Internationalisation (POCI) and PORTUGAL2020, and national funds through FCT UIDB/04423/2020 and UIDP/04423/2020.



Molecular screening of cyanotoxins from cyanobacteria isolated along Moroccan Atlantic Coast

Raquel Silva¹, João Morais^{1,3}, Flavio Luis de Oliveira¹, Brahim Sabour², Vitor Vasconcelos^{1,3}

¹Blue Biotechnology and Ecotoxicology, CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Porto, Portugal

²Biology, UCD – Faculty of Science, Université Chouaib Doukkali, El Jadida, Morocco

³Biology, FCUP – Faculty of Science, University of Porto, Porto, Portugal

Presenting author email: araqssilva@gmail.com

Abstract

Cyanobacteria are microorganisms with a long evolutionary history and a remarkable adaptability that can be found both in aquatic and terrestrial ecosystems, including extreme environments (e.g. freshwater, marine, terrestrial, hot springs, deserts, etc.). This group of organisms are considered a rich source of secondary metabolites with potential biotechnological applications. Also, they have the capability to produce some potent toxins (cyanotoxins) that can induce consequences to environment and human health. Cyanotoxins can be found in freshwater and marine ecosystems and are widely distributed in almost every country and different regions of the planet. They can be classified into five functional groups according to their primary target organ or effects being designated as hepatotoxins, neurotoxins, cytotoxins, dermatotoxins and irritant toxins. Biosynthetic pathway of these toxins are a matter of great interest due to their varied bioactivities like antitumor, antiviral, antibacterial, antimalarial, immunosuppressive agents and multi-drug resistance reversers. In this work, the presence of genes involved in the biosynthesis of cyanotoxins (microcystin, saxitoxin, cylindrospermopsin, and anatoxin) will be screened in more than 150 strains of cyanobacteria previously isolated from different samples obtained along Moroccan Atlantic coast (several sampling sites from El Jadida to Essaouira). There is lack of studies regarding the presence of cyanotoxins in cyanobacteria in this region and some of the isolated strains belong to genera (e.g. *Phormidium* sp., *Pseudanabaena* sp., *Leptolyngbya* sp., *Lyngbya* sp., *Geitlerinema* sp., etc.) that have the potential to produce cyanotoxins.

Keywords - Cyanobacteria; toxins; biotechnological applications



A bioguided approach on the discovery of nucleosides with antifouling properties

Sandra Pereira^{1,2}, Leonor Ferreira¹, Vitor Vasconcelos^{1,2}, Mariana Reis¹ and Joana R. Almeida¹

¹Blue Biotechnology and Ecotoxicology Group, CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, Matosinhos, Portugal

²Department of Biology, FCUP – Science Faculty, University of Porto, Porto, Portugal

Presenting author email: sandra.pereira@ciimar.up.pt

Corresponding author email: jalmeida@ciimar.up.pt

Abstract

Prevention of marine biofouling continues to cause worldwide problems, due to the antifouling biocidal agents currently in use. Natural products have the potential to provide solutions for antifouling compounds that are effective and ecologically compatible. The diversity of secondary metabolites produced by cyanobacteria, make these organisms a promising source of bioactive compounds, especially when antifouling activity has been already documented. The aim of this study was to explore the metabolic diversity of a range of cyanobacterial strains from LEGE Culture Collection in search for environmentally friendly bioactive compounds for antifouling purposes. A library of fractions, derived from methanolic extracts, belonging to different cyanobacterial strains were tested towards a prominent macrofouling organism (*Mytilus galloprovincialis* larvae). Promising fractions were submitted to a bioassay guided sub-fractioning that led to the isolation of two compounds. Their structure elucidation was determined by spectroscopic techniques such as 1D and 2D nuclear magnetic resonance and by mass spectrometry. Analysis of the data indicated the compounds to belong to the nucleoside class. Their antifouling bioactivity will be further assessed on other levels of biological organization such as microfouling organisms (marine bacteria and microalgal biofilms), as well as their potential environmental toxicity to the marine environment.

Keywords - Natural products; Cyanobacteria; Biofouling; Antifouling

Acknowledgments - This research was supported by national funds through FCT – Foundation for Science and Technology within the scope of UIDB/04423/2020 and UIDP/04423/2020, under the projects NASCEM PTDC/BTA-BTA/31422/2017 (POCI-01-0145-FEDER-031422) financed by FCT, COMPETE2020 and PORTUGAL2020, and CYANCAN (PTDC/MED-QUI/30944/2017), co-financed by NORTE 2020, Portugal 2020, and the European Union through the ERDF, and by FCT. This work was also funded by the structured program of R&D&I ATLANTIDA - Platform for the monitoring of the North Atlantic Ocean and tools for the sustainable exploitation of the marine resources (reference NORTE-01-0145-FEDER-000040), supported by the North Portugal Regional Operational Program (NORTE2020), through the European Regional Development Fund (ERDF). SP is supported by the FTC grant SFRH/BD/145380/2019.



Applying the circular economy concept to wastewater management by using constructed wetlands as nature-based solutions: a case study at the Macao hospitality industry

Sara Justino^{1,2}, Cristina S. C. Calheiros¹, Paula Lima Castro³, David Gonçalves²

¹Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Portugal

²Institute of Science and Environment (ISE), University of Saint Joseph, Macao SAR

³CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Portugal

Presenting author email: sara.justino@usj.edu.mo

Corresponding author email: sara.justino@usj.edu.mo

Abstract

The purpose of the current study is to apply the circular economy concept to wastewater management by designing a nature-based solution that can shift the wastewater treatment paradigm from central to decentralized solutions in highly urbanized areas, such as Macao city.

The need of this study is related to urbanization pressure, increasing water demands, and the lack of new central wastewater treatment solutions in highly urbanized areas. More specifically, how the hospitality industry in Macao, partially responsible not only for the depletion of freshwater resources, but also for an increase in environmental pollution, can help to change this paradigm.

The circular economy approach can provide new pathways towards a more sustainable water management for this industry. Unfortunately, in the case of wastewater reuse, its potential has not been untapped outside water scares regions, with research and efforts put to water conservation, but not into a circular economy perspective.

Taking in consideration the above, by designing an aerated vertical flow constructed wetland with microbial fuel cells coupled to treat wastewater to recycled water, we can not only reduce the wastewater load at Macao, but also create a unique solution with high treatment efficiency and low area footprint, a necessity for historical highly dense urban areas. In addition, our solution will help to further extend the knowledge about emerging pollutants removal by constructed wetlands, while expanding the information about wastewater reuse risks without proper legislation plus maintenance guidelines for long term operation of nature-based solutions.

Keywords - Nature-based solutions; urban; water management; circular cities; climate change.



Trace metals dynamics after digestate amendment on soil columns – first results

Sofia Correia^{1,2}, Inês Ribeiro^{2,3}, Andreia Braga-Henriques^{4,5}, Ralph Urbatzka², Maria F. Carvalho^{2,3}

¹FCUP- Faculty of Sciences of the University of Porto, Porto, Portugal

²Ecobiotec Group, CIIMAR- Interdisciplinary Centre of Marine and Environmental Research, Matosinhos, Portugal

³ICBAS - Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, Portugal

⁴MARE - Marine and Environmental Sciences Centre & OOM - Oceanic Observatory of Madeira, Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação (ARDITI), Piso 2, Caminho da Penteada, 9020-105 Funchal, Madeira, Portugal

⁵Regional Directorate for Fisheries, Regional Secretariat for the Sea and Fisheries, Government of the Azores, Rua Cônsul Dabney - Colónia Alemã, 9900-014 Horta, Azores, Portugal

Presenting author email: sofia_fariacorreia@hotmail.com

Corresponding author email: sofia_fariacorreia@hotmail.com

Abstract

Actinobacteria are Gram-positive bacteria typically containing a genome rich in guanine and cytosine. These microorganisms are capable of producing secondary metabolites with a great structural diversity and are known to inhabit very diverse environments, including the deep sea.

Due to the fact that Actinobacteria contain a high number of biosynthetic genes that may not be expressed under common cultures conditions, the strategy known as “one strain many compounds” (OSMAC) has emerged as an important approach to enhance the chemical diversity of actinobacterial metabolites. This strategy enables the activation of silent gene clusters for natural product biosynthesis, by varying culture conditions.

In this study, the OSMAC approach was applied to 10 actinobacterial strains isolated from deep-sea samples collected at Madeira and Azores islands (NE Atlantic), in order to find novel bioactive compounds with pharmaceutical relevance.

Six different conditions were applied to each isolate: i) heat shock; ii) addition of *E. coli* lysate to growth medium; iii) growth in the culture medium glucose- yeast autolysate- peptonized milk (GPA); iv) growth in the culture medium glucose-yeast extract-malt extract (GYM); v) addition of N-acetylglucosamine to growth medium and vi) addition of *E. coli* culture supernatant to growth medium. Crude extracts resulting from the different culture conditions were screened for antimicrobial and anticancer activity and compared to a control grown under normal conditions.



The antimicrobial assays allowed the identification of 10 actinobacterial extracts with ability to inhibit the growth of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhimurium*. The MTT assay showed eight extracts able to reduce the viability of the breast cancer cell line.

Future steps will include screening of anti-inflammatory and anti-obesity activities and investigating if novel secondary metabolites are responsible for the observed bioactivities.

Keywords - Actinobacteria; OSMAC; deep-sea; secondary metabolites; antimicrobial; anticancer

Acknowledgements - I. Ribeiro would like to thank a PhD grant from Foundation for Science and Technology (FCT) - SFRH/BD/136357/2018. AB-H was partly supported by the Oceanic Observatory of Madeira Project (M1420-01-0145-FEDER-000001-Observatório Oceânico da Madeira-OOM). This work was financially supported by the project ACTINODEEPSEA (POCI-01-0145-FEDER-031045) co-financed by COMPETE 2020, Portugal 2020 and the European Union through the European Regional Development Fund (ERDF) and by FCT, Portugal, through national funds. This research was also supported by national funds through FCT within the scope of UIDB/04423/2020 and UIDP/04423/2020.



Characterization of natural products in cone snails from the Cabo Verde archipelago

Sónia Ribeiro^{1,2}, Ralph Urbatzka², Vitor Vasconcelos^{1,2}, Jorge Neves²

¹Department of Biology, FCUP, Faculty of Science, University of Porto, Porto, Portugal

²CIIMAR - Interdisciplinary Center of Marine and Environmental Research, University of Porto, Matosinhos, Portugal

Presenting author email: up201604362@fc.up.pt

Corresponding author email: jorge.neves@ciimar.up.pt

Abstract

The genus *Conus*, also known as cone snails, form one of the largest known genera of venomous marine animals. These tropical marine gastropods belong to the family Conidae and the superfamily Conoidea (=Toxoglossa). They can be found in various locations worldwide, with a more significant presence in tropical and subtropical waters such as Indo-Pacific, Western Pacific, and Macaronesian regions, like Cabo Verde. These marine invertebrates can be classified into three groups: (1) molluscivorous if they prey on other mollusks; (2) vermivorous, when they prey on worms like polychaete; (3) piscivores, when they prey on fishes. As poor sight animals, cone snails developed several strategies, such as potent venoms (chemical defenses) containing conopeptides (conotoxins) used as defense and predation strategies. Here, we study the venoms of two vermivorous cone snails endemic from Cabo Verde archipelago (*Conus venulatus* and *Conus ateralbus*). Conotoxins diversity and venoms profile were evaluated by RP-HPLC, and fractions tested for different bioactivities. Zebrafish Nile red fat metabolism (obesity) was performed, and glucose uptake (diabetes) and reduction of lipid content in fatty acid-overloaded liver cells (steatosis) assay will soon be carry out. LC-MS method will be used to qualitative and quantitative characterize conotoxins found in the venoms. Molecular masses obtained with this method will be compared with the already described for conotoxins present in the ConoServer database. RP-HPLC profiles suggest that *Conus ateralbus* has more conotoxins/peptides in its venom than *Conus venulatus*. Anti-obesity assays showed that *Conus ateralbus* fractions could lead to a significant lipid reduction (~50%) by fraction C.ate R2.6. On the other hand, *Conus venulatus* showed no statistical differences from controls. In summary, the present study revealed that *Conus ateralbus* might produce conotoxins able to modulate lipid metabolism, and the work is now focus on the identification of peptides in the bioactive fraction C.ate R2.6, as well as the screening for other bioactivities.

Keywords - *Conus ateralbus*; *Conus venulatus*; conotoxins; vermivorous; metabolic diseases



Trace metals dynamics after digestate amendment on soil columns – first results

Veronica Baldasso^{1,2}, Neus Bonet^{1,2}, Carlos R. Gomes^{1,2}, Ana P. Mucha^{1,3}, C. Marisa R. Almeida^{1,2}

¹CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal;

²Chemistry and Biochemistry Department, Faculty of Sciences, University of Porto, Portugal

³Biology Department, Faculty of Sciences, University of Porto, Portugal

Presenting author email: veronica.baldasso@fc.up.pt

Corresponding author email: veronica.baldasso@fc.up.pt

Abstract

Digestate is the end product of anaerobic digestion. It is an organic matter - nutrient rich matrix that can be utilized as soil amender depending on waste origin and compliance to national legislations. Most of the produced digestate does not meet the quality standards of legislations and is treated as waste discarded in landfills. Within a circular economy approach, this project aims at considering digestate as a resource for marginal soil restoration after appropriate pollutant removal. This present work focuses on the dynamics of trace metals (TM) in the soil profile after digestate application, analyzing the potential interactions between TM and recalcitrant microorganic pollutants and the effects of contaminants on soil microbial communities. Short-term (28 day, starting 19/04/2021) soil mesocosm experiments were set up in columns (diameter 19 cm; height 60 cm) filled with 11 L of non-contaminated nutrient poor soil collected in Porto city district (NW Portugal) and a top layer with 4 L of digestate mixture (1:1 (v/v) digestate: soil). Digestate was collected from an anaerobic digestion plant (Mafra, Portugal). Two experimental conditions were tested: (E1) soil amended with digestate, and (E2) soil amended with digestate doped with metformin (antidiabetic drug). For each experimental condition duplicates were assembled. From each column soil samples were taken at 5 depths at specific time periods. All soil samples were analyzed for total metal concentration by atomic absorption spectroscopy after acid digestion, metal bioavailability via BCR sequential extraction method¹ and organic contaminants by liquid-chromatography coupled to mass spectrometry after solid-phase extraction. Characterization of soil microbial communities was assessed by next generation sequencing. The results of this ongoing work will be presented at the Blue Think Conference, showing that circular economy waste management strategies can be expanded allowing metal recovery while improving soil health and decreasing society's waste footprint.

Keywords - Trace metals; metal interactions; metal bioavailability; Municipal Solid Waste digestate; soil restoration



BLUE THINK CONFERENCE

Share Science, Spread Knowledge

Acknowledgments - This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 861088.

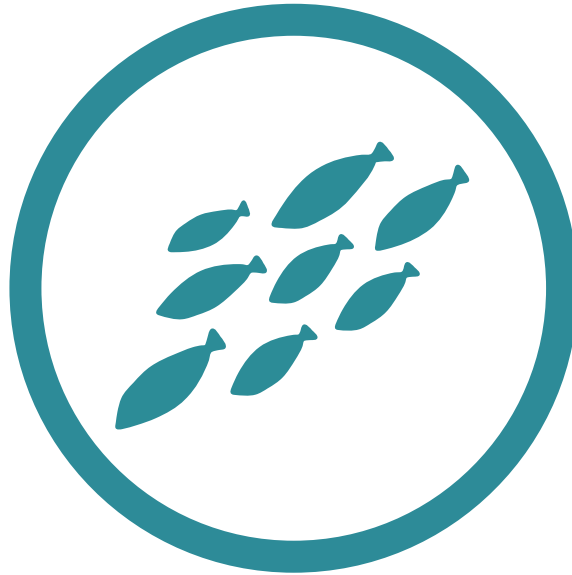
References

¹Ruaret et al., 2000. J. Environ. Monit. 2, 228–233.



BLUE THINK CONFERENCE

Share Science, Spread Knowledge



SESSION 3: AQUATIC RESOURCES AND AQUACULTURE

MODERATION: SABRINA RODRIGUES AND CRISTINA VELASCO

KEYNOTE SESSION – JORGE FERNANDES



Jorge M.O. Fernandes is a Professor in Genomics and Molecular Biology at Nord University, Norway. He has also held honorary professorships at the University of Messina (Italy) and the University of Tokyo (Japan). Prof. Fernandes obtained his BEng in Chemical Engineering with specialization in Biotechnology from Instituto Superior Técnico (Portugal), where he graduated with distinction in 1997.

His passion for Biology led him to pursue a doctoral degree in Comparative Immunology at the University of St Andrews. After his PhD graduation in 2003, he took a post-doc position until 2007, studying the molecular mechanisms that regulate muscle growth in fish. Prof. Fernandes has over 15 years' experience as an independent researcher and is a recognized expert in aquaculture genomics.



BLUE THINK CONFERENCE

Share Science, Spread Knowledge

His current research fields are muscle growth and innate immunity in teleosts, with focus on the epigenetic regulation (DNA methylation and miRNAs) of gene networks by environmental factors in commercially important species. Prof. Fernandes has an established track record of high-standard research, with 113 publications in international journals with peer review and an h-index of 35. He is a regular reviewer for various scientific journals and funding bodies and serves on the editorial board of PLoS One and Scientific Reports. He has won the Nord University research prize in 2014 and 2016. Prof. Fernandes has been awarded an Outstanding Young Investigator grant by the Research Council of Norway (2008-2013), an ERC Consolidator grant (EPIFISH, 2016-2021) on epigenetics of Nile tilapia domestication and an ERC Proof of Concept grant (EPIMARK, 2018-2020) to discover epigenetic markers of growth in fish.



BLUE THINK CONFERENCE

Share Science, Spread Knowledge



ABSTRACTS

ORAL COMMUNICATIONS



The network of prokaryotic interactions in a recirculating aquaculture unit of sole (*Solea senegalensis*)

Diana Bastos Almeida^{1,2,3}, Miguel Semedo², Catarina Magalhães^{2,4,5}, Isidro Blanquet³, Ana Paula Mucha^{2,4}

¹ICBAS – Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, Portugal

²CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Porto, Portugal

³SEA8 - Safiestela Sustainable Aquafarming Investments, Lda.

⁴FCUP – Faculty of Sciences, University of Porto, Porto, Portugal

⁵School of Science, University of Waikato, Hamilton, New Zealand

Presenting and corresponding author email: diana.almeida@ciimar.up.pt

Abstract

Recirculating aquaculture systems (RAS) allow water reuse by managing waste and nutrient recycling, consequently making intensive fish production compatible with environmental sustainability, and improving fish welfare. A key aspect of these systems is the beneficial bacterial community of the biofilter that improves water quality, for example through nitrification and/or pathogen resistance. Biotic relationships between microorganisms present in these communities may play a crucial role in driving their functional potential. However, these interactions are often neglected in engineered environments. The aim of this work is to detect prokaryotic interactions in RAS, through the definition of relevant taxa, and with cluster identification using co-variance and co-occurrence tools. Taxa were obtained by 16S rRNA gene sequencing using Illumina MiSeq® and the DADA2 pipeline with the SILVA database. The selection was based on the taxonomic attribution and includes potentially pathogenic bacteria, belonging to either the *Tenacibaculum* or *Vibrio* genus, and nitrifying bacteria, those belonging to the *Nitrosococcaceae*, *Nitrosococcaceae* or *Nitrospinaceae* family and the *Nitrososphaeria* class. Other taxa that have significant interactions with the groups selected were also included. Two independent systems of an aquaculture facility were studied (Pre-Ongrowing and Weaning). In the Pre-Ongrowing, nine subcommunities were identified in the correlation network, while 19 were found in the Weaning. Correlations between nitrifying taxa were found in both systems. Correlations between *Tenacibaculum* and *Vibrio* were found only in the Weaning system. Cases of individual nitrifying taxa were found, as well as *Nitrospira* taxa interacting with *Nitrosomonas*. Since *Nitrospira* are commonly associated with nitrite oxidation and *Nitrosomonas* with ammonia oxidation, the later result is to be expected. As for the first, this could be an indication of some taxa that perform the complete nitrification process. Future work will include an elucidation into the individualist/communitarian behavior of these groups, links with habitat (biofilm or water) and phylogeny.

Keywords – Network; correlation; microbiome; Recirculating Aquaculture Systems



BLUE THINK CONFERENCE

Share Science, Spread Knowledge

Acknowledgments – The authors acknowledge Fundação para a Ciência e Tecnologia (FCT) for the PhD fellowship ref. PD/BDE/135542/2018 and Safiestela Sustainable Aquafarming Investments, S. A. (SEA8). This research was supported by the project 39948_FeedMi, supported by Portugal and the European Union through FEDER/ERDF, CRESC Algarve 2020 and NORTE 2020, in the framework of Portugal 2020; and by national funds through FCT—Foundation for Science and Technology within the scope of UIDB/04423/2020 and UIDP/04423/2020.



Identification of antimicrobial peptides in oysters selected for bacterial and virus disease resistance.

Gabriella Vasconcelos Vilella¹, Ana García^{2,3}, André Cunha², Benjamin Costas^{2,3}, Lionel Dégrémont⁴, Sergio Fernández-Boo¹

¹FCUP – Sciences Faculty of Porto University, Porto, Portugal

²CIIMAR – University of Porto, Matosinhos, Portugal.

³ICBAS – University of Porto. Portugal

⁴IFREMER – La Tremblade Station. France

Presenting author email: gabivilella@yahoo.com.br

Corresponding author email: sboo@ciimar.up.pt

Abstract

The Pacific oyster is the main oyster's species cultivated worldwide and, in addition to that, in Portugal exists an increasing industry of oyster production. For example, France, the largest producer of *Crassostrea gigas* has ranged from 100.000 to 150.000 tons for several decades. Unfortunately, it begun to decrease due to degradation of the environment by anthropogenic actions and infectious disease outbreaks, more specifically by two pathogens – OsHV-1 μ Var virus and the bacteria *Vibrio aestuarianus*. The mortality excess has been reported for many years and affected all the oyster farming basins with a high mortality of their stocks. As the resources to minimize the effects of a parasite introduction on oyster populations are very limited, breeding programs become a great perspective to increase oyster's resistance.

This study aimed to evaluate the differences in resistance of different selected *C. gigas* oyster families against infection by *V. aestuarianus* and also evaluate their molecular signature to try to unveil the characteristics that confers this resistance.

A breeding selection program was performed by selecting families with different susceptibility to OsHV-1 and *V. aestuarianus* at IFREMER facilities (France). Six of those families were subjected to a *V. aestuarianus* infection trial and sampled at 24, 48 and 72 hours period to evaluate their haemolymph, mucus and tissue gene expression and immune performance. Mortality was followed during 30 days after infection and three families presented a mortality around 29 to 36% while others reach up to 84%.

A gene expression analysis of the main antimicrobial peptides and immune related genes was done in resistant and susceptible families showing great differences in gene expression between them that could explain the different resistance to infection. Two particular families called OVA46 and OVA56 have had the lowest mortality, being the most promissory families for intensive aquaculture production.

Keywords – Oyster; pathogens; breeding program; gene expression; aquaculture



Effects of dietary histidine and plant lipids on the Atlantic salmon (*Salmo salar*) health status following transfer to seawater

Inês Carvalho^{1,2}, Sofie Remø³, Diogo Peixoto^{1,2}, Nini H. Sissener³, Per Gunnar Fjellidal⁴, Rune Waagbø³, Benjamín Costas^{1,2}

¹Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Universidade do Porto, Avenida General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal.

²Instituto de Ciências Biomédicas Abel Salazar (ICBAS-UP), Universidade do Porto, Rua de Jorge Viterbo Ferreira nº 228, 4050-313 Porto, Portugal.

³Institute of Marine Research (IMR), Boks 1870 Nordnes, 5817 Bergen, Norway.

⁴Matre Research Station, Institute of Marine Research (IMR), N-5984 Matredal, Norway.

Presenting author email: mariainesrcarvalho97@gmail.com

Corresponding author email: mariainesrcarvalho97@gmail.com

Abstract

With the aquaculture expansion, there is great urgency in reducing the industry's dependence on finite-marine ingredients, such as fish oil. However, plant lipid-based diets might markedly deviate from fish natural feeding habits and negatively impact their health. In the particular case of Atlantic salmon, this issue acquires major relevance during the seawater transfer (SWT), a challenging production moment often associated with great losses. To improve the overall fish health, supplementing diets with immuno-modulators, such as certain amino acids, is becoming a common approach. The present study aimed to investigate whether dietary lipid source and histidine (His) supplementation could influence the non-specific immune response and antioxidant system in Atlantic salmon after SWT.

The fish were given four experimental diets differing in lipid source (100% fish oil or blend of vegetable oils) and His content (10 or 14 mg His/g diet) in freshwater (FW), and a cross-over was done after SWT to investigate the effect of supplying His supplementation in FW vs SW. At the end of the FW phase and 2 and 4 weeks after SWT, fish were sampled for blood plasma and liver to assess immune parameters and oxidative stress biomarkers, respectively.

Regardless of dietary treatment, post-smolts appeared to be under oxidative stress two weeks after SWT. Even so, they seemed to manage to protect themselves from oxidative damage, since no increase in LPO levels were observed between FW and following SWT. Additionally, all groups appeared to recover well from the transfer. Nevertheless, post-smolts fed fish oil-based diets displayed higher plasma bactericidal and alternative complement activities than those fed vegetable oil feeds. Apart from a stimulating effect of His supplementation on plasma IgM levels four weeks after SWT, it did not seem to significantly influence the post-smolt Atlantic salmon immunity and oxidative status, independently of the dietary lipid source.

Keywords – Atlantic salmon; histidine; vegetable oils; immunomodulation; oxidative stress



Can seaweeds and microalgae, single or blended, modulate the gut microbiota of European seabass (*Dicentrarchus labrax*)?

Mariana Ferreira^{1,2}, Yousri Abdelhafiz³, Viswanath Kiron³, Luisa M.P. Valente^{1,2}

¹LANUCE; CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, University of Porto, Matosinhos, Portugal

²ICBAS – Instituto de Ciências Biomédicas Abel Salazar, University of Porto, Porto, Portugal

³Faculty of Biosciences and Aquaculture, Nord University, Bodø, Norway

Presenting author email: marianaipf@gmail.com

Corresponding author email: marianaipf@gmail.com

Abstract

Nutritional manipulation of the intestinal microbiota can have a positive impact on fish health. The seaweed *Gracilaria gracilis* and microalga *Nannochloropsis oceanica* have been recently explored by the feed industry, but the impact of such products on gut microbiota of European seabass is poorly understood. We evaluated the impact of *G. gracilis* and *N. oceanica*, single or blended, on the composition of European seabass intestinal microbial community.

European seabass were fed four diets: commercial-based diet (CTRL), and three experimental diets with the inclusion of 8 % *G. gracilis* (GRA), 8 % *N. oceanica* (NAN), and a blend of 4 % of each alga (NANGRA). After 15-weeks, posterior intestine mucus was collected; V3-V4 region of the 16S rRNA was amplified and sequenced using Illumina® MiSeq platform. After quality filtering, taxonomic assignment was performed using RDP classifier. R packages “iNEXT” and “phyloseq” were used to calculate alpha diversity. Packages “microbiome” and “DESeq2” were employed to determine relative abundance of core taxa and to identify OTUs that were differently abundant in the study groups.

Inclusion of *G. gracilis* and *N. oceanica* had a significant impact on alpha diversity: species richness was significantly lower in GRA fish compared to CTRL; while Shannon and the Simpson diversity were significantly reduced in fish fed both GRA and NAN diets. When algae were included in a blend (NANGRA), the alpha diversity measures remained similar to those of CTRL fish. Core microbial taxa were composed of genera *Flavobacterium*, *Parcubacteria*, and *Lactobacillus*. The most abundant phyla were Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes. Compared to CTRL fish, the inclusion of algae, single or blended, led to a decrease/increase of specific groups of bacteria and clarification on their function will allow for a better understanding of the impact of these algae on the gut microbiota of European seabass.

Keywords – Seaweeds; microalgae; gut microbiota; european seabass



BLUE THINK CONFERENCE

Share Science, Spread Knowledge

Acknowledgments – This work was funded by the structured program of R&D&I ATLANTIDA - Platform for the monitoring of the North Atlantic Ocean and tools for the sustainable exploitation of the marine resources (reference NORTE-01-0145-FEDER-000040), supported by the North Portugal Regional Operational Programme (NORTE2020), through the European Regional Development Fund (ERDF). M. Ferreira acknowledges Fundação para a Ciência e a Tecnologia (FCT) for grant SFRH/BD/144843/2019 (FCT/FSE).



BLUE THINK CONFERENCE

Share Science, Spread Knowledge



ABSTRACTS

FLASH COMMUNICATIONS



The effects of inducing swimming activity in the physiology and welfare of gilthead seabream (*Sparus aurata*)

Carlos Espírito Santo^{1,2}, Francisco Guardiola³, Rodrigo Ozório¹, Leonardo Magnoni¹

¹LANUCE, CIIMAR – Interdisciplinary Centre for Marine and Environmental Research, University of Porto, Matosinhos, Portugal.

²Faculty of Sciences, University of Porto, Porto, Portugal.

³Department of Cellular Biology and Histology, Faculty of Biology, University of Murcia, Murcia, Spain.

Presenting author email: carlos.esanto91@gmail.com

Corresponding author email: lmagnoni@ciimar.up.pt

Abstract

Swimming includes both physiological and behavioural components and is a fundamental activity contributing to fitness in several fish species. Establishing suitable swimming conditions that can lead to enhanced performance and to the improvement of the welfare of farmed fish (e.g., by reducing stress) is of outmost importance. Whether exercise can promote immunity is an area to be explored, with potential application in aquaculture. The aim of this study was to evaluate if different swimming conditions may modulate both metabolic and immune responses in gilthead seabream (*Sparus aurata*). Briefly, fish were subjected to 5 different treatments during 6 h (n=8-10): control (no induced swimming), continuous low intensity swimming at 0.8 body length per second (BLs⁻¹) (L), continuous high intensity swimming at 2.3 BLs⁻¹ (H), discontinuous low intensity swimming (DL, 0.8-1.6 BLs⁻¹) and discontinuous high intensity swimming (DH, 0.8-2.3 BLs⁻¹). After applying the different treatments, skin mucus and blood were collected and, after euthanized, liver, skeletal muscle and head-kidney were sampled. Haematological and metabolic parameters were analyzed in blood and plasma, as well as oxidative stress markers in liver and immune parameters in plasma and skin mucus. Preliminary results showed that the different swimming conditions applied did not have a significant effect in immune parameters in plasma and skin mucus of fish. On the other hand, several oxidative stress markers in the liver were modulated by the different swimming conditions. Total glutathione activity was significantly higher in H group compared to control and DH groups whilst the glutathione reductase (GR) showed lower activity in L, H and DH groups compared to control one. Furthermore, the analysis of the impact of different types of swimming in other relevant tissues will provide new insights on the benefits and drawbacks of inducing swimming in cultured gilthead seabream.

Keywords – Gilthead seabream (*Sparus aurata*); induced swimming; oxidative stress; immune system; metabolic enzymes



Search for biomarkers of resistance in *R. decussatus* to *P. olseni* parasite infection

João Estêvão¹, Hugo Osorio², Benjamin Costas¹, Andreia Cruz³, Sergio Fernández-Boo¹

¹Animal Health and Aquaculture (A₂S), CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, University of Porto, Porto, Portugal.

²I3S - Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal.

³Oceano Fresco S.A, Nazaré, Portugal.

Presenting author email: jestevao@ciimar.up.pt

Corresponding author email: jestevao@ciimar.up.pt

Abstract

The grooved carpet shell (*Ruditapes decussatus*) is a bivalve mollusc species with a distribution from NE-Atlantic coast and Mediterranean Sea. This species is highly desirable and sold at high prices being Portugal the top-seller (FAO).

A decline on the species happened since the 90's due to biotic and abiotic factors, such as parasite infection and environment degradation. Infection by *Perkinsus olseni* parasite is the main biotic factor that causes decrease of populations. It was first diagnosed in the 1980s, after introduction of the invasive species *Ruditapes philippinarum* from Asia for intensive production in Europe. It has been observed that *Perkinsus* causes changes in humoral and cellular responses and rise of mortality in infected individuals. Despite of lacking an adaptive immune system, this species has demonstrated to thrive in highly affected areas, reaching adult phase and surviving high parasite prevalence.

Thus, this study seeks to identify markers of resistance in populations of *R. decussatus* affected by *P. olseni* by looking at the hemolymph's proteome profile of tolerant individuals in comparison with susceptible ones. Five populations with high prevalence of the parasite across Europe namely, Pontevedra (Spain), Algarve (Portugal), Naples (Italy), Venice (Italy), and Turkey were considered. A *Perkinsus*-free population (Noia, Spain) was used as control.

A substantial number of expressed proteins was identified (771, Abundance Ratio>1.5), being 206 found in tolerant (t) individuals and 565 on susceptible (s) ones. Moreover, 395 exclusive proteins were identified, from which t=87 and s=308. Functions related to metabolism were most represented in susceptible than in tolerant individuals suggesting a higher production of energy to counteract the infection. Also, six proteins were differentially expressed in all tolerant individuals and seems to be possible markers of resistance to *P. olseni* infection.

These results points towards a host generated tolerance suggesting an existence of adaptation mechanisms to parasite.

Keywords – *R. decussatus*; *P. olseni*; host-parasite interaction; proteomics; innate immunity; adaptation



Can the inclusion of *Dunaliella salina* in cold extruded diets result in sea urchin gonads with high consumer acceptance?

Inês Garrido^{1,2}, Tiago Sá¹, Luís F. Baião^{1,2,3}, Helena M. Amaro¹, Tânia Tavares⁴, F. Xavier Malcata^{4,5}, Isabel Costa⁶, A. Catarina Guedes^{1,6}, Luísa M.P. Valente^{1,2}

¹CIIMAR/CIIMAR, Interdisciplinary Centre of Marine and Environment Research, University of Porto, Matosinhos, Portugal

²ICBAS, School of Medicine and Biomedical Sciences, University of Porto, Porto, Portugal

³Sense Test, Vila Nova de Gaia, Portugal

⁴LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Porto, Portugal

⁵FEUP – Faculty of Engineering of University of Porto, Porto, Portugal

⁶ISS, Ínclita Seaweed Solutions, CIIMAR, Matosinhos, Portugal

Presenting author email: garridoines44@gmail.com

Corresponding author email: lvalente@icbas.up.pt

Abstract

Sea urchin gonads have been increasingly demanded and marketed in Europe due to its organoleptic features. Studies have shown echinoculture resulting in high gonad yields by using formulated extruded diets for *Paracentrotus lividus*, however producing gonads with a pale-yellow colour, whilst Mediterranean consumers seek a bright orange/orange-reddish colour. Five isonitrogenous and isoenergetic diets were formulated by inclusion of *Dunaliella salina* of different levels (D1 and D2), *D. salina* of different levels and *Porphyra* (D1P and D2P) and glycine (GLY) to a CTRL diet. All diets were cold extruded, softly dried and distributed every 48h, during 8 weeks, to quadruplicates of *P. lividus* (initial body weight 52.32 ± 13.23 g). At the end of the trial all sea urchins were individually weighted and measured and gonads of 8 animals per tank were sampled for further analysis. All tested diets were able to enhance gonad yield (from 6.4 to 15.8% and from 8.9 to 17.0%, for males and females, respectively), with females having larger gonads than males, but without differences between diets. Diets significantly affected all free amino acids in gonads with urchins fed the experimental diets presenting higher concentrations of arginine, valine, methionine and glycine than the ones fed with the CTRL diet. Differences were observed between sexes for carotenoid content: males contained higher concentrations of total carotenoids, echinenone (most abundant pigment), α - and β -carotene than females, but practically undetected lutein and zeaxantin. Female's most abundant carotenoids were lutein, echinenone and zeaxantin. Males' gonads presented more lightness (higher L^*) and higher h^* (hue angle), but less redness, yellowness and colour contrast (lower a^* , b^* and C^* , respectively) than females. Sea urchins fed *Dunaliella*-diets were able to produce redder and less luminous gonads than the CTRL group, resulting in a colour improvement ranging from bright orange/red gonads with high acceptance for consumers.



BLUE THINK CONFERENCE

Share Science, Spread Knowledge

Keywords – *Paracentrotus lividus*; sea urchin; *Dunaliella salina*; gonad colour; carotenoids; cold extrusion



BLUE THINK CONFERENCE

Share Science, Spread Knowledge



ABSTRACTS

POSTER PRESENTATIONS



Does the inclusion of defatted *Tenebrio molitor* larvae meal in diets for European sea bass (*Dicentrarchus labrax*) have impact on central regulation of food intake?

Ana Basto^{1,2,3}, Luisa M.P. Valente^{1,2}, Marta Conde-Sieira³, José L. Soengas³

¹LANUCE, CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal

²ICBAS, Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, Portugal

³Department of Functional Biology and Health Sciences, Animal Physiology Laboratory – Faculty of Biology and Marine Research Center, University of Vigo, Spain

Presenting author email: anabasto@gmail.com

Corresponding author email: anabasto@gmail.com

Abstract

Since the use of insect protein in aquafeeds was approved by European Union in 2017, its use as fishmeal (FM) alternative has been increasingly explored in several fish species. Understanding the underlying mechanisms responsible for feeding behavior in fish is pivotal for ensure the most adequate dietary formulations for aquaculture. This study aimed to evaluate the impact of partial replacement of FM by defatted TM (dTM) on the expression of orexigenic and anorexigenic neuropeptides of European sea bass. Three isonitrogenous diets were formulated to replace increasing levels of FM by dTM: 0, 40 and 80 (CTRL, TM40 and TM80, respectively). Each diet was assigned to triplicate groups of 25 fish (55 ± 5g) fed 3 times daily, in a recirculating saltwater system (35‰, 22 ± 1°C). After 10 weeks of feeding, plasma, hypothalamus and telencephalon were collected at 2, 6 and 24h after feeding to evaluate metabolites and mRNA relative abundance of neuropeptides involved in the regulation of food intake. All diets were equally accepted by fish, resulting in similar food intake. Plasma cholesterol levels decreased 24h after feeding in fish fed TM40; fish fed dTM diets had higher cholesterol levels, 2 and 6h after feeding, than those fed CTRL. Increased non-esterified fatty acids levels occurred in plasma of fish fed TM80, regardless of the sampling time. At central level, no changes occurred in the mRNA abundance of neuropeptide Y, agouti-related protein 2, pro-opio melanocortin a or cocaine- and amphetamine-related transcript 2. The results obtained suggest that dietary replacement of FM by 40-80% of dTM for 10 weeks does not affect food intake and its homeostatic regulation in sea bass at central level. However, a short-term study is needed to fully characterize the response of mechanisms involved in food intake regulation immediately after exposure to dTM rich diets.

Keywords - Aquafeeds; hypothalamus; insect meal; neuropeptides; telencephalon



Cyanobacteria and actinobacteria – a source of novel bioactive molecules with biotechnological applications for obesity and appetite

Ana C. Fonseca^{1,2}, M. Fátima Carvalho¹, Vítor Vasconcelos^{1,2}, Mariana Alves Reis¹, Ralph Urbatzka¹

¹Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Porto, Portugal

²Faculty of Sciences, University of Porto, Porto, Portugal

Presenting author email: anacatarinasfonseca@gmail.com

Corresponding author email: anacatarinasfonseca@gmail.com

Abstract

Metabolic diseases, such as obesity, characterizes one of the most challenging health issues worldwide, due to their associated morbidity and mortality. Currently, the drugs available to help in the treatment of this metabolic disease are scarce. Natural products (NPs) synthesized by microorganisms are a wealthy source of valuable biological active molecules. This study aims to bioprospect microorganisms – a library of 117 cyanobacterial fractions from 39 strains obtained previously in “Cyanobesity” project, and 82 actinobacterial strains isolated from marine sponge *Hymeniacidon perlevis* collected in Praia da Memória, in Northern Portugal - for promising novel compounds such as nutraceuticals and/or compounds with pharmaceutical properties on obesity and appetite. Bioactive screenings were performed for appetite-reducing (using liposomes and *Paramecia bursaria*) and anti-obesity activity using zebrafish as a whole animal model for the screening of novel natural products from actinobacterial crude extracts and cyanobacterial fractions. The anti-obesity activity was assessed by Nile Red fat metabolism assay and appetite reduction was evaluated using feeding bioassays with fluorescent stained liposomes or paramecia. Two actinobacterial extracts showed promising anti-obesity activity, belonging to the genus *Streptomyces* and *Nocardiopsis*. In the appetite assay with liposomes, sixteen extracts revealed appetite reduction activity belonging to the genus *Nocardiopsis*, *Streptomyces*, *Nocardia*, *Gordonia*, *Rhodococcus*, *Micrococcus* and *Micromonospora*. The screening of cyanobacteria fractions is ongoing for both appetite assays. In the future, the responsible metabolites for the bioactivities expressed will be identified through metabolomics (LC-MS/MS) and growth manipulation strategies. Subsequently, it is intended to elucidate the chemical structure of the metabolites and to decipher the molecular mechanism of the isolated compounds.

Keywords - Metabolic diseases; zebrafish larvae; natural products; anti-obesity; appetite



Searching for antimicrobial peptides from sea urchin *Paracentrotus lividus* coelomic fluid

Ana Garcia^{1,3}, Rafaela Santos^{1,2}, Joana Azevedo¹, Pedro Leão¹, Benjamín Costas^{1,3}, Cláudia Serra^{1,2}, Sergio Fernández-Boo¹

¹CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Porto, Portugal

²Department of Biology, Faculty of Sciences, University of Porto, Porto, Portugal

³ICBAS - Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, Portugal

Presenting author email: afgarcia@ciimar.up.pt

Abstract

Antimicrobial peptides from natural sources are being explored as an alternative to conventional antibiotics to fight the ongoing problem of microbial resistance. Sea urchins are exposed to challenging environments, where they have to face pathogens like virus and bacteria. As so, they have an effective innate immune system, which relies on a varied population of immune cells – coelomocytes, found on the coelomic fluid. This study aimed to search for antimicrobial peptides from coelomocytes and coelomic fluid of the sea urchin *Paracentrotus lividus* by testing sea urchin extracts against bacterial strains known as etiological agents responsible for diseases in aquaculture. Therefore, sea urchin individuals were sampled from the coast of Vila Chã. Both perivisceral fluid and coelomocytes from coelomic fluid were subjected to liquid-liquid extraction, with 60 % acetonitrile. The aqueous phase was further subjected to solid phase extraction with growing ACN concentrations. The resulting fractions were tested against five bacterial strains: *Aeromonas hydrophyla*, *Vibrio anguillarum*, *Vibrio parahaemolyticus*, *Photobacterium damsela* subsp. *piscicida* and *Tenacibaculum maritimum*. The fractions which showed a higher activity in inhibiting the bacterial growth were fractionated by HPLC with a continuous growing gradient of ACN and the main peaks were collected and tested again against the above-described five bacterial strains. The peptides of the most promising fractions were characterized by shotgun proteomic. Results show that the higher inhibition rates were observed for the fractions obtained after elution of the SPE column with 40% ACN. However, when these elutes were fractionated by HPLC, the resulted purified fractions seem to have lost their effect on inhibiting bacterial growth. Shotgun proteomics showed the presence of several proteins and peptides, as toposome and metallothionein. Overall, these results indicate that the presence of some proteins and peptides involved in response to pathogens may be responsible for the antimicrobial activity of sea urchin extracts.

Keywords - Antimicrobial peptides; sea urchin; antibacterial activity; shotgun proteomics



Tryptophan modulatory role in European seabass (*Dicentrarchus labrax*) juveniles immune response to acute inflammation under stressful conditions

A. Ricardo^{1,2}, I. Duarte^{1,2}, D. Peixoto^{1,2}, P. Santos^{1,3}, R. Azeredo¹, M. Machado¹, B. Costas^{1,2}

¹CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Matosinhos, Portugal.

²ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal.

³MARE – Politécnico de Leiria – Centro de Ciências do Mar e do Ambiente, Peniche, Portugal.

Presenting author email: up201407773@up.pt

Corresponding author email: bcostas@ciimar.up.pt

Abstract

Introduction

Amino acids (AA) play several functions on key metabolic pathways with central roles in immune and neuroendocrine responses. Tryptophan in particular is an essential AA with recognized participation on the modulation of stress, antioxidant and immune systems. Therefore, the aim of this study was to evaluate the effect of dietary tryptophan supplementation on acute stress response of European seabass immune status, inflammatory mechanisms to *Photobacterium damsela* piscicida (*Phdp*).

Methodologies

Fish were randomly distributed in 2 recirculated seawater systems each comprised by 8 tanks. After acclimatization, fish from one of the systems were submitted to stressful crowding conditions. Afterwards, fish were fed for 15 days with control diet (CTRL) or the CTRL supplemented with L-tryptophan at 0.3% DM (4 tanks per stress situation/diet). Fish were then sampled and the remaining fish were intraperitoneally injected with *Phdp* and the inflammatory response evaluated at 4, 24, 48 and 72h. The haematological profile, immune response and oxidative stress were evaluated.

Results/Discussion

Few changes were observed regarding the evaluated parameters. Stressed fish fed TRP increased peripheral lymphocyte concentration from 4 to 72h post-infection and the same occurred in fish fed CTRL from 4 to 48 and 72h. Neutrophils increased in all treatments after 4 and 24h and decreased after 48 and 72h. Nevertheless, plasma cortisol level dropped in stressed fish fed TRP compared to their counterparts fed CTRL 4h following inoculation, suggesting that this AA can prevent stress-induced cortisol production, thereby potentially reverting cortisol-mediated immunosuppressive effects.



Conclusion

Preliminary data from this study suggests that dietary tryptophan supplementation could work as a promising nutritional strategy to counteract seabass cortisol rising under acute stressful conditions upon an inflammatory response. Such effect could be key for the improvement of disease resistance, therefore additional information regarding the neuroendocrine-immune network will be gathered.

Keywords - Amino acids; functional feeds; immunomodulation; inflammation



Text mining from titles: a screening tool to unravel seaweed's valorization potential.

Aires Duarte¹, Isabel Sousa Pinto¹ and Isabel Costa¹

¹Laboratório de Biodiversidade Costeira (LBC), Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Matosinhos, Portugal.

Presenting author email: airesmduarte@gmail.com

Corresponding author email: airesmduarte@gmail.com

Abstract

Fish-farms produce as byproducts nutrients that can be used by seaweeds to grow and, consequently, produce biomass that can be valorized in different applications. The seaweed species to cultivate with fish aquaculture must be chosen wisely since they must have high productivities, be adapted to local environmental conditions and be commercially valuable. Choosing the seaweed from hundreds of species might be a difficult and extensive task, but nowadays there are tools, such as text mining, which might ease this task. Text mining is a very useful tool to acquire high quality information from articles and to identify relevant information, useful knowledge and interesting traits for these species.

The text mining tool was developed using R software and its fulltext package to search for DOI, title and other information related to seaweed species present in Portugal. These data were searched in BMC, Crossref, EuropePMC, Plos and Scopus data sources. Articles that do not contain the species name on the title were excluded. Title's words retrieved from each species-specific articles were counted on tidytext package. Outputs were performed using ggplot2 and wordcloud2 package.

The total number of articles found in this search were 169902. After selecting the articles that contained the species name in the title, the total number of articles found were 5388. For 105 seaweed species, no articles were found with their names in the title. The most relevant outputs of this tool are the word cloud plot which shows every word mentioned in the titles by species and the heatmap which shows the frequency of specific title's words by species. This analysis will allow to gather relevant information on seaweed species (more and less studied), useful for species selection to be further studied as a nutrient extractive component associated with fish aquaculture.

Keywords - Text mining; screening; IMTA; seaweeds; R; nutrients



Effects of Ygeia+ on European seabass (*Dicentrarchus labrax*) health condition and disease resistance

André Cunha^{1,2}, Paulo Santos^{2,3}, Inês Ferreira^{2,4}, Carla Teixeira^{1,2,5}, António Afonso^{1,2}, Sara Magalhães⁶, Tiago Aires⁶, Elisabete Matos^{5,7}, Benjamin Costas^{1,2}

¹ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal

²CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Porto, Portugal

³IPL – Instituto Politécnico de Leiria, Peniche, Portugal

⁴I3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

⁵SPAROS Lda., Olhão, Portugal

⁶Sorgal S.A, São João de Ovar, Portugal.

⁷B2E - Associação para a Bioeconomia Azul – Laboratório Colaborativo, Leça da Palmeira, Portugal.

Presenting author email: andre.cunha.96@gmail.com

Corresponding author email: andre.cunha.96@gmail.com

Abstract

The present study aimed to: i) study the effects of Ygeia+ short-term feeding on immune-condition, oxidative stress and disease resistance of European seabass (*Dicentrarchus labrax*); and ii) assess the interactive effects of short-term feeding with Ygeia+ and vaccination in the immune response and disease resistance of European seabass.

Two trials were performed with European seabass weighing 12-36 g in recirculating seawater systems. In both trials fish were fed for 5 and 10 days a control (commercial) diet or the Ygeia+ diet, both produced and provided by Sorgal. In the first trial, fish were inoculated with an inactivated pathogen after each feeding time and the inflammatory response was assessed for 4, 24 and 48 h. In the second trial, fish were vaccinated with AVAC VR/PD/TM (HIPRA) or sham injected after each feeding period. Following vaccination procedures, fish were fed the control diet for 3 weeks and sampled for the assessment of immune parameters and oxidative stress biomarkers. Fish were also bath challenged with *Tenacibaculum maritimum* to evaluate if Ygeia+ can induce protection and its synergistic effects with AVAC VR/PD/TM.

The first trial pointed to a positive effect of Ygeia+ with a tendency to increase circulating monocyte and neutrophil numbers following inflammation in fish fed Ygeia+ for 5 days. Neutrophilia and monocytosis correlated well with the increase of plasma lysozyme at 4 h following an inflammatory insult. During the second trial, specific IgM augmented in all vaccinated groups, which showed improved survival. Moreover, vaccinated seabass fed Ygeia+ for 10 days tended to increase total glutathione levels in liver, compared to their counterparts fed the control diet. In summary, functional diets such as Ygeia+ are important sustainable prophylactic strategies that deserve further attention. In particular, other feeding times should be considered to



BLUE THINK CONFERENCE

Share Science, Spread Knowledge

fine-tune Ygeia+ as a feeding strategy to improve European seabass robustness.

Keywords - European seabass; Ygeia+; functional feeds; immunomodulation; vaccination



Development of disruptive wave energy technologies towards improved autonomy and sustainable powering of marine activities

Daniel Clemente¹, Paulo Rosa Santos¹, Francisco Taveira Pinto¹, Cátia Rodrigues², José Correia³, Ricardo Esteves³, André Pereira², João Ventura²

¹Departamento de Engenharia Civil, FEUP - Faculdade de Engenharia da Universidade do Porto, Porto, Portugal; Núcleo de Estruturas Hidráulicas e Energia do Mar, CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Porto, Portugal

²Departamento de Física e Astronomia, FCUP - Faculdade de Ciências da Universidade do Porto, Porto, Portugal; IFIMUP - Instituto de Física dos Materiais da Universidade do Porto, Porto, Portugal

³InanoE - inanoEnergy, Universidade do Porto, Porto, Portugal

Presenting author email: up201009043@edu.fe.up.pt

Corresponding author email: up201009043@edu.fe.up.pt

Abstract

The advent of promising “Blue Economy” markets, such as aquaculture, marine exploration and green hydrogen, implies an energy demand to be met with sustainable supply solutions. Current technologies include wind turbines and solar panels, which suffer from resource intermittency and high exposure to the ocean environment. A more predictable and denser alternative, regarding its resource, comes from harvesting wave energy. To that end, a hybrid solution is here proposed and discussed: the E-Motions with integrated triboelectric nanogenerators (TENGs). While the E-Motions was conceived to convert wave/wind induced roll oscillations of multipurpose offshore floating platforms into electricity, TENGs are capable of highly efficient energy conversion from any relative mechanical motion. The combined approach enables sharing of the same platform (including buoys or vessels), dual wave energy conversion, protection of sensitive equipment and stacking of multiple Power Take-Off systems and/or TENG layers. Recently, a 1:20 geometrical scale experimental study was conducted, within the wave basin at FEUP’s Hydraulics Laboratory, featuring three E-Motions hull designs – Half-Cylinder, Half-Sphere and Trapezoidal Prism – with integrated TENG structures. A thorough parametric analysis, with regular waves, was followed by irregular waves tests. Results point towards a strong hydrodynamic response on the part of the Half-Cylinder (up to 40° of roll peak-to-peak amplitudes) and the Half-Sphere (up to 30°), while the Trapezoidal Prism’s performance was poorer. In terms of power output, the Half-Cylinder variant achieved average values of up to 9.96 kW (prototype values), with low variability across the considered irregular wave conditions. As for the TENG, power density outputs of up to 1.51 W/m² were measured. Upcoming studies include the application of numerical models, towards an optimization strategy of the design variables, and complementary experimental studies to further demonstrate this hybrid approach.

Keywords - Marine Renewable Energy; E-Motions; TENGs; hybridization; composite modelling



Swine blood hydrolysates for European seabass functional diets: impact on fish response to stress

Daniela Resende^{1,2,3}, Ricardo Pereira^{1,2,3}, Cristina Velasco^{1*}, David Domínguez¹, Miguel Pereira³, Carlos Pereira⁴, Bianca Marques⁵, Cristina Rocha⁵, Manuela Pintado³, Luísa M.P. Valente^{1,2}

¹CIIMAR UP – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal

²ICBAS UP – School of Medicine and Biomedical Sciences, University of Porto, Porto, Portugal

³CBQF, Laboratório Associado, ESB-UCP – Centro de Biotecnologia e Química Fina, Escola Superior de Biotecnologia da Universidade Católica Portuguesa, Porto, Portugal

⁴Politécnico de Coimbra/ESAC – Escola Superior Agrícola de Coimbra, Coimbra, Portugal

⁵CEB, UM – Centre of Biological Engineering, University of Minho, Braga, Portugal

Presenting author email: danielaresende@outlook.com

*Corresponding author email: cvelasco@ciimar.up.pt

Abstract

In aquaculture farms, fish are subjected to stress situations, which induce oxidative stress through an imbalance between reactive oxygen species (ROS) generation and antioxidants' scavenging activity. Diet supplementation with functional ingredients to modulate oxidative stress after air exposure was investigated, using bioactive peptides from swine blood hydrolysates (BH), within a circular economy context. Three BH were obtained by autohydrolysis (AH) or enzymatically. The enzymatically produced BH were further submitted to micro- (MF) or nanofiltration (NF). Five isolipidic and isoproteic diets for European seabass (*Dicentrarchus labrax*) were developed: one fishmeal-based (positive control, PC); one commercial-based, with 50% of fishmeal replacement by plant proteins (negative control, NC); and three where 3% of each BH was added to the NC. Diets were assigned to triplicates of 71 juveniles (initial weight 12.3 ± 1.4 g) and fed to apparent satiation in a recirculating saltwater system. After 12 weeks, 9 fish/treatment were immediately sampled or air-exposed for 1 minute and let to recover for 6 hours prior to sampling, in which plasma and liver were collected. Plasmatic cortisol and lactate were elevated after air exposure, without differences among diets. Regarding liver oxidative stress, lipid peroxidation (LPO) tended to increase in all diets after air exposure. Considering non-stressed fish, MF led to the lowest LPO, albeit only significantly lower than AH. Protein oxidation decreased in stressed fish but remained unchanged among diets. Catalase was significantly lower for NC, NF and AH compared to PC, and increased after stress. Superoxide dismutase activity was lower for AH and PC than for MF, and was reduced in stressed fish, compared to non-stressed. In conclusion, the NF, with smaller peptides than the other BH, may modulate European seabass antioxidant defenses, lowering catalase without increasing LPO, suggesting it could provide exogenous antioxidants to counteract ROS-induced oxidative stress.



BLUE THINK CONFERENCE

Share Science, Spread Knowledge

Keywords - Blood hydrolysates; Bioactive peptides; European seabass; oxidative stress; lipid peroxidation; antioxidants

Acknowledgements - Work supported by Project MOBFOOD, POCI-01-0247-FEDER-024524•LISBOA-01-0247-FEDER-024524, cofounded by PORTUGAL2020, Lisb@a2020, COMPETE 2020 and the EU. DR thanks FCT, SANFEED and SenseTest© for her PhD grant (PD/BDE/150524/2019). RP thanks FCT for his PhD grant (SFRH/BD/144631/2019).



Dietary tryptophan supplementation and its modulatory role in juvenile European seabass (*Dicentrarchus labrax*) during chronic inflammation

I. Duarte^{1,2}, A. Ricardo^{1,2}, D. Peixoto^{1,2}, P. Santos^{1,3}, M. Machado¹, R. Azeredo¹, B. Costas^{1,2}

¹CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Matosinhos, Portugal.

²ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal.

³MARE – Politécnico de Leiria – Centro de Ciências do Mar e do Ambiente, Peniche, Portugal.

Presenting author email: up201605556@up.pt

Corresponding author email: bcostas@ciimar.up.pt

Abstract

Introduction

Amino acids (AA), in addition to their functions in protein synthesis, are recognized to regulate central metabolic pathways, which are crucial in the establishment of a successful immune response. Tryptophan (TRP) complex metabolism results in several metabolites that, direct or indirectly, modulate a wide array of physiological functions, including immune-related processes. Therefore, this study will gather new insights on the modulatory effects of TRP during chronic inflammation in European seabass (*Dicentrarchus labrax*).

Methodologies

A total of 192 fish were randomly distributed in 12 tanks and maintained in two recirculated seawater systems. Two dietary treatments were evaluated: a control diet, meeting the nutritional requirements of seabass (CTRL) and the CTRL-based diet supplemented with TRP. After one week of acclimatization, fish were intraperitoneally injected with either an inflammatory insult (Freund's Incomplete Adjuvant, FIA) or a sham saline solution (Hank's Balanced Salt Solution, HBSS). Fish were fed twice a day for 28 days and were sampled at 7, 14, 21 and 28 days post-injection and the immune response and oxidative stress were assessed.

Results / Discussion

Regarding response to inflammation (i.e. regardless time or diet), both gut peroxidase and bactericidal activities were inhibited in FIA- relative to HBSS-injected group. In contrast, FIA induced cell recruitment, with higher peritoneal cells numbers than in the sham group. Inhibition of gut immunity while leucocyte response is locally enhanced, might reflect a redistribution of energy allocation. Catalase and total glutathione increased over time in FIA-injected fish. Despite non-significantly, humoral bactericidal activity was lower in FIA-injected fish fed TRP-supplemented diet, compared to those fed CTRL.

Conclusion

Preliminary data showed few TRP-mediated effects, suggesting TRP dietary supplementation in



BLUE THINK CONFERENCE

Share Science, Spread Knowledge

this context does not significantly modulate fish immune response. Nevertheless, additional data on immune and haematological profile, as well as information on neuroendocrine-immune interactions will be conducted.

Keywords - Amino acids; Chronic inflammation; Immunomodulation; Tryptophan



Improving agar properties of farmed *Gracilaria gracilis* by using filtered sunlight

Francisca Silva-Brito^{1,2}; Sara G. Pereira³; Cristina M.R. Rocha³; Elisabete da Costa^{4,5}; M. Rosário Domingues^{4,5}; Andreina Azevedo⁶, Anake Kijjoa²; Helena Abreu⁶; Leonardo Magnoni¹

¹CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal.

²ICBAS - Institute of Biomedical Sciences of Abel Salazar, University of Porto, Porto, Portugal.

³CEB - Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

⁴Centro de Espetrometria de Massa, Departamento de Química & QOPNA, Universidade de Aveiro, Aveiro, Portugal;

⁵Departamento de Química & CESAM & ECOMARE, Universidade de Aveiro, Aveiro, Portugal

⁶ALGApplus - Produção e Comercialização de Algas e seus Derivados, Ílhavo, Portugal.

Presenting author email: fisbrito@gmail.com

Corresponding author email: fisbrito@gmail.com

Abstract

Although *Gracilaria* sp. is the most important raw material for the extraction of agar, this seaweed appears to generate gels with low strengths, reducing its commercial value. The effects of different light spectra on agar yield, composition and gel properties, pigment content and antioxidant activity of *Gracilaria gracilis* (GRA) cultivated in a land-based IMTA-system were investigated. The antioxidant activity in the residue obtained from GRA agar extraction (agar waste, AW) were also investigated. Seaweed was cultivated for 4 weeks applying neutral (NT), blue (BL) and red (RD) spectral filters in outdoor tanks (duplicates). The agar yield in the BL condition (13%) was 1.3 and 2 times higher than in NT and RD conditions, respectively. BL light produced an agar gel strength (1037.9 g cm⁻²) similar to the NT condition (960.0 g cm⁻²). Light spectra had no significant effect on melting (~93.5 °C) nor gelling temperatures (~44.1 °C) of agar. The productivity of GRA farmed under the BL condition (0.70% day⁻¹) decreased when compared to the NT condition (1.14% day⁻¹), while RD condition (0.93% day⁻¹) presented a middling growth. BL condition increases the total carbohydrate content of GRA compared to NT and RD conditions (78.72, 68.48 and 27.41 mg Glc Eq. g⁻¹ SW, respectively). The content of pigments was similar among different treatments. The antioxidant activity of GRA was unchanged by the different light spectra. The total phenol content and the antioxidant activity of AW were higher than in the GRA (31.76 mg vs. 7.52 mg PGE g⁻¹ E and 1.67 vs. 2.92 mg mL⁻¹, respectively). Results showed that the agar yield and gel strength are improved when applying BL filters despite a decrease in growth. Also, this study showed that AW could have potential applications due to its antioxidant activity (e.g., as a feed additive).

Keyword - Agar gel strength; agar yield; antioxidant activity; *Gracilaria gracilis*; IMTA-system



Molecular, biochemical and functional impact of suspended sediments in the model species *Mytilus galloprovincialis* under hyperbaric conditions

Marlene Pinheiro^{1,2}, Adriana Oliveira², Susana Barros^{1,3,4}, Nélson Alves^{1,2}, Joana Raimundo^{5,6}, Miguel Caetano^{5,6}, João Coimbra⁷, Teresa Neuparth¹, Miguel M. Santos^{1,2}

¹ Endocrine Disruptors and Emergent Contaminants, CIIMAR – Interdisciplinary Centre for Marine and Environmental Research, University of Porto, Porto, Portugal

² Biology Department, FCUP – Faculty of Sciences of the University of Porto, University of Porto, Porto, Portugal

³ Centre for the Research and Technology of Agro-Environmental and Biological Sciences, UTAD – University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

⁴ Research Institute on Chemical and Biological Analysis, USC – University of Santiago de Compostela, Santiago de Compostela, Spain

⁵ Environmental Oceanography and Bioprospection, IPMA – Portuguese Institute for Sea and Atmosphere, Lisbon, Portugal

⁶ Contaminant Pathways and Interactions with Marine Organisms, CIIMAR – Interdisciplinary Centre for Marine and Environmental Research, University of Porto, Porto, Portugal

⁷ Ecophysiology, CIIMAR – Interdisciplinary Centre for Marine and Environmental Research, University of Porto, Porto, Portugal

Presenting author email: bpinheiro@ciimar.up.pt

Corresponding author email: bpinheiro@ciimar.up.pt

Abstract

The increasing scarcity of land based mineral deposits is driving the rising interest in deep-sea mining as the next source for minerals and rare earth elements. Deep-sea environments, although mostly unknown and inhospitable, are subjected to anthropogenic stressors and will be affected by mining operations, with the release of sediment plumes regarded as one of the most immediate and broad effects of mining the seabed.

The effects of suspended sediments of different grain sizes (63-125µm; 125-250µm; 250-500µm) at 1 g/L were studied in the model species *Mytilus galloprovincialis* under hyperbaric conditions (4 Bar). Molecular (gene expression of actin – ACTN, glutathione S-transferase alpha – GSTA, superoxide dismutase 2 – SOD2, catalase – CAT, heat shock protein 60 – HSP60 and DNA mismatch repair protein – MSH6), biochemical (catalase – CAT, glutathione S-transferase – GST, lipid peroxidation – LPO) and functional (filtration rate – FR) endpoints were analyzed in juvenile mussels. The FR decreased significantly for all treatments with a more pronounced effect in the smaller grain sizes. Significant alterations were also found for all tested biomarkers and gene expression was downregulated for CAT and ACTN.

These findings showed that smaller sized particles are the ones causing more adverse effects and



that, due to their high distribution and suspension potentials, particular attention should be given to the release and resuspension of particles, in the seabed and the water column, as a result of mining practices. Furthermore, establishing safety and preservation guidelines and regulations is vital in order to protect sensitive habitats and allow for responsible and sustainable mining in deep-sea environments.

Keywords - Hyperbaric chamber; *Mytilus galloprovincialis*; suspended sediments; gene expression; biomarkers; filtration rate



Chronic life-cycle studies of the priority pharmaceutical Metformin with *Danio rerio*: molecular and biochemical assessment

Marta Ribeiro^{1,2,3}, Susana Barros^{1,4,5}, Miguel M Santos^{1,3}, Teresa Neuparth¹

¹ Endocrine Disruptors and Emerging Contaminants Group, CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal

² ICBAS – Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, Portugal

³ FCUP – Faculty of Sciences, University of Porto, Porto, Portugal

⁴ CITAB – Centre for the Research and Technology of Agro-Environmental and Biological Sciences, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal

⁵ IAQBUS – Research Institute on Chemical and Biological Analysis, University of Santiago de Compostela, Santiago de Compostela, Spain

Presenting author email: martadcribeiro@gmail.com

Corresponding author email: martadcribeiro@gmail.com; tneuparth@ciimar.up.pt

Abstract

More than 451 million people worldwide have diabetes mellitus and the vast majority of whom are affected by type 2 diabetes (T2DM). Metformin (MET) is the first-line pharmaceutical to treat patients with T2DM, since it has the ability to reduce hyperglycemia. MET has become one of the most prescribed and consumed pharmaceuticals worldwide and consequently, a compound of emerging concern in aquatic ecosystems.

The main objective of this research is to integrate up-to-date biochemical and molecular methodologies to address the mode(s) of action of MET on the freshwater fish *Danio rerio* (zebrafish) after a full life-cycle exposure. Zebrafish were exposed to three environmentally relevant concentrations of MET (361 ng/L, 2166 ng/L and 13000 ng/L). In this study, the underlying toxicity mechanisms of MET were determined in zebrafish larvae and adults by the characterization of ecological endpoints, biochemical markers (cholesterol and triglycerides) and the transcription level of key genes involved in energy and lipid metabolism.

The results revealed that MET was able to increase both length and weight of 9 mpf exposed fish. Growth indexes, such as Fulton's condition factor and the hepatosomatic index were significantly higher in exposed animals. The results of the biochemical parameters showed a significant decrease of cholesterol levels in 9 mpf males and females and a significant decrease of triglyceride levels in 9 mpf males. Transcription levels of target genes, of 20 dpf larvae and adult zebrafish (9 mpf), were also altered in a non-monotonic dose response, after exposure to MET.

Results show that a full life-cycle exposure to environmentally relevant concentrations of MET impacted different metabolic pathways in the aquatic vertebrate *D. rerio*. These findings provide important environmental data for risk assessment, given that aquatic organisms are chronically exposed to MET during multiple generations.



BLUE THINK CONFERENCE

Share Science, Spread Knowledge

Keywords - *Danio rerio*; metformin; chronic effects; mechanisms of action; energy metabolism; lipid metabolism



Effects of surface water samples from Northern Portugal on zebrafish embryonic development

Nélson Alves^{1,2}, Teresa Neuparth¹, Miguel M. Santos^{1,2}

¹Endocrine Disruptors and Emerging Contaminants, CIMAR/CIIMAR, LA – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Porto, Portugal

²Department of Biology, Faculty of Sciences of University of Porto, University of Porto, Porto, Portugal

Presenting author email: nalves@ciimar.up.pt

Corresponding author email: nalves@ciimar.up.pt; miguel.santos@fc.up.pt

Abstract

In the last decades, several legal instruments and innovative technologies were developed to improve the quality of environmental matrices, however emerging contaminants are only partially removed by Wastewater Treatment Plants and their presence in the environmental matrices have been detected. In this context, the project Nor-water was created with the aim to identify the main emerging contaminants and their sources in the hydrographic basins of northern Portugal and Galicia and develop high throughput tools to assess and reduce the environmental risks associated with these contaminants.

In this framework, the goal of this work was to perform a screening of the potential toxicity of water samples collected in several rivers in the north of Portugal, using the standardized OECD 236 test guideline with embryonic development of *Danio rerio*.

The water samples were collected in July 2020 in nine sites in Minho, Lima, Ave and Cávado Rivers. Zebrafish embryos were exposed to the collected samples for 120 hours according with the OCDE 236 Test. Mortality, abnormalities in embryonic development and heartbeat were assessed at several time-points. At the end of the exposure, larvae length, area of yolk sac and sensorimotor reflexes were evaluated.

The exposure of zebrafish embryos to Lima5, Lima8 and Minho6 samples induces changes on larvae length, area of yolk sac and heartbeat. An increase of developmental abnormalities was also observed at 120hpf for embryos exposed to Lima8 and Cávado. No statistical differences were observed in sensorimotor reflexes and mortality.

Overall, these results show evidences of a potential toxicity of several water river samples, thus supporting the need for a more detailed characterization to identify the most toxic chemicals present.

Keyword: Risk assessment; *Danio rerio*; water bodies; emerging contaminants



BLUE THINK CONFERENCE

Share Science, Spread Knowledge

Acknowledgements: This study was developed under the project NOR-WATER - Poluentes emergentes nas águas da Galiza-Norte de Portugal: novas ferramentas para gestão de risco, (Reference: 0725_NOR_WATER_1_P), financed by Programa de Cooperação Interreg V-A Espanha/Portugal (POCTEP) 2014–2020.



***Patella ulyssiponensis*: a species surviving of the messinian crisis? Its historical dispersal may help to understand the migration routes of benthic organisms and its relationship with the rhodolith *Lithophyllum hibernicum*?**

Nuno Gomes¹, Carlos Antunes¹, Dimítri de Araújo Costa¹

¹Estuarine Ecology and Biological Invasions Group, CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal

Presenting author email: Nuno@ciimar.up.pt

Corresponding author email: Nuno@ciimar.up.pt

Abstract

The genus *Patella* from the SubClass Patellogastropoda is represented by a group of species exclusive of the Northeast Atlantic Ocean (including Macaronesian archipelagos) and Mediterranean Sea. The species *Patella ulyssiponensis* and *Patella aspera* are common at European waters, with the first inhabiting continental coast and the second one being endemic for Macaronesian archipelagos. However, the acceptance of these two lineages as separate species is still highly debated. In the juvenile stages, *Patella* spp. benefit from the substrate formed by the algae (for settlement) and feeding on it. The red coralline species algae *Lithophyllum hibernicum*, distributed from Northeast Atlantic to the Mediterranean, usually is found as epilithic crusts or unattached forms (named rhodoliths beds) although it also forms epizoic crusts on other organisms, e.g. shell surfaces. In order to study the historic dispersal and migration routes of the *Patella ulyssiponensis-aspera* complex, taxonomic, genetic and biogeographic approaches were employed based on haplotype network analyses and estimations for the most common recent ancestor (TMRCA), using Cytochrome Oxidase I. With the presence of a shared haplotype between the continental (*P. ulyssiponensis*) and insular (*P. aspera*) lineages, and with basis of morphological and nomenclatural data, a synonymy for these two species is proposed, as well as an evolutionary scenario for its dispersal based on a high haplotype diversity for the Mediterranean regions, indicating its survival during the Messinian Salinity Crisis (6 Mya), followed by a colonization of the Proto-Macaronesian archipelagos. The epizoic association of *L. hibernicum* on adult *P. ulyssiponensis* shell surface is recorded in this study, likewise the promotion of settlement conditions provided by these coralline algae to *P. ulyssiponensis* larvae, may explain the reach of *P. ulyssiponensis* distribution through rhodolith transportation.

Keywords - Interspecific relationship, *Lithophyllum hibernicum*, *Patella aspera*, Macaronesia, Northeast Atlantic, Principle of Priority.



Cellular, humoral and molecular responses of gilthead seabream (*Sparus aurata*) juveniles to bacterial infection with *Photobacterium damsela* subsp. *piscicida*

Paulo Santos^{1,2,3}, Diogo Peixoto^{1,2}, Inês Ferreira^{1,2,4}, Ricardo Passos³, Pedro Pires³, Teresa Baptista³, Benjamin Costas^{1,2}

¹CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal

²ICBAS, Institute of Biomedical Sciences Abel Salazar - University of Porto, Portugal

³MARE, Polytechnic of Leiria, Portugal

⁴IBMC, Instituto de Biologia Molecular e Celular, University of Porto, Portugal

Presenting author email: paulo.santos@ciimar.up.pt

Corresponding author email: paulo.santos@ciimar.up.pt

Abstract

The current development of aquaculture industry is being challenged by the occurrence of several bacterial outbreaks causing massive animal and monetary losses. Since health biomarkers definition is crucial for disease detection, the present study was designed to evaluate haematological, humoral and molecular innate immune responses of gilthead seabream (*Sparus aurata*) within the first days of infection with *Photobacterium damsela* subsp. *piscicida* (Phdp).

A time-course study was performed at CETEMARES (Politécnico de Leiria, Peniche, Portugal) facilities with 72 seabream juveniles (9.8 ± 2.2 g). Among them, 12 fish were selected and sampled before infection (time 0). Thereafter, the remaining animals were intraperitoneally (i.p.) injected with 100 μ l of exponentially growing Phdp (106 CFU/ mL) and distributed in 3 recirculating seawater systems. Two animals per tank (n=6) were sampled at 3, 6, 9, 24 and 48 h after i.p. injection. At each sampling point, fish were anaesthetized, and blood samples were collected for haematological procedures. Afterwards, the remaining blood was centrifuged, and plasma was collected for humoral parameters evaluation. Head kidney was also collected for gene expression analyses.

Significant differences were found in several parameters analyzed. Red blood cell levels decreased in infected animals 9 h after infection, whereas haematocrit, haemoglobin and mean corpuscular haemoglobin concentration were found diminished in infected animals 48 h post-infection. Even though total peripheral leucocytes did not change along the experiment, circulating neutrophil and monocyte populations showed augmented numbers since the first hours of infection. Regarding humoral responses, infected animals presented increased antiproteases activity 48 h post-infection. Molecular findings corroborate the results above, with increased expression of genes related to inflammation and phagocytic activity such as Interleukin1 β ,



BLUE THINK CONFERENCE

Share Science, Spread Knowledge

Interleukin-34 and Interleukin-10.

Further studies must be performed with different pathogenic species in order to provide information for a better comprehension of infection development and host immune machinery.

Keywords - Animal health; infection; immune response; red blood cells; neutrophils; Interleukin-34



Stock structure of the Brazilian sardine *Sardinella brasiliensis* from Southwest Atlantic Ocean inferred from otolith elemental signatures

Rafael Schroeder^{1,2}, Paulo Ricardo Schwingel¹, Edgar Pinto^{3,4}, Agostinho Almeida³, Alberto Teodorico Correia^{2,5,6,*}

¹Laboratório de Ecossistemas Aquáticos e Pesqueiros. Escola do Mar, Ciência e Tecnologia. Universidade do Vale do Itajaí (UNIVALI). Rua Uruguai 458, Centro 88302-901, Itajaí, Brasil.

²Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR). Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos S/N, 4550-208 Matosinhos, Portugal.

³Faculdade de Farmácia da Universidade do Porto (FFUP). Laboratório Associado para a Química Verde (LAQV/REQUIMTE). Rua Jorge Viterbo Ferreira 228, 4050-313, Porto, Portugal

⁴Escola Superior de Saúde do Instituto Politécnico do Porto (ESS-IPP). Rua Dr. António Bernardino de Almeida 400, 4200-072 Porto, Portugal.

⁵Faculdade de Ciências da Universidade Fernando Pessoa (FCS-UPF). Rua Carlos da Maia 296, 4200-150 Porto, Portugal.

⁶Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto (ICBAS-UP). Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal.

Presenting author email: schroederichthys@gmail.com

Corresponding author email: atcorreia.ciimar@gmail.com

Abstract

The Brazilian sardine (*Sardinella brasiliensis*) sustains the most important pelagic fishery off Brazilian waters. The earliest studies about the stock structure of *S. brasiliensis* suggested the existence of two subpopulation units taking into account distinct regional somatic growth rates and spawning areas. However, further works have been unable to confirm this scenario, and for fisheries management purposes a single population unit is considered in Brazil. In order to unravel the stock structure of *S. brasiliensis*, a total of ninety adult individuals of two years old were collected in three fishery grounds of Brazil in its area of distribution [Rio de Janeiro (RJ), Santa Catarina (SC) and Rio Grande do Sul (RS)], between February and October 2018. Multi-elemental signatures of whole otoliths were determined using inductively coupled plasma mass spectrometry and combined with somatic and reproductive basic data. A generalized linear model found significant different somatic growth rates among regions. Whole otolith chemical signatures exhibited distinct regional patterns, mainly driven by differences in Sr/Ca, Ba/Ca, Li/Ca, Mg/Ca, Mn/Ca, Cu/Ca and Zn/Ca. Combining somatic and reproductive data with multi-elemental signatures, the leave-one-out classification re-assigned individuals to their area of origin with an accuracy of 100% (RJ), 93% (SC) and 87% (RS). The hereby results indicate that the connectivity between the local population of *S. brasiliensis* in the RJ region (23°S) with the southern populations is limited. However, spatially structured semi-discrete groups were found between



BLUE THINK CONFERENCE

Share Science, Spread Knowledge

26°S and 32°S. Despite the possibility of intermixing of *S. brasiliensis* populations in the Brazilian Southwest Atlantic coast, they should be regarded as different stocks for fisheries management purposes.

Keywords - Purse Seine Fishery; Pelagic Fish; Clupeidae, Population structure; Natural Tags



Spatiotemporal dynamics of parasites and diseases affecting cockles from the Ria de Aveiro

Simão Correia^{1,2}, Sergio Boo², Luísa Magalhães¹

¹Departamento de Biologia, CESAM – Centro de Estudos do Ambiente e do Mar, Universidade de Aveiro, Aveiro, Portugal

²A2S, CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Matosinhos, Portugal

Presenting author email: simaocorreia@ua.pt

Corresponding author email: luisa.magalhaes@ua.pt

Abstract

The Ria de Aveiro coastal lagoon, an ecosystem from the northwest Portuguese coast, is the assembly of several biotopes resulting in a complex environment with unique characteristics, high biodiversity and utmost importance for human settlement. Among many marine resources, the Ria de Aveiro is home to one of the most important European natural beds of cockles, *Cerastoderma edule*. Cockles play a central role in the ecosystem functioning with supply of many provisioning and regulating ecosystem services. Besides, in the Ria de Aveiro and other coastal communities of the Atlantic coast, cockles frequently represent the main livelihood of the population, generating a strong socio-cultural footprint. Despite this ecological and socio-economic importance, there is still a lack of knowledge about local main threats and drivers of cockle population dynamics, hindering the implementation of effective management plans. In this sense, through a one-year field monitoring, the present study aimed to identify the pathogenic agents (micro and macroparasites) affecting cockles in the Ria de Aveiro with estimate of spatiotemporal dynamics. The survey was conducted in eighteen sampling sites, covering the entire cockle distributional range of the Ria de Aveiro. The biodiversity of parasites found was high; more than 15 species belonging to different clades, from arthropods to Alveolata. Trematodes were the parasites detected in higher prevalence and intensity. Results showed a general spatial and temporal homogeneity of the parasites communities with no clear seasonality. Nevertheless, hot spots of infection were identified with respective identification of the correlated environmental drivers (i.e., abiotic conditions, namely sediment mean grain size and host density). This study contributed to update the information regarding cockles' parasites and diseases occurring in the Ria de Aveiro and may support decision-makers towards the sustainable management of this resource and assist a better description of the ecological structure of this ecosystem.

Keywords - *Cerastoderma edule*; Pathogenic agents; Host-parasite interactions; Coastal systems; Bivalve



Can a marine fish with limited swimming proficiency and strong homing behaviour display increased dispersal potential and lack population genetic structure? The curious case of the worm pipefish *Nerophis lumbriciformis* (Teleostei: Syngnathidae).

Tito Mendes¹, Cidália Gomes¹, Nuno Monteiro^{2,3,4}, Agostinho Antunes^{1,2}

¹EGB, CIIMAR/CIMAR – Interdisciplinary Centre of Marine and Environmental Research, Porto, Portugal

²Department of Biology, Faculty of Sciences, University of Porto, Porto, Portugal

³CIBIO (InBio), Research Center in Biodiversity and Genetic Resources, Porto, Portugal

⁴Faculty of Health Sciences, CEBIMED, University Fernando Pessoa, Porto, Portugal

Presenting author email: tmendes@ciimar.up.pt

Corresponding author email: tmendes@ciimar.up.pt

Abstract

The family Syngnathidae encompasses a group of teleost fishes – including seahorses, seadragons, pipehorses, and pipefishes – that exhibit limited dispersal capabilities and strong habitat dependency. Consequently, population genetics studies performed with syngnathids often report the occurrence of high levels of population differentiation. The worm pipefish, *Nerophis lumbriciformis*, presents the same reduction of swimming elements and homing behaviour as other syngnathids. However, and contrarily to the expected, this pipefish displays an opposite demographic pattern, evidencing a high dispersal potential and a lack of demarked population structuring. The analysis of three mitochondrial (cytochrome b, 12S and 16S rDNA) and a nuclear (rhodopsin) markers, amplified from 119 individuals collected throughout its native range, showed that this species maintains a genetically homogeneous population with indications of extensive gene flow. We hypothesize that the unusually high dispersal potential exhibited by *N. lumbriciformis* may be related to its pelagic larval stage which, supported by marine currents, may act as major dispersal vectors, thus allowing for population admixture and preventing genetic segregation among individuals from distinct geographic locations. This findings reveal a very rare demographic behaviour for marine species with limited swimming proficiency, especially considering syngnathids, and highlight the potential impact of pelagic larval stages and marine currents in their dispersal capacity.

Keywords - Phylogeography; haplotypes; population expansion; genetic diversity; North Atlantic Drift

Acknowledgements: Fundação para a Ciência e Tecnologia (FCT) and European Regional Development Fund (ERDF)



BLUE THINK CONFERENCE

Share Science, Spread Knowledge



SESSION 4: OCEAN DECADE

MODERATION: MARIANA OLIVEIRA AND RITA MENDES

MODERATOR – SANDRA RAMOS



Sandra is a researcher at CIIMAR (University of Porto, Portugal) and coordinator of CMIA Matosinhos. The main focus of her research is marine ecology, with special emphasis on early life stages of fishes and impacts of human activities, including emergent pollutants as microplastics. Her research also includes marine ecosystem services and new approaches for environmental assessments, using participatory approaches to capitalize local stakeholder's knowledge to promote the conservation of marine resources and ecosystems.

MINISTER OF THE SEA - RICARDO SERRÃO SANTOS



Doctor Ricardo Serrão Santos has a degree in Psychology and Behavioral Ecology at the Higher Institute of Applied Psychology and a Ph.D. in Environmental and Evolutionary Biology at the Faculty of Science, University of Liverpool, United Kingdom. He is Principal Investigator at the University of the Azores where he was Director of the Department of Oceanography and Fisheries between 1997 and 2011. He is currently the Vice President of the Scientific Council of the Oceanographic Institute of Paris. He was an MEP from 2014-2019 and an effective member of the Committee on Fisheries and the Committee on Agriculture and Regional Development. He was the S&D Group coordinator for the Committee on Fisheries. In the European Parliament he was also Vice-President of two Intergroups “Climate Change, Biodiversity and Sustainable Development” and “Seas, Rivers, Islands and Coastal Zones”. He is a member of several advisory boards of international organizations, including the EOOS-European Ocean Observing System, or BiodivERsA. In 2009 he was elected Corresponding Member of the Lisbon Academy of Sciences.

INVITED SPEAKER - ISABEL SOUSA PINTO



Professor Isabel Sousa Pinto holds a PhD in Marine Biology (phycology) from the UCSB, USA. She is a Professor at the University of Porto and head of the Laboratory of Coastal Biodiversity with about 30 researchers at the CIIMAR-UP.

She researches ecology and marine biodiversity: a) distribution patterns and biology b) the role of biodiversity in the functioning and services of the ecosystems, and the effects of multiple drivers including climate change and invasive species, fisheries and harvest. Professor Isabel has an international science policy background, participating in several global networks. Professor Isabel is also member of the Steering/Executive Committees of several European and international programs: EUROMARINE (2014-present), EMBOS (2011-2015), EOOS (2019-present), EKLYPSE (2016-2020) Member of the Implementing Committee of GEO BON from the intergovernmental program and Co-chair of its Marine Biodiversity Observation Network (MBON) (2010-present).



INVITED SPEAKER - JOANA LARANJEIRA



Joana holds a PhD in Biotechnology from the Catholic University of Porto, a MsC Biology from the Coimbra University and a BsC in Microbiology from the Catholic University of Porto.

She is the R&D and Plant Manager at Allmicroalgae, a company that grows and commercializes microalgae on a large scale.

Joana holds more than 15 years of experience in microalgae biotechnology. Currently, she is involved in several national (ALGAVALOR, ALGAESOLUTIONS, PERFORMALGAE and VALORMAR) and international (PROFUTURE, MAGNIFICENT, SIMBA, ZEROwaste) projects.

INVITED SPEAKER – RAQUEL GAIÃO SILVA



Raquel Gaião Silva holds a BSc in Biology and an International MSc in Biodiversity and Marine Conservation. She is currently working at BlueBio Alliance (BBA), where she manages all the organization's daily tasks.

Raquel has been involved in the development and implementation of stakeholder engagement projects related to the sustainable use of blue bioresources. She has also been involved in the organisation and dissemination of networking events and projects of BBA.

Her main areas of interest stand on the bridge between marine science and society, especially knowledge transfer for the sustainable Blue Entrepreneurship.

FINAL REMARKS

We, from the PhD students committee of CIIMAR, are thankful for all the participants of the Blue Think Conference 2021, especially Professor Vitor Vasconcelos, Doctor Felix Figueroa and Professor Jorge Fernandes, who kindly accepted our invitation to share their knowledge and expertise with all of us. We are very grateful for all the help of the Scientific Committee offered during the organization of this conference and to the communication office of CIIMAR who helped us reach so many and such a diverse group of people.

Last year we promised more, so we tried to take our conference a step forward and included a Roundtable focused on the Ocean Decade! Our Roundtable Invitees Professor Isabel Sousa Pinto, Doctor Joana Laranjeira, Raquel Gaião Silva and, the special participation of the Minister of the Sea Doctor Ricardo Serrão Santos, moderated by Doctor Sandra Ramos, provided us with an invaluable input and enriched our discussion beyond expectation. It was an honor and a pleasure to share the screen with all of them! We reserve a special thank you for Doctor Joana Xavier who gave us important feedback regarding the organization of the roundtable.

Again, this conference surpassed all our expectations with 83 abstracts submissions, more than 200 registrations and a sponsorship from Santander, we could not be happier!

We hope next year will bring us closer together, allowing us to share knowledge but also human connections.

Thank you for attending,
CIIMAR's PhD Students Committee

BLUE THINK CONFERENCE

Share Science, Spread Knowledge

SPONSORS



CONTACT

For more information and suggestions: phd_committee@ciimar.up.pt