

BOOK OF ABSTRACTS

BLUE THINK CONFERENCE

Share Science, Spread Knowledge



Matosinhos, Portugal

17th September, 2020

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THE CONFERENCE

The Blue Think Conference (BTC) 2020 was the first meeting organized by the Interdisciplinary Centre of Marine and Environmental Research (CIIMAR) PhD Students' Committee. The aim was to gather all students affiliated with CIIMAR in a casual and relaxed environment to share the outputs of our works.

The presentations were divided into three sessions framed in the scope of CIIMAR three main research lines, which aim to accomplish developments in **Marine Biotechnology, Global Changes and Ecosystem Services** and **Biology, Aquaculture and Seafood Quality**. Each session covered one of the three areas and counted with the presence of renowned scientists who talked about their scientific careers, professional experiences and most important work achievements.

We hope that this day has expanded our scientific knowledge but also allowed CIIMAR students who work in different groups and laboratories, scattered through several faculties and institutes of the University of Porto, to create bonds and collaborations.

Furthermore, we urged to extend the invitation to the general public. Everyone was welcome to join and discuss 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)

MESSAGE TO PARTICIPANTS

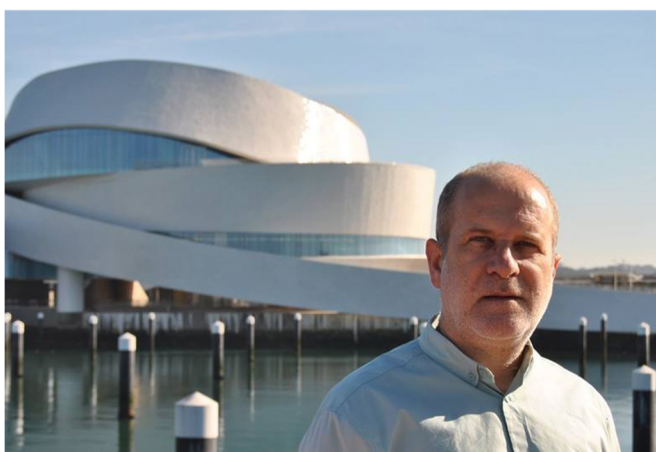
The first Blue Think Conference organized by the PhD students committee of CIIMAR is here. During this day, students will have the opportunity to show their works, discuss their ideas, and plan their future. It will be a great serendipity environment that certainly will make you all evolve and will incubate new projects for the future.

In this weird period of the covid-19 pandemic, this compulsory virtual conference with more than 100 presentations and 200 registrations all over the globe will be a great opportunity to increase its impact since no boundaries exist; no travel expenses will be required and no need to ask your supervisor a week off to attend the conference. I hope that in 2021 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 your careers.

Professor Vitor Vasconcelos

Director of CIIMAR



ORGANIZING COMMITTEE

Ana Matos

André Santos

Diana Almeida

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Joana Martins

Maria Paola Tomasino

Miguel Semedo

Rita Azeredo

Sergio Fernández Boo

INVITED SCIENTIFIC COMMITTEE

Alicia Toranzo

Rodrigo Costa

Nuno Queiroz



SESSION 1: BIOLOGY, AQUACULTURE AND SEAFOOD QUALITY

MODERATION: DIANA ALMEIDA AND SERGIO FERNANDEZ

KEYNOTE SPEAKER – ALICIA TORANZO



Alicia E. Toranzo is Professor of Microbiology at the University of Santiago de Compostela (USC) since 1991, where she leads a research group on pathology of aquatic animals (Competitive Reference Group of the Xunta de Galicia, Spain).

She is Ph.D. in Biology and Graduate in Pharmacy from USC and she was a pre and post-doctoral fellow at the University of Maryland and University of Oregon (USA) from 1980 to 1982. She was Chairman of the Microbiology and Parasitology Department of the USC from 2004 to 2013, and from 1999 she coordinates the PhD program on "Advances in Microbial and Parasitic Biology" at the USC. Some of her main research topics are: phenotypic and molecular characterization of bacterial pathogens, epizootiological analysis, molecular studies of virulence mechanisms of bacterial pathogens and development of strategies for the prevention and control of fish diseases.

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She is the author of more than 300 articles, most of them published in SCI indexed journals, as well as 22 book chapters. She is inventor of 8 patents for use in aquaculture (vaccines, probiotics and diagnostic tools), has participated in more than 65 National, European and Autonomic Research and Development Projects funded by public and private bodies and maintains a continuous consulting and service collaboration with companies from the veterinary and aquaculture sectors. In addition, she is participating in research networks related to environmental Microbiology and fish Immunology.

She has supervised 17 PhD Theses, has been invited to give more than 50 conferences in National and International meetings and workshops, is advisor of different international journals in Microbiology and Aquaculture, is member of the experts Committee for the evaluation of national and international programmes, has been part of the scientific and/or organizer Committee of Meetings of Aquaculture and Microbiology and she is active member of 10 Scientific Societies. She was recognized with the "Jaime Ferrán" National Award for young investigators (1993) of the Spanish Society of Microbiology, the "Galician Title of Excellence in Research" (2006) and the "María Wonenburger Award" of the Xunta de Galicia (2018). Since 2014 and 2017 she belongs to the "Real Academia Gallega of Sciences" (RAGC) and "Real Academia Gallega de Farmacia", respectively.



SESSION 2: MARINE BIOTECHNOLOGY

MODERATION: DIOGO ALEXANDRINO AND JOANA MARTINS

KEYNOTE SPEAKER – RODRIGO COSTA



Since April 2016, Rodrigo Costa is Assistant Professor at the Department of Bioengineering (DBE) of Instituto Superior Técnico (IST), University of Lisbon. He graduated in Biological Sciences at the Federal University of Rio de Janeiro, Brazil, in 1998 and obtained his M.Sc. degree in Ecology from this same University in 2001. He acquired his Ph.D. degree in Life Sciences from the Technical University of Braunschweig, Germany, in 2006, and was a post-doctoral fellow at the University of Groningen, The Netherlands, from 2006 to 2008.

He was then awarded the Research Scientist contracts “Ciência” (2008) and “FCT Investigator” (2015) from the Portuguese Foundation for Science and Technology (FCT). During this period, he founded and led the “Microbial Ecology and Evolution Research Group” (MicroEcoEvo) at the Centre of Marine Sciences (CCMar), Algarve University, Portugal.

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Professor Rodrigo Costa is an invited affiliate (Research Scientist) of the Lawrence Berkeley National Laboratory (LBNL), University of California at Berkeley, USA, and of the Centre of Marine Sciences (CCMAR), Portugal.

He is the ambassador of the International Society for Microbial Ecology (ISME) in Portugal, an invited member of the Editorial Board of Scientific Reports, and member of three academic societies. In 2019, he chaired the International Symposium on Bacterial Genetics and Ecology (BAGECO) in Lisbon, Portugal.

Professor Costa authored more than 70 scientific publications encompassing original research articles in international journals, conference proceedings, book chapters and theses. He participated in over 150 communications (60 oral) in scientific conferences, workshops, and colloquia. Since 2010, Professor Costa was involved in 16 R&D projects (8 as PI / Co-PI).

His research addresses the diversity and function of microorganisms in natural and fabricated biomes - with emphasis on Eukaryote-Prokaryote symbioses studied with hi-end metagenomics approaches -, their implications to host/ecosystem health and climate regulation, and potential use as renewable sources of innovative biotechnological appliances.



SESSION 3: GLOBAL CHANGES AND ECOSYSTEM SERVICES

MODERATION: FERNANDO PAGELS AND ANA MAFALDA CORREIA

KEYNOTE SPEAKER – NUNO QUEIROZ



Nuno Queiroz has 15 years' research experience of field studies on the behavioural ecology of sharks and tunas and currently holds an assistant research position at the Research Center in Biodiversity and Genetic Resources (CIBIO/InBIO), in Porto.

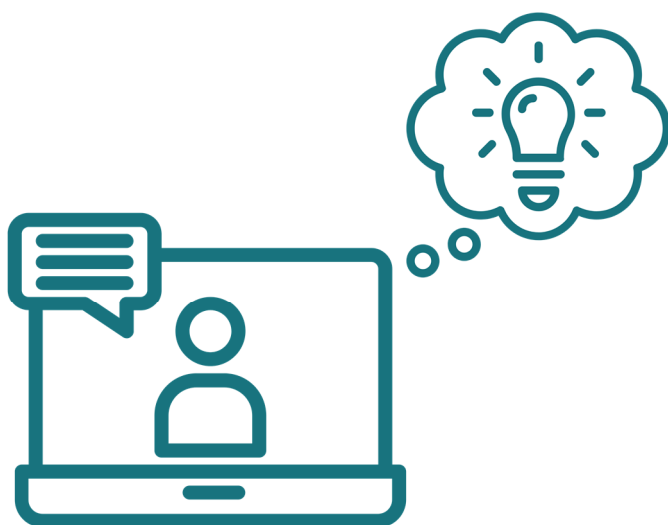
He has published more than 45 papers in ISI indexed journals with over 3000 citations, including manuscripts in Nature, Science and PNAS.

His research focuses on the ecology of predatory fish movements and spatial distributions, the environmental constraints, and the consequences for conservation. He has also innovated new approaches to the way movement and environmental big-data are analysed for testing behavioural models that help understand the causes of observed movement patterns.

A current interest is in development of novel bio-logging tags for sharks and tunas.

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ABSTRACTS

ORAL COMMUNICATIONS

Isolation, Identification and Study of the Bioactive Potential of Actinobacteria Associated with the Marine Sponge *Hymeniacidon perlevis*

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Abstract

Antimicrobial resistance and oncological diseases are two representative examples of health problems that cause most concern worldwide. Natural products from microbial origin are recognized as a rich source of biologically active molecules with potential pharmaceutical applications. In this work, we studied the cultivable actinobacterial community associated with a marine sponge common in the Portuguese coast, *Hymeniacidon perlevis*, and investigated the capacity of the recovered isolates to produce compounds with antimicrobial and anticancer properties. A sample of *H. perlevis* was collected at Praia da Memória, in Northern Portugal. For the isolation of actinobacterial strains, fragments of the sponge were subjected to three different pre-treatments and inoculated on three selective culture media. 16S rRNA gene analysis revealed 82 isolates associated with the phylum Actinobacteria, with the largest fraction being affiliated with the genus *Streptomyces*. Isolates belonging to the genera *Gordonia*, *Nocardia*, *Nocardiopsis*, *Dietzia*, *Tsukamurella*, *Micromonospora*, *Micrococcus*, *Brachybacterium*, *Glutamicibacter*, *Rhodococcus* and *Paenoarthrobacter* were also recovered.

Fifty-seven actinobacterial organic extracts were tested for antimicrobial activity, using the agar-based disc diffusion method and minimal inhibitory concentration (MIC) was subsequently evaluated for extracts showing relevant bioactivity. Seven extracts (all obtained from isolates of the genus *Streptomyces*) showed antimicrobial activity against *Bacillus subtilis* and/or *Candida albicans*, with MIC values between 15.62 and 125 µg mL⁻¹. Anticancer activity was also evaluated and was tested in two human cancer cell lines (human liver cancer HepG2 and human colorectal cancer HCT-116) using the MTT method. Forty-one extracts were able to reduce the cell viability of at least one cancer cell line. Of these, 6 extracts had strong activity against the cell line HepG2, reducing its viability in more than 80% after 48 h of exposure, and 4 extracts reduced in more than 40% the viability of the cell line HCT-116. The results obtained in this study showed that *H. perlevis* is rich in Actinobacteria with promising antimicrobial and anticancer activities, opening good perspectives for the discovery of novel bioactive molecules.

Keywords – marine actinobacteria; marine sponges; bioactive compounds; *Hymeniacidon perlevis*; antimicrobial; anticancer

Alga epibiont on mussel beds – Co-engineering effects

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Abstract

On the rocky shore of the intertidal, organisms such as macroalgae and mussels share the same habitat. Both have a fundamental role in terms of biodiversity, as they can contribute to the increase of diversity, not only due to their activity, but also due to their lifestyle, being considered as ecosystem engineers. Specifically, in the intertidal zone of the north of Portugal, red algae of the genus *Caulacanthus* occasionally live as epibiont above mussel beds of *Mytilus galloprovincialis* Lamarck, 1819. Although the importance of each engineer separately in the ecosystem is recognized, knowledge concerning the interactive effects of these two organisms is still limited. Thus, the objective of this study is to explore how the presence of *Caulacanthus* spp. as co-engineer on *M. galloprovincialis* beds can affect their attributes and associated macrofauna. For this purpose, four dates were sampling and in each date, two different sites were selected from a rocky shore in the north of Portugal and, at each site, five quadrat (10 x 10 cm) replicates of mussels with and without alga were collected. Univariate and multivariate analyses were used to test the effect of presence of alga on the density, size and condition index of mussels and on the total number of individuals, taxa number, Shannon index, Pielou index and the structure of their associated macrofauna assemblages. Concerning mussel attributes, results showed that the presence of *Caulacanthus*, as a co-engineer on mussel beds, only affected the condition index, with a significant interaction between date and condition. Regarding mussel-associated fauna, analyses revealed significant differences between conditions for the diversity, Shannon index and Pielou index; for the structure of the whole assemblage, a significant interaction between date and condition was also observed. Overall, it can be concluded that *Caulacanthus* spp., acting as co-engineer of *M. galloprovincialis*, played an important role on mussels' attributes and associated macrofauna.

Keywords – Co-engineer; *Caulacanthus* spp.; *Mytilus galloprovincialis*; mussel attributes; associated macrofauna

Acknowledgments – This research was developed under the Project No. 30181, co-financed by COMPETE 2020, Portugal 2020 and the European Union through the ERDF, and by FCT through national funds. PhD grant: SFRH/BD/114935/2016 by FCT

Three Ascidians Tunic from a Metaproteomic Perspective

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Abstract

Marine ecosystems encompass a huge biodiversity, being exceptional reservoirs of natural compounds. In the last decade, marine invertebrates of the extremely diverse Ascidacea class (Chordata, Tunicata) gained the attention of the scientific community. Beyond their proximity to vertebrates, this interest lies on their associated bioactive potential – highly related to the richness of organisms embedded in their tunic (*i.e.* Actinobacteria, Cyanobacteria, Proteobacteria phyla), with benefits for the involved host-symbiont organisms.

Considering these studies, this work aims to overview the symbiotic relationships described worldwide in ascidians and their benefits to the involved organisms. Moreover, in this work was explored the tunic of three ascidian specimens – *Ciona* sp., *Molgula* sp. and *Microcosmus* sp. – collected in Portugal using a shotgun proteomic approach. Briefly, the extracted proteins were processed with a nano LC-MS/MS system and the resulting raw data was searched against the Bacteria and Metazoa sections of the UniProtKB, using the Proteome Discoverer software. The proteins identified were combined with a non-redundant Antimicrobial Peptides (AMPs) database and further analysed with MaxQuant freeware.

The results of this work highlight that ascidians take several advantages from symbiotic associations but also contribute to the survival and life cycle of other species. Furthermore, the shotgun proteomic analysis revealed that ascidians tunic is very dynamic by uncovering a total of 443 proteins (337 Metazoan and 106 Bacterial) and 37 AMPs, namely bacteriocins. Allowed, also, to detect host-microbe interactions by detecting proteins with antagonistic activities in the same ascidian sample but from different sections; *e.g.* lysyl endopeptidase found in Bacteria section and aprotinin with serine-type endopeptidase inhibitor activity in Metazoa section. In addition, “Biosynthesis of antibiotics” was one of the most highlighted pathways detected.

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Overall, this exploratory study gave insights about host-microbe interactions and revealed the high potential of shotgun proteomics approaches in the characterization and biodiscovery of the ascidians' tunic and its microbiome.

Part of the work that will be presented was published at Mar. Drugs 2020, 18, 362; doi:10.3390/md18070362.

Keywords – Ascidiacea; Symbionts; Shotgun proteomics; AMPs; Biotechnological potential

Evaluation of the effects of dietary tryptophan on growth performance, feed utilization, biochemical parameters, gene expression and locomotor behavior of zebrafish (*Danio rerio*) juveniles

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Abstract

Tryptophan (Trp) is an essential amino acid, used for protein synthesis and several metabolic functions. Also, it is the only serotonin precursor, a neurotransmitter with relevant physiological and behavioral roles. Serotonin synthesis follows a dose-response mechanism with dietary Trp. This study aimed to evaluate the effects of increasing levels of dietary on growth performance, feed utilization, biochemical parameters, gene expression, and locomotor behavior in zebrafish juveniles.

A growth trial was conducted with zebrafish juveniles fed isoproteic (40%DM) and isolipidic (8%DM) diets supplemented with five graded levels of Trp at 0.19, 0.38, 0.76, 1.52, 3.04%DM (diets, Trp0, Trp2, Trp4, Trp8, Trp16, respectively). Diets were tested in quadruplicate, with fish being fed twice a day, six days a week for six weeks. At the end of the trial, brain, muscle and liver from fish of groups fed Trp0, Trp4 and Trp16 were for serotonin analysis (in brain), acetylcholinesterase activity (in brain and muscle) and expression of genes (in liver) involved in protein accretion and degradation pathways (IGF1, TOR, UBE2H), the serotonin pathway (serotonin receptors: Htr2cl1, Htr1aa; serotonin transporter: Slc6a4a; Trp hydroxylase: TPH1a, TPH1b, TPH2a, TPH2b) and regulation of cellular protective responses against electrophilic and oxidative stresses (Keap1, Nrf2). Also, fish from each of the referred dietary treatments were analyzed in a video tracking system for locomotor behavior. Finally fish from all dietary treatments were sampled for whole-body composition analysis.

Results showed that dietary Trp levels did not influence growth performance nor feed utilization. However, increasing levels of Trp linearly increased brain serotonin levels. Moreover, a quadratic response was found for brain acetylcholinesterase activity in relation to Trp levels, although muscle activity was not affected. Dietary treatment also influenced gene expression, Trp4 lowering the relative expression of Htr2cl1, and Trp4 and Trp16 lowering the relative expression of Htr1aa;

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additionally, increasing linear tendencies with the Trp dietary level were found in TOR and Keap1 relative expression, and a negative linear expression was found for TPH1a.

Locomotor behavior was also affected by dietary treatment with Trp4 associated to a less stressful behavior, while Trp0 and Trp16 to a more stressful behavior.

Keywords – Tryptophan; Zebrafish; Acetylcholinesterase; Serotonin; Growth; Gene Expression; Locomotor Behavior

Flavonoid derivatives as a sustainable strategy to prevent marine biofouling: synthesis and antifouling studies

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Abstract

Marine biofouling, caused by the accumulation of marine micro and macroorganisms on submerged surfaces, has huge material and economic impacts for maritime industries, as well as environmental problems due to the spread of invasive species. Antifouling paints with tributyltin (TBT) were extensively used to prevent the attachment of fouling organisms but, due to their toxicity, these paints were banned. Following, antifouling paints with copper and booster biocides were introduced, but even these compounds have proven to be toxic to the environment. Therefore, the research for new antifouling compounds is an urgent request [1,2].

Previous studies performed by our group identified some flavonoids with antifouling activity [3,4]. Based on these results, a series of sixteen flavonoids was synthesized and evaluated for their antifouling activity. Chalcones were prepared through Claisen-Schmidt condensation of appropriately substituted acetophenones with benzaldehydes. The synthetic approach for the synthesis of flavones was based on the reaction between phloroglucinol and β -ketoesters, the Mentzer Pyrone synthesis [5]. Antifouling activity was investigated using the *in vivo* anti-settlement bioassay with *Mytilus galloprovincialis* larvae [3,4]. Afterwards, the most potent compounds were tested for ecotoxicity to non-target marine organisms using the brine shrimp *Artemia salina* nauplii lethality test. The promising antifouling compounds were also non-toxic at 50 μ M, while ECONEA®, a commercial antifouling agent, showed 100% lethality at the same concentration [3,4]. Moreover, some studies to evaluate the mechanism of action were performed.

At the end of this study, structure-activity relationship studies were carried out that allowed us to conclude that chalcones showed higher capacity to inhibit the adhesion of mussel larvae than flavones, being potential lead compounds for the discovery of new non-toxic antifoulants.

Keywords – Biofouling; Flavones; Chalcones; Synthesis; Antifouling 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 and under the projects NASCEM- PTDC/BTA-BTA/31422/2017 (POCI-01-0145-FEDER-031422) financed by FCT, COMPETE2020 and PORTUGAL2020, PTDC/SAU-PUB/28736/2017 (reference POCI-01-0145-FEDER-028736) and PTDC/AAG-TEC/0739/2014 (reference POCI-01-0145-FEDER-016793 (Project 9471-RIDTI)) co-financed by COMPETE 2020, Portugal 2020 and the European Union through the ERDF, and by FCT through national funds. Daniela Pereira and Catarina Gonçalves acknowledge for her grants (SFRH/BD/147207/2019 and NASCEM/BI-Lic/2019-53, respectively).

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Swine blood hydrolysates as promising ingredients for European seabass (*D. labrax*) diets

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Abstract

In this work, we hypothesised that peptide-rich swine blood hydrolysates (BH) could be beneficial ingredients for European seabass diets, due to their potential antioxidant, mineral-binding, immunomodulatory or antimicrobial activities. Furthermore, this inclusion could address *T. maritimum* infections that occur in aquaculture farms, with high impact on fish well-being and aquaculture production, while promoting circular economy.

Three fractions of swine BH were studied: one obtained by autohydrolysis (AH); two obtained enzymatically and submitted either to a micro- (MF) or nanofiltration (NF). Dried hydrolysates were included in isolipidic and isoproteic diets for European seabass: a fishmeal (FM) based diet (positive control, PC), a commercial-based diet where 50% of FM was replaced by vegetable proteins (negative control, NC) and three diets where 3% of each BH was added to the NC. Diets were assigned to triplicate groups of 71 juveniles (initial weight 12.3 ± 1.4 g), fed three times daily *ad libitum* in a recirculating saltwater system. Growth, nutrient utilisation and whole-body composition were evaluated after 12 weeks. At the end of the trial, ten fish per tank were infected with *Tenacibaculum maritimum* (3.5×10^5 cfu/L), in a two-hour water bath, and mortality was assessed for 8 days.

Fish fed PC had the highest final weight, followed by NC and NF. NC had a significantly higher weight than the MF and AH groups. Specific growth rate (SGR) and feed conversion ratio (FCR) of fish fed NF showed no significant differences from PC and NC. Fish fed AH also displayed similar SGR to NF and NC but significantly higher than MF. Despite no differences regarding final body composition, lipid and energy retention and gain were lowest in fish fed MF, which also had the lowest condition factor. Protein retention was unaffected by dietary treatments. Regarding the infectious challenge, NF had the lowest mortality rate, differing significantly from the NC and MF

groups. Mortality for AH and PC groups did not differ from NF. MF showed the highest mortality. Overall, results indicate that dietary supplementation with NF improves European seabass resistance to *T. maritimum* without affecting growth rate and can hence become a valuable ingredient for this species.

Keywords – Blood hydrolysates; fish robustness; circular economy; bioactive peptides; *Tenacibaculum maritimum*

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. We would also like to thank the scientific collaboration under the FCT project UID/Multi/50016/2019. DR thanks FCT, SANFEED and SenseTest© for her PhD grant (PD/BDE/150524/2019).

Isolation of chitinolytic bacteria from the gut microbiota of European sea bass fed with insect meals

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Abstract

Aquaculture growth has rendered fish meal (FM) economically and ecologically unviable. Among the alternative ingredients identified, insect meal (IM) is one of the most promising. IM has high protein and lipid contents and a balanced amino acid profile. However, insects are also rich in chitin, which may negatively interfere with fish performance and nutrient digestibility. To overcome potential chitin drawbacks, this study aimed to isolate, from the European sea bass gastrointestinal tract, chitinolytic bacteria capable of improving the use of high IM-containing diets. Based on the enhanced adaptability of gut microbial communities, selective pressure was applied through the use of chitin-enriched diets. Five isoproteic (45%) and isolipidic (18%) diets were formulated: a control FM based-diet (CTR), the CTR diet supplemented with 5% of chitin (CHIT5), and 3 diets with the inclusion of 25% of *Hermetia illucens* (HI) larvae meal (HM25), *Tenebrio molitor* larvae meal (TM25) or *Hermetia illucens* exuviae meal (HEM25) as partial FM substitute. Aerobic sporulating bacterial isolates were targeted resulting in 366 isolates. These isolates were screened for sporeforming and chitin metabolism abilities, resulting in 40 putative probiotic (PRO) isolates. Taxonomic identification of the isolates followed by hemolytic activity characterization narrowed the isolate number to 21. These isolates will be screened for antibiotic resistance, gut-survival, and bioreactors upscale suitability. The best PRO candidates will thereafter be assessed *in vivo* for their ability to improve dietary chitin utilization and fish disease resistance.

Keywords – Aquaculture; Insect meal; Sporeformers; Chitin; Probiotics

Effects of chemical modulators in rearing water on growth performance, digestive enzyme activity and innate immune response in European seabass (*Dicentrarchus labrax*)

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Abstract

Intensive aquaculture production increases the risks of disease outbreaks. Prophylactic antibiotics, high dosages of UV radiation and/or ozonation are the most common methods used to control pathogenic bacteria. These methods may negatively affect fish performance, interfering as well with the existing bacterial community. The use of chemical modulators in the water is presented as an alternative to the referred disinfection methods, especially because they may improve the antioxidant capacity and immune function in fish.

Humic acids are organic substances found in natural water systems that has been identified as a chemical modulator that may interfere with fish disease treatment and/or immune status improvement. In a similar way, some “in vitro” studies using *Ecklonia bicyclis* extracts, also indicates that this edible marine brown algae (natural from Japan, Korea and China) may contribute to a better fish health state.

Seabass juveniles were maintained in seawater in four conditions: with dissolved Humic Acid (HA) (2.5 mgL⁻¹), with *E. bicyclis* extract (2.5 mgL⁻¹) (EB) or with a mixture of both 1:1 (1.25 mgL⁻¹ each) (MIX). For comparison, there were a control group that were kept under similar experimental conditions, but without the addition of chemical modulators in the water. At the end of the 56-day trial, several growth (final body weight, feed conversion ratio, daily growth index and protein efficiency rate), innate immune (alternative complement, peroxidase and lysozyme), oxidative stress (catalase, lipid peroxidation, glutathione peroxidase, glutathione s-transferase, glutathione reductase, total glutathione, oxidized glutathione) and digestive enzymes parameters (amylase, lipase, chymotrypsin and trypsin) were evaluated. Total glutathione and glutathione peroxidase decreased in all chemical modulator groups, when compared with CTRL. Glutathione s-transferase showed higher activity in HA and EB groups compared to CTRL and MIX groups. The HA and EB groups showed higher glutathione reductase and catalase activities, respectively,

when compared to the other groups. The innate immune parameters were not affected by the chemical modulators. Trypsin showed higher activity in all groups, except the CTRL group. Chymotrypsin showed lower activity in HA and EB treated groups, while MIX group presented similar results to the CTRL. The MIX group showed higher lipase activity than the other groups. HA and MIX groups showed higher amylase activities than the CTRL and EB groups. Overall results indicate that chemical modulators may improve the antioxidant capacity of the fish, which will allow the fish to respond better to environmental challenges, such as temperature variations. The digestive enzymes activity was also stimulated improving the fish capacity to digest dietary nutrients.

Keywords – Humic acids; *Ecklonia bicyclis*; Antioxidant capacity; Digestive enzymes

Diversity and Biotechnological Potential of Deep-sea Actinobacteria from Madeira and Azores Islands

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Abstract

Natural products from marine microorganisms have been explored to meet the urgent need for new drugs against antibiotic-resistant bacterial infections, cancer diseases and obesity disorders. Actinobacteria are a large group of morphologically and physiologically diverse bacteria well-known for their production of biotechnologically relevant compounds. Bioprospection of Actinobacteria in underexplored and/or extreme environments, like the deep-sea, constitute one of the last frontiers for the discovery of novel valuable bioactive molecules. In this study, 63 deep-sea samples (sediments, corals and sponges) were collected from Azores and Madeira archipelagos, at depths from 150 to 3199 m, with the aim of isolating Actinobacteria and evaluate their capacity to produce bioactive metabolites. Two pre-treatments and 5 selective culture media supplemented with different antibiotics were used. Up to the moment, 126 actinobacterial strains belonging to 14 genera were isolated. Bioactivity assays conducted with crude extracts obtained from 43 actinobacterial strains, revealed 10 extracts with antimicrobial activity against one or more of the test microorganisms - *Candida albicans* (extracts from six *Streptomyces* strains), *Staphylococcus aureus* (extracts from three *Streptomyces* strains), *Bacillus subtilis* (extracts from two *Streptomyces* strains and one from a *Brevibacterium* strain) and *Escherichia coli* (extracts from two *Streptomyces* strains). Cytotoxicity assays revealed six extracts capable of reducing the cellular viability of the cancer cell lines HepG2 (three *Streptomyces* strains, one *Nocardiopsis* strain and one *Microbacterium* strain), HCT 116 (two *Streptomyces* strains and one *Microbacterium*), SH5Y-SH (one *Rhodococcus* strain) or T-47D (one *Rhodococcus* strain), in at least 40% after 48h of exposure. Anti-obesity screening assays allowed to identify six extracts (three from *Brevibacterium* strains, two from *Streptomyces* strains and one from a *Dietzia* strain) exhibiting significant neutral lipid-reducing activity after 48h of exposure.

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The next steps of this work will focus on identification of other strains, screening of their bioactive potential and investigation if novel secondary metabolites are responsible for the observed bioactivities.

Keywords – Marine Actinobacteria; Deep-sea; Antimicrobial; Anticancer; Anti-obesity

Mitogenomic Phylogeny of Freshwater Mussels (Bivalvia: Unionida)

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Abstract

Freshwater mussels (order Unionida) are a critically imperilled group of molluscs that play key ecological roles and provide important services to humans. These taxa present two divergent mitochondrial DNA molecules in males (Female or F-type and Male or M-type) as a result of Doubly Uniparental Inheritance (DUI). The available number of mitogenomes for Unionida is low, particularly for M-type genomes. A further shortcoming is that published mitogenomes are restricted to only a few higher Unionida taxa, with no mitogenomes being available for several families and subfamilies. For the subfamily Ambleminae, two out of five tribes have not had published complete mitogenomes. *Pleurobema oviforme*, *Amblema plicata* and *Popenaias popeii* each belong to tribes Pleurobemini, Amblemini and Popenaidini, respectively and have not had published mitogenomes. The mitogenomes were sequenced by Next-Generation Sequencing (NGS) using Miseq Illumina runs. Quality control of the obtained reads, assembly and annotation was carried out using an established pipeline. Phylogenetic Analyses were carried out on the mitogenomes using standard methods, e.g. Maximum Likelihood and Bayesian Inference. The newly sequenced mitogenomes all share the same gene order (UF1), and the tree produced confirms the same phylogeny of the most recent tribal classification within Ambleminae.

Keywords – Freshwater Mussels; Mitophylogenomics; Next-generation Sequencing

Quantification of chiral drugs in environmental in Douro river estuary by LC-MS/MS

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Abstract

Chiral drugs (CD) have been identified as one of the main environmental concerns due to the different forms present in the environment. The particularity of these drugs is that both metabolism and biodegradation can be enantioselective. Therefore, CDs can be detected in the environment with different values of the enantiomeric fraction (EF) [1]. In addition, the enantiomers of CDs can have differences in pharmacokinetics, pharmacodynamics and (eco) toxicity, which can affect aquatic organisms in an enantioselective way [2]. Consequently, it is important to determine the EF of these compounds using environmental monitoring programs for an accurate risk assessment. This work presents the development of an enantioselective method to quantify CDs in surface waters in the Douro River estuary. Different classes of CD were selected, such as adrenergic beta-blockers, antidepressants, stimulants, non-steroidal anti-inflammatory drugs (NSAIDs) and some illicit drugs such as cocaine and its metabolites, accounting for twenty-three target compounds. The analytical method was based on solid phase extraction (SPE) followed by mass spectrometry in tandem liquid chromatography (LC-MS / MS) with a triple quadrupole analyzer. Enantioseparations were performed using the Chirobiotic V® and Whelk-O® columns. Samples collected from five sampling points in the Douro River estuary were analyzed for seven consecutive days. Nine of the compounds were found every day, including illicit drugs such as amphetamine and methamphetamine. Tramadol and its metabolites *N*-desmethyltramadol (NDT) and *O*-desmethyltramadol (ODT) were also found, with tramadol and NDT being found at all sampling points, but NDT at a concentration about six times higher than tramadol.

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Polyoxygenated xanthenes with skin anti-aging potential

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Abstract

The marine environment is extremely rich and holds a variety of organisms capable of producing substances with potent biological activity. Xanthenes are heterocyclic polyphenolic compounds that can be obtained from terrestrial and marine sources, as well as by synthesis [1]. *In silico* and *in vitro* studies have suggested that simple hydroxylated xanthenes with vicinal diol groups have promising antioxidant activity [2]. In the cosmetic industry, antioxidants from marine sources have long been used to prevent or minimize skin aging, making the search for new compounds in this area a high priority [3]. Considering that, trioxxygenated xanthenes were hypothesized as promising antioxidants with potential as skin anti-aging agents.

Herein, a trioxxygenated xanthone, 1,4-dihydroxy-3-methoxy-9*H*-xanthen-9-one, was successfully synthesized through a route involving acyl radical cyclizations [4]. The referred xanthone and four other polyoxygenated xanthenes synthesized in previous works were studied for their potential as skin anti-aging agents. The antioxidant activity of the xanthenes was evaluated by the DPPH assay and two of the tested compounds showed IC₅₀ values in the same range as the ascorbic acid. Regarding their capacity to inhibit skin-degrading enzymes, almost all the compounds were excellent tyrosinase inhibitors, more active than kojic acid, and were weak to moderate collagenase inhibitors. Additionally, the metal chelating effect (FeCl₃ and CuCl₂) as well as the

stability at different pH values were studied to understand their potential to be used as future cosmetic agents. 1,2-Dihydroxyxanthone exhibited a dual ability to protect the skin against UV damage by combining antioxidant/metal chelating properties with UV-filter capacity and revealed to be more stable in the pH range that is closest to the pH of the skin. Lastly, the phototoxicity of 1,2-dihydroxyxanthone was evaluated in a human keratinocyte cell line and no phototoxicity was observed in the concentration range tested.

Keywords – skin; photoaging; antioxidants; xanthenes; 1,2-dihydroxyxanthone

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Algae-associated Actinobacteria: unveiling their diversity and potential to produce novel bioactive compounds

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Abstract

Bioprospecting microorganisms from untapped marine sources for the discovery of chemical novelty may represent a solution for clinically relevant problems, such as infections caused by antibiotic multi-resistant bacteria, cancer pathologies and metabolic diseases. Actinobacteria, well-known prolific producers of bioactive secondary metabolites, often live in association with marine organisms such as macroalgae, representing a promising - yet poorly explored – resource of novel bioactive compounds. In this work, cultivable Actinobacteria associated with Chlorophyta, Rhodophyta and Phaeophyceae macroalgae, collected in rocky shores in northern Portugal, are being studied and screened for their potential to produce compounds with antimicrobial and/or cytotoxic properties. The actinobacterial strains were isolated using selective pre-treatments and culture media, followed by the taxonomic identification of the isolates. According to 16S rRNA analysis, isolates recovered so far are mainly affiliated with the genus *Streptomyces*, but 10 other genera – including some known as rare - were also retrieved: *Gordonia*, *Isoptericola*, *Kitasatospora*, *Microbacterium*, *Microbispora*, *Nocardia*, *Nocardiopsis*, *Nonomuraea*, *Rhodococcus* and *Verrucosipora*. Antimicrobial activity against *Candida albicans* and/or *Staphylococcus aureus* was detected for some isolates, with minimum inhibitory concentration (MIC) values ranging from 0.487 to 1000 µg mL⁻¹. The cytotoxic activity of some actinobacterial strains was also tested on two human cancer cell lines (breast carcinoma T-47D and neuroblastoma SH-SY5Y) and in a non-carcinogenic endothelial cell line (hCMEC/D3). Seven extracts were found to only compromise the viability of cancer cells, translating into a possible anticancer asset. Although dereplication data revealed the presence of antimycins in most active extracts, it also unveiled the likely presence of unknown bioactive compounds that will be further studied by bioactivity-guided protocols. Results obtained so far uncovered not only the rich algae-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; macroalgae

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Biogeographic patterns, diversity and distribution of Arctic's protists community

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Abstract

Climate change is affecting the Arctic Ocean through the decrease of the sea ice extent and thickness. As sea ice gets thinner and with the replacement of multi-year ice (MYI) with first-year ice (FYI), the dynamics of phytoplankton and primary productivity has been changing. These changes are radically redefining the biogeography of marine Arctic ecosystem, leading to a redistribution of species, however the consequences of this biological reorganization are not yet known. In our ongoing research we proceed with a comprehensive comparison between the 18S metabarcoding approach, using next generation sequencing techniques (NGS), and the classic microscope cell identification. Our aim, is to study the biogeographic patterns of Arctic phytoplankton diversity and distribution along two oceanographic transects in the Marginal Ice Zone (MIZ) around Svalbard. Microbial phytoplankton samples were concentrated on board through filtration, during an Arctic oceanographic campaign, led by Norwegian Polar Institute (NPI), and sent to CIIMAR (Portugal, Porto) for following environmental DNA extraction and metabarcoding analysis. Microscope phytoplankton counts and identification were performed on board in fresh samples, then preserved samples were also later analysed at IO PAN labs (Sopot, Poland). Results, revealed that the Eukaryotic data set comprises highly complex and diverse protists community structure, with a marked biogeographic pattern of the protists communities along the Svalbard Marginal Ice Zone with strong links identified between protists communities distribution and environmental gradients along the transects. This research is relevant to fill critical gaps concerning the evaluation of the response of the changing eukaryotic microbiome in response to climate change impacts.

Keywords – Climate Change; Arctic Ocean; Arctic Phytoplankton; 18S Metabarcoding; Microscopic cell counts

Fish-gut *Bacillus* spp. harbour promising Quorum-Quenching molecules to control aquaculture bacterial pathogens

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Abstract

Bacterial disease outbreaks are one of the biggest constraints to the development of aquaculture. The pathogenicity of bacterial organisms that infect aquaculture systems is closely related to the expression of virulence factors and the formation of biofilms, both regulated by quorum-sensing (QS). Hence, disruption of bacterial QS, known as Quorum-Quenching (QQ), has been proposed as a novel approach to control bacterial fish pathogens. *Bacillus* spp., sporeforming gram-positive bacteria, have recognizable biotechnology applications, namely as probiotic health-promoting agents or as a source of natural antimicrobial molecules. Here we characterized the potential of fish-gut *Bacillus* spp. in producing QQ compounds capable of suppressing fish pathogens QS. *Bacillus* spp. QQ-potential was evaluated by using *Chr. violaceum* biosensors that produce a purple violacein pigment in response to QS-related molecules, such as Acyl-homoserine lactones (AHLs). We found out that ~12% of *Bacillus* spp. isolated from the gut of aquaculture fish species have the potential to interfere with AHLs. We further selected 9 isolates as producers of extracellular molecules capable of enzymatically degrade AHLs. Moreover, 8 of these isolates amplified putative QQ genes – *aiiA* and *ytnP*, encoding an acyl-homoserine lactone metallo- β -lactamase. To evaluate the isolates' extracellular QQ capacity against fish pathogens QS, we targeted the main bacterial organisms known to cause disease in aquaculture, some considered emerging zoonotic agents, including *Aeromonas hydrophila*, *A. salmonicida*, *A. bivalvium*, *A. veronii*, *Vibrio anguillarum*, *V. harveyi*, *V. parahaemolyticus*, *V. vulnificus*, *Photobacterium damsela*, *Tenacibaculum maritimum*, *Edwardsiella tarda*, and *Shigella sonnei*. Since gram-negative bacteria, including fish pathogens, regulate their QS through AHLs, we tested these fish pathogens for AHLs production using the same biosensor. We observed that *E. tarda* produce AHLs molecules detectable by the biosensor, and when subjected to the *Bacillus* spp. extracellular QQ molecules, the *E. tarda* QS-related molecules were enzymatically degraded by

FI314, FI436 and FI464, identified as *B. subtilis*, *B. velezensis* and *B. pumilus*, respectively. Taken together, our results identified three promising candidates to become the source of bioactive molecules to be used in the fight against aquaculture bacterial diseases. The identification of these molecules is currently under study.

Keywords – Aquaculture; Fish diseases; Sporeformers; Quorum-Quenching; N-acyl-homoserine lactones

Differential Cetacea Circadian Rhythmicity is associated with the molecular erosion of Cortistatin

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Abstract

The ancestors of Cetacea underwent profound morpho-physiological alterations. By displaying an exclusive aquatic existence, cetaceans evolved unique patterns of locomotor activity, vigilant behaviour, thermoregulation and circadian rhythmicity. Deciphering the molecular landscape governing many of these adaptations is key to understand the evolution of phenotypes. Here, we investigate Cortistatin (*CORT*), a neuropeptide displaying an important role mammalian biorhythm regulation. This neuropeptide is a known neuroendocrine factor, stimulating slow-wave sleep, but also involved in the regulation of energy metabolism and hypomotility inducement. We assessed the functional status of *CORT* in 361 mammalian genomes (25 orders), including 30 species of Cetacea. Our findings indicate that cetaceans and other mammals with atypical biorhythms, thermal constraints and/or energy metabolism, have accumulated deleterious mutations in *CORT*. In light of the pleiotropic action of this neuropeptide, we suggest that this inactivation contributed to a plethora of phenotypic adjustments to accommodate adaptive solutions to specific ecological niches.

Keywords – gene loss; mammals; circadian; sleep

Occurrence, abundance, and characteristics of microplastics along the northern coastal waters of Portugal

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Abstract

Microplastics (MPs - plastic particles with less than 5 mm) are considered one of the most emerging aquatic pollutants, attracting significant concern and interest in the scientific community. Due to their size and ability to adsorb pollutants, microplastics pose a concerning threat at the individual and ecosystem level of biological organization. To evaluate the current situation on the northern coast of Portugal, samples from aquatic environments with different levels of human impacts were collected, including coastal marine protected area, urban estuary, a submarine wreck, and a recreational marina. An optimized protocol was used for the quantification of microplastics and visual identification, followed by FTIR spectroscopy analysis to confirm and characterize microplastics (polymer, type, size, color). A total of 183 samples were collected between December 2016 and July 2020. One hundred eighty-two of those contained microplastics and a total of 2 692 MPs particles have been identified and classified. Microplastics concentration ranged from 0.0021 to 42000 MPs particles/m³, showing very different levels of microplastic pollution among sites. Microplastics observed included fibers, fragments, and film and granules or foam were absent from our samples. Up to 51% of microplastics had a size range between 1-3 mm. Microplastics were found in a variety of colors with transparent being the dominant one, followed by white, black, and blue. Polyethylene and polypropylene were the main types of microplastic polymers detected. This study showed that microplastics were present in all the aquatic environments surveyed, however higher concentrations were observed in areas with more anthropogenic activities emphasizing the need for measures to properly manage plastic litter and mitigate plastic pollution.

Acknowledgements – NetTag project financed by EMFF-EASME and national funds through FCT - Foundation for Science and Technology within the scope of UIDB/04423/2020 and UIDP/04423/2020 and a PhD fellowship to S. Rodrigues (SFRH/BD/145736/2019).

Keywords – Plastic pollution; surface waters; anthropogenic activities; plastic debris.

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ABSTRACTS

POSTER COMMUNICATIONS

Biosynthetic diversity of Arctic ocean microbiomes

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Abstract

Polyketide synthase (PKSs) and Non-ribosomal peptides synthetase (NRPSs) are two mega enzymes responsible for the biosynthesis of a large fraction of relevant natural products (NPs). Molecular markers targeting biosynthetic genes, such as ketosynthase (KS) domain of PKSs have been used to assess the diversity and distribution of biosynthetic genes of bacterial isolates and complex microbial communities. More recently, metagenomics studies have complemented this approach by allowing the recovery of complete biosynthetic gene clusters (BGCs) from environmental DNA. The Arctic ocean is one of the least exploited ecosystems regarding bioprospection. In this study, the biosynthetic diversity from water samples collected at north of Svalbard during N-ICE2015 expedition was assessed using PCR-based strategies coupled with high-throughput sequencing and metagenomics analysis. Obtained results have revealed the samples assessed are mostly rich NRPS, lantipeptides, saccharides and fatty acids. From the 74 recovered Metagenome assembled genomes (MAGs) it was possible to extract 179 BGCs using anti-SMASH. A network analysis performed using BiG-SCAPE identified potential new NP families, such as those belonging to non-ribosomal peptides and polyketides. A detailed description and comparison of results obtained using both approaches (amplicon and metagenomics) and of the most promising samples in study will be presented.

Keywords – natural products; metagenomics; Arctic; ocean

‘Bellow the eye’: using eDNA for the evaluation of environmental water quality in a tropical urban estuary

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Abstract

The Sergipe River's estuary is located within a densely populated area of the city Aracaju, Sergipe State, Brazil. High levels of anthropogenic activities are observed in this area. These activities lead to an increased volume of raw domestic sewage and wastewater from industrial processes. Besides industrial activity, shrimp aquaculture and deforestation of mangroves contribute to poor water quality. Domestic sewage inputs across Brazil lead to measurable environmental changes in the coastal aquatic ecosystems. Environmental DNA (eDNA) is a relatively novel tool that uses genetic material harvested from the environment to provide specific information when morphological identification becomes unreliable. An eDNA approach to monitoring bacterial diversity in freshwater courses can alert to environmental changes due to anthropogenic impacts and pollution, and even to provide a water quality assessment.

For this study, seven points (numbered 1 to 7) along the Sergipe river estuary (SE) were sampled, and two points (numbered 1 and 2) at the Real river estuary (RE) were used as control sites. Water samples were filtered through Strivex filters, followed by DNA extraction. The extracted DNA was amplified for 16S gene and sequenced in Illumina platform (PE 250bp). For data analysis, the QUIME2 software was used. The results indicate that RE2 presents a clear separation from the remaining samples, unlike RE1 that is relatively aligned with all the impacted sites, except for SE4. The two most abundant phyla found, both in sampling and reference sites, belonged to Proteobacteria and Terrabacteria and are known to be core bacteria in the population present in lotic waters. As expected, sewer indicator bacteria such as *Acinetobacter* and *Arcobacter* genera were present in the sites impacted by anthropogenic activity. Species from the *Bifidobacterium* genera, commonly found in human intestinal microflora, were also found in the impacted sites. These results indicate that action should be taken in order to safeguard the estuarine/marine environments and the local population. The presence of harmful bacteria may

also impair local fishing and aquaculture activities. In conclusion, the present data, when allied with data from algae and fish diversity, will allow a better understanding of the estuary of the Sergipe river.

Acknowledgements – This project and Dr Nuno Ferreira was supported by an MSCA COFUND Fellowship (H2020-COFUND-SIRCIW>MINT-512202) through the European Union, the Welsh Government and Cardiff University. The project was also partially supported by the EU and FCT/UEFISCDI/FOR-MAS in the frame of the collaborative international consortium REWATER, financed under the ERA-NET Cofund WaterWorks2015 (Water JPI), and by national funds through FCT (Portuguese Foundation for the Science and Technology) within the scope of UIDB/04423/2020 and UIDP/04423/2020.

Keywords – eDNA; *Bifidobacterium*; Sergipe river; sewage

Past Chemicals, present problems: Does TBT alter behaviour and transcriptional pattern in *Triops longicaudatus*?

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Abstract

Tributyltins (TBT) are organotins introduced in the '60s in the environment as biocides that are forbidden in almost every country since 2008. Due to its persistence, it can be found in almost every type of aquatic ecosystems. It is considered an endocrine disruption, mainly through the activation of the retinoic acid receptor α (RXR α) and the complex with the proliferator-activated receptor γ (PPAR γ -RXR). In a previous study performed by Pedro *et al.* (2019), Tadpole shrimps from the species *Triops longicaudatus* exposed to a sublethal dose of TBT (0.243 μ g/L), showed a distinct behaviour pattern from the non-exposed ones. *T. longicaudatus* are small crustaceans considered living fossils. Their physiology, easy culture/maintenance, short life cycle, among other characteristics, make them a perfect ecotoxicological model. As so, the study of the mechanisms behind the effects of TBT in these organisms can be a useful tool for toxicodynamic research. In order to identify the toxicological effects of TBT on these organisms, Tadpole shrimps were exposed to TBT at a concentration of 0.243 μ g/L, and collected after 1 hour to related the impacted genes with the observed behaviour parameters and after 6 hours to evaluate metabolic changes such as the activation or deactivation of pathways that indicate a response by the animal to the stress. The behaviour pattern was registered by video-tracking. A whole-body RNA extraction was performed and obtained molecules sequenced by PE 150bp, 6Gb using Illumina NovaSeq 6000. Reads were mapped against a draft genome and transcriptional differences were calculated between the control reads and the ones exposed to TBT for further evaluation. The behaviour alterations observed in a previous study performed by Pedro *et al.* (2019) could be linked to specific altered metabolic pathways. After the 6th hour of exposure the impact of TBT showed a different pattern mainly through an intensification of pathways related to detoxification. These results can be used to develop further research into the mechanism of TBT toxicity but also for mechanisms of metal handling and detoxification or even reproductional effects in *T. longicaudatus*.

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Keywords – Tadpole shrimp; RNAseq; TBT; RXR; PPAR

Different extraction methodologies reveal anti-obesity and anti-inflammatory properties of microalgal species

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Abstract

Microalgae are known as producers of proteins and lipids, but also of valuable compounds for human health benefits (e.g. polyunsaturated fatty acids PUFA's; minerals, vitamins, or even pharmaceutical compounds). The overall objective of this research is to develop novel products or nutraceuticals from microalgae for application on human health, and concretely on metabolic diseases.

Chlorella sp, *Chlorococcum sp* and *Nannochloropsis sp* were grown under autotrophic conditions, and *Chlorella sp* additionally under heterotrophic conditions. Microalgae biomass was extracted by various organic solvents and performing different conditions of ohmic treatment. Those extracts were evaluated for their bioactivities, toxicity, and metabolite profiles. Some of the extracts reduced the neutral lipid content using the zebrafish larvae fat metabolism assay or decreased the LPS-induced inflammatory reaction in RAW264.7 macrophages. Furthermore, anti-hepatic steatosis activity was evaluated in fat-overloaded HepG2 liver cells. Toxicity was not observed in the MTT nor the SrB cellular assays *in vitro*, or by the appearance of lethality or malformations in zebrafish larvae *in vivo*. Differences of metabolite profiles of microalgal extracts obtained by UPLC-LC-MS/MS and GNPS analyzed will be presented, linked to observed bioactivities.

In conclusion, microalgae species demonstrated anti-obesity and anti-inflammatory activities *in vitro* and *in vivo*, and could be valuable resources for the development of future nutraceutical products.

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 Internationalization (POCI) and PORTUGAL2020, and national funds through FCT UIDB/04423/2020 and UIDP/04423/2020.

Keywords – Microalgae; zebrafish; bioassay; lipids; inflammation; metabolites

Does the total substitution of fishmeal by *Tenebrio molitor* larvae meal in diets for European seabass have impact on muscle growth?

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Abstract

In 2017, the use of insects as protein source for aquafeeds was authorized by European Union. *Tenebrio molitor* larvae meal (TM) is a valuable protein source (up to 70% DM) with well-balanced amino acid profile, and a well-established industrial production. The success of aquaculture not only depends on fish growth, but also on the ability to produce high quality products for consumers. Muscle tissue represents the edible part of fish, being flesh firmness one of the most appreciated characteristics. Fillet texture is largely dependent on muscle cellularity which also determines fish growth potential. Thus, this study aimed to evaluate the impact of defatted TM (TMd), to substitute fishmeal (FM) in diets for European seabass. Four isonitrogenous diets were formulated to replace increasing levels of FM by TMd: 0, 40, 80 and 100% (CTRL, TM40, TM80 and TM100, respectively). Each diet was assigned to triplicate groups of 25 fish (55 ± 5 g) fed 3 times daily, in a recirculating saltwater system (35‰, $22 \pm 1^\circ\text{C}$). After 10 weeks of feeding, fish were individually weighed and measured. Muscle from 3 fish/tank was collected for evaluation of gene expression and cellularity. Overall, fish tripled their weight. Feed intake was lowest in fish fed TM100, but these fish had the best FCR resulting in similar final body weight and final condition factor between treatments. Fish fed TM80 and TM100 had lower expression of myogenic factor 2 (*myod2*) than those fed CTRL, while myostatin (*mstn*) expression only was lower in fish fed TM100 when compared to CTRL. A compensatory expression of muscle atrophy F-box/atrogenin 1 (*mafbx/atrogenin1*) and myomaker, myoblast fusion factor (*mymk*) was observed, being the expression of these genes higher in fish fed TM100 than CTRL. Muscle cellularity was similar between fish fed different treatments. These results indicate that the observed gene expression modulation was not enough to produce a significant alteration in muscle phenotype, suggesting a high potential of TMd as protein source for aquafeeds.

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Keywords - *Dicentrarchus labrax*; Insect protein; muscle growth; *Tenebrio molitor*

Preliminary biocolonization data of Artificial Reef samples in Matosinhos (North of Portugal)

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Abstract

The northeast Atlantic coastal marine environments are the place of many economic activities, including exploitation of natural resources and recreation (e.g. fisheries, aquaculture, tourism). These activities have a direct impact on biodiversity, possibly leading to its loss, and contribute to the establishment of invasive marine species. It is also known that coastal reefs are natural hotspots for biodiversity, which can be compromised due to anthropogenic pressure. As such, actions to preserve and restore coastal marine ecosystems are urgently needed. An ecological tool with potential positive effects on biodiversity is the use of artificial reefs (AR). The successful deployment of AR depends on several factors, namely location, design and materials employed. Therefore, best materials to use and biodiversity and habitat features, pre and post deployment, must be evaluated to assess its success. Common bioassessment tools include non-destructive methods (underwater visual census and remote underwater video) and destructive methods such as substrata scraping for posterior biocolonization assessment in laboratory.

The present work is included in the framework of the European funded project *3DPARE - Artificial Reef 3D Printing for Atlantic Area* which aims to develop a new generation of artificial reefs, built using 3D printing technologies, innovative designs and materials, contributing to the sustainable management and recovery of the marine ecosystems of this region. In Portugal, samples of these new AR materials are being tested in Matosinhos bay area. So far, three sampling periods were done and the preliminary results of biocolonization are shown.

Furthermore, assessment of socioeconomic impact of the AR concept and possible deployment in Matosinhos area is being prepared through a survey, based on questionnaires.

Acknowledgements – Project 3DPARE - Artificial Reef 3D Printing for Atlantic Area. Funding INTERREG Atlantic Area (EAPA_174/2016)

Keywords – Artificial Reef; Environment; Ecology; Biocolonization; Biodiversity

Symbiotic chemical signals from plants as inducers of the production of cryptic compounds in *Nostoc* spp.

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Abstract

Symbiotic cyanobacteria from the genus *Nostoc* have been pinpointed as prolific producers of structurally unique natural products. However, when grown under standard laboratory conditions, these cyanobacteria may not render as prolific as the environmental congeners since most of the metabolites encoded in the biosynthetic gene clusters (BGC) are not expressed in these conditions. Alteration of the cultivation conditions, co-cultures or use of chemical cues can be used to “awake” these BGC, leading to the production of novel bioactive compounds.

In an attempt to unlock the production of cryptic compounds, it was proposed to investigate the effects of chemical cues related with the mechanisms of host plant-cyanobacteria symbiosis (hormogonium inducing factor -HIF- and hormogonium repressing factor -HRF-) in the growth, morphology and metabolomic profiles of *Nostoc* sp. LEGE 12450 and *Nostoc punctiforme* PCC 73102. Thus, DAG (HIF) and naringin (HRF) were added to the cyanobacterial cultures along the growth curve: 4 timepoints during 35 days of culture. The effects of DAG and naringin were compared to untreated controls.

In *Nostoc* sp. LEGE 12450, DAG caused delay in the start of the exponential phase and naringin limited the growth. In *Nostoc punctiforme* PCC 73102, the chemical cues did not provoke any alteration of the growth pattern. However, differences in the vegetative cells differentiation were observed in both strains: in the group treated with DAG, there was increase of hormogonia filaments twenty-one days after seeding (t_{21}). Whereas, in the group treated with HRF, there was increase in heterocysts fourteen days after seeding (t_{14}).

The biomass and the medium were also extracted, analyzed comparatively: variations in the metabolomic profile were detected. At the end, the extracts were tested for their cytotoxic activity in HCT 116 colon adenocarcinoma cells and compared to non-treated cancer cells: the methanolic extract of the biomass with DAG retrieved twenty-one days after seeding (Dt21P) led to a decrease of more than 50% of cell viability.

Keywords – *Nostoc*; Cryptic compounds; Symbiosis chemical cues; Metabolomics; Cytotoxic compounds

Nature-based solutions to support the management of aquatic ecosystems: potential application of floating islands to Fervença river

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Abstract

Accordingly to The United Nations World Water Development Report 2018, “Nature-based solutions (NBS) are inspired and supported by nature and use, or mimic, natural processes to contribute to the improved management of water”. They delivery a wide range of ecosystem services depending on their configurations and purpose of application, being widely applied for ecosystems restoration. The restoration of ecosystems is a top priority has declared by the United Nations General Assembly, being 2021 - 2030 the UN Decade on Ecosystem Restoration. To meet the current challenges, it is thus important to look closely to the threatened ecosystems such the ones found in urban rivers and streams, that are under severe anthropogenic pressures. The Fervença River, located into Portuguese part of the Douro basin (latitude: 41°47'N; longitude 6°46'W), is about 25 km long and it is regarded as an urban river, because flows through the city of Bragança. Along its course it encounters nonpoint sources of pollution, originated by agricultural activities, and point sources of pollution from some villages located in the vicinity. The introduction of nature-based solutions, such floating wetland islands, to support the ecosystem rehabilitation of this heavily arterialized and modified river section, can contribute to (1) reduce nutrient concentrations in the water and thus, the algal blooms; (2) recreation of lost fluvial habitats, contributing to the increase of the biodiversity levels. The objective of the present approach is to evaluate the technical and economic viability of the installation of Floating islands - one of bioengineering technologies considered nature-based - in this highly modified section of the Fervença River. If viable, this solution could assist the improvement of ecological and visual quality of the surrounding urban area.

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.

Keywords – ecosystem services; water management; water quality; biodiversity; river restoration; urban river

Systemic immune responses of gilthead seabream *sparus aurata* juveniles fed microalgae-derived beta-glucans

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Abstract

This work aimed to evaluate the effects of both short- and long-term dietary supplementation with microalgae (*Phaedactylum tricornutum*) extracted β -glucans on immune genes expression, oxidative stress biomarkers and plasma parameters in gilthead seabream (*Sparus aurata*).

A practical diet was used as CTRL, whereas 3 others based on CTRL were further supplemented with a constant dose of β -glucans, derived from *Saccharomyces cerevisiae* (diet YEAST) and different extracts of *P. tricornutum* (diets PH21 and PH37). Diets were randomly assigned to quadruplicate groups of 200 gilthead seabream (initial body weight: 4.1 ± 0.1 g) that were fed to satiation three times a day for 8 weeks in a pulse feeding regimen. Fish were first fed the different experimental diets intercalated with the CTRL diet every 2 weeks. After 2 and 8 weeks of feeding, 3 fish/tank were sampled for blood and tissues collection.

All groups showed equal feed conversion ratio (FCR) and relative growth rate (RGR) values (1.2 and 3.8 %/day, respectively) attaining similar final body weight (FBW) (CTRL: 41.4 ± 1.6 g; C+: 42.5 ± 0.9 g; PH21: 42.1 ± 1.1 g and PH37: 41.9 ± 1.9 g). No changes were observed in plasma bactericidal and anti-protease activities and IgM levels among different dietary groups. However, the relative proportion of circulating lymphocytes increased in seabream fed the diet PH21 compared to fish fed the CTRL diet after 2 weeks. In contrast, seabream fed the 3 supplemented diets decreased the relative proportion of peripheral lymphocytes compared to those fed CTRL at the end of the feeding period. Liver lipid peroxidation, catalase and superoxide dismutase activities showed no differences among dietary treatments. β -glucan supplementation did not affect fish growth performance. The absence of clear effects on plasma immune parameters and oxidative stress biomarkers may suggest that dietary treatments did not activate a humoral immune response. However, dietary treatments appeared to modulate peripheral lymphocyte

numbers with opposite effects. Results pointed to an immunostimulatory effect of diet Ph21 after 2 weeks of feeding, but a long term feeding (i.e. 8 weeks) with β -glucan supplementation seems to negatively affect the adaptive arm of the immune system with a drop in circulating lymphocytes. Still, and having in mind the nature of β -glucan stimulus, it is reasonable to expect a more evident local immune modulation in the gut. Further analysis on gut samples are currently underway.

Keywords – Microalgae β -glucan; Pulse feeding; immunostimulatory

Genes from the pseudoautosomal region (PAR1) of the mammalian X chromosome: synteny, phylogeny and selection

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Abstract

Sexual chromosomes recombination is restricted to a homologous area common to both, the pseudoautosomal region (PAR), composed by PAR1 and PAR2, which behaves like an autosome in both pairing and recombination. The PAR1 region, common to most of the eutherian mammals, including humans, is located at the termini of the short arm and presents a recombination rate 20 times higher than the autosomes. In order to gain insight into the evolution and the interspecific differences in PAR1, 15 PAR1 genes were collected from 41 mammalian genus and used to perform phylogenetic and selection analyses. Differences between the expected and the produced phylogenetic trees were found, suggesting an unexpected increase in substitution rate in rodents, that can be due to a change in chromosomal location of the PAR1 genes in this order. Additionally, three genes (ASMT, PLCXD1 and ZBED1) exhibited positive selection signatures, however this result was in only one of the two selection analyses performed. The synteny of the PAR1 genes in Primates, Artiodactyla, Carnivora and Perissodactyla was also analysed revealing differences among species, like genes translocated into different positions and genes absent, including an overlapping of the genes ZBED1 and DHRSX in various taxa. We found that genomic migration may modulate the evolution of PAR1 genes.

Keywords – sexual chromosomes; pseudoautosomal region; phylogenetics; selection; synteny

Dietary oxytetracycline treatment can modulate gut health condition in the Nile tilapia (*Oreochromis niloticus*)

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Abstract

Bacterial richness and diversity in the gut microbiota are extremely important for normal metabolic and immunological functions. Antibiotics, psychological and physical stress, and certain dietary components can contribute to intestinal dysbiosis, which is an imbalance between commensal and pathogenic bacteria, that can contribute to inflammatory diseases. This study was carried out to evaluate the immunomodulatory effects of oxytetracycline (OTC) dietary treatment on gut gene expression in Nile tilapia (*Oreochromis niloticus*) juveniles. A group of 315 tilapia (ABW: 118 ± 0.8 g) was randomly distributed among 9 tanks (35 fish each tank), in a freshwater recirculating system (23 °C, 10 h light /14 h dark) at UTAD (University of Trás-os-Montes e Alto Douro) facilities. All animals were acclimatized to the experimental conditions and fed with a control diet (CTRL) that fulfilled the known nutritional requirements for Nile tilapia, during 15 days. Thereafter, animals from 6 tanks were fed with a medicated feed (OTC) at a therapeutic dose ($55 \text{ mg Kg}^{-1} \text{ body weight day}^{-1}$), whereas fish from the other 3 tanks were fed with the control diet (CTRL). During OTC treatment, water from those tanks was not shared and freshwater was continuously added daily to allow water renewal ($50 \% \text{ d}^{-1}$). After 10 days, fish from OTC treatment shifted to the control diet (OTC-CTRL) or an extreme diet (OTC-ED). The feeding trial lasted for 55 days. Nile tilapia juveniles were fed twice a day, until apparent satiety. Several samplings ($n = 9$ per tank) were performed: i) at 15 days to determine gut health condition before OTC treatment; ii) at 25, 26 and 33 days to evaluate the effects of the medicated feed; iii) at 40 and 55 days to evaluate recovery after OTC treatment. Anterior and posterior portions of the intestinal tissue were collected to evaluate the expression of immune-related genes, as described by Machado *et al.* (2020). Among the 3 treatments, fish from OTC-ED group showed an up-regulation in the expression levels of genes related to innate immune, inflammatory and anti-oxidant responses in the posterior intestine, at 26 days (CAT), at 33 days (TNF α , IL1 β , CAT, PNCA, IL10), at 40 days (IL1 β , CAT), and at 55 days (G6PD, CASP3). Further analyses will be performed to evaluate the effects of oral administration of OTC in the gene expression of the anterior intestine.

Keywords – Oxytetracycline; Immunity; Intestinal inflammation; Gene expression; *Oreochromis niloticus*

Development of the Impact Assessment of Offshore Wind Farms on Biodiversity: Protocols, Analysis of Potential Areas and Environmental Restrictions for the Brazilian Coast

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Abstract

In the Paris Agreement on climate change, one of Brazil's commitments is to increase the use of renewable sources. On the Brazilian coast, wind energy has a generation potential of around 700 GW in locations with depths of the Atlantic Ocean of up to 50 m. The expansion of offshore wind farms (OWFs) and their insertion on the Brazilian coast has already been announced, with six OWF projects in the initial federal environmental licensing process. This PhD Thesis aims to improve the environmental impact assessment activities, as well as to analyze the best strategies to mitigate the impacts generated on biodiversity by the OWF energy generation. The specific objectives are to conduct an international survey on the status of protocols for biodiversity studies, the results of which can be used to assess environmental impacts and mitigation strategies for offshore projects in Brazil; develop basic protocols for inventory, monitoring and conservation of biodiversity, as well as the initial identification of landscape units. Initially, interview scripts will be conducted, with semi-structured questionnaires applied to the stakeholders. These results will be weighed against the main aptitudes and weaknesses of the physical, biotic and anthropic means in the Brazilian areas with the greatest wind potential. Maps of potential areas, restrictions and sensitivities for the installation of OWFs will be elaborated, through the development of scenarios, generated by the crossing of thematic information from a multicriteria matrix and paired comparison. It is also intended to establish, in the form of a Term of Reference, the best methodologies for inventories and monitoring of biodiversity on the Brazilian ocean to assess environmental impact.

Keywords – Offshore Wind Farms; Environmental Impact Assessment; Multi-criteria analysis; Biodiversity; Brazil.

Didactic model of floating islands as learning tool in schools

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Abstract

Floating wetland islands is a technology that comprises a floating platform with vegetation, whose operation is inspired by physical-chemical and biological processes that occur in natural wetlands. It is based on the interaction between plants, microorganisms and surface water. These systems can be applied to different water bodies such as lakes, ponds, rivers and marinas. This technology can be used with the purpose of improving the water quality, through phytoremediation processes, promoting biodiversity and rehabilitation of ecosystems.

The present work aims to use a didactic model of floating islands as a tool of teaching-learning in water purification processes and ecosystem services for primary school students, being also aligned with the Sustainable Development Goals 6 (clean water and sanitation) and 14 (life below water) of the Agenda 2030. For this purpose, a didactic model is being developed based on scientific knowledge and observation of the process of implementation and monitoring floating islands, which will be transposed into a protocol to be used in the school context.

We opted for the participatory methodology, in order to integrate students to the pedagogical contents and thus make them meaningful. The floating island model was designed with low-cost materials, in order to make it inclusive in the most diverse school contexts. With the construction and implementation of the didactic model that involves complex content, it is expected that the student at the end will be able to relate the socio-environmental context in his / her surroundings with scientific knowledge.

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.

Keywords – Floating Islands; Learning; Didactic Model, Environmental Education; water management; biodiversity

Development and validation of HILIC method for quantification of a sulfated synthetic antifouling compound

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Abstract

Marine organisms are extremely rich in antifouling (AF) sulfated metabolites, as is the case of zosteric acid, used as a defence mechanism against adherent organisms [1]. As such, the search for environmentally friendly AF products has led many researchers to synthesize marine inspired sulfated AF compounds [2]. However, due to the high polar properties of these sulfated compounds, chromatographic analysis has always been a difficult task due to the lack of retention in conventional C₁₈ columns. Hydrophilic interaction liquid chromatography (HILIC) is the most appropriate alternative mode for analysis of high polar compounds by high performance liquid chromatography (HPLC) [3]. The main goal of this study was to develop and validate a HPLC with UV-detection method using HILIC elution mode and its application in several environmental fate studies of gallic acid persulfate (AGS), a powerful compound with AF activity against the larval settlement of one of major fouling species, *Mytilus galloprovincialis*. AGS was quantified by a HILIC column with aqueous solution with acetonitrile:water (78:22 v/v) containing 20 mM of ammonium acetate buffer and the final pH adjusted to 5.6 as a mobile phase; flow rate of 0.8 mL/min; column oven was maintained at 28 °C; injection volume was 10 µL and the detection wavelength was set at 236 nm. This HILIC method was properly developed and validated according to the ICH Guidance for Industry Q2 (R1) through several parameters, namely specificity/selectivity, linearity, precision, accuracy, range, limits of detection (LOD) and quantification (LOQ) [4]. The assay was found to be precise, sensitive, and the calibration curves were linear within the studied concentration range, allowing the quantification of AGS.

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Keywords – Biofouling; Antifouling agents; Sulfated compounds; HILIC column; Validation

Biodiversity and biogeographic patterns of deep-sea glass sponges (Porifera, Hexactinellida) of the Atlantic

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Abstract

Remote and poorly investigated deep-sea 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 deep-sea 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. Sound knowledge and understanding of large-scale distribution patterns of deep-sea species and habitats is therefore of critical importance for the development and effective implementation of national and international agreements for conservation and sustainable management. A fundamental requirement to guarantee a successful approach is the delineation of ecologically and biologically meaningful regions, that reflect patterns of species diversity and distribution and their underpinning ecological and evolutionary processes. The objective of this work is investigating the biogeographic and (phylo-)diversity patterns of the Atlantic hexactinellids integrating data on species distributions and a robust phylogeny. To accomplish that goal, new glass sponge species from remote areas sampled across the Atlantic will be described, bio- and phylodiversity patterns investigated combining advanced statistics and phylogenomic approaches, and the conservation status of habitat-forming species assessed across the Atlantic.

Keywords – deep-sea; Atlantic; biogeography; conservation; multidisciplinary.

Optimization of a cetacean occurrence dataset: controlling methods for data bias, verification, and validation

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Abstract

Long-term monitoring datasets can provide a baseline to better understand physical and ecological responses to ocean environmental changes. These datasets can play an important role in marine management and conservation, so it is imperative that they become public following FAIR Data Principles in order to enhance their reusability by stakeholders from scientific community to decision-makers. To take full advantage of the datasets, it is necessary that the data is reliable, and the sources of bias are identified and quantified. CETUS Project is a cetacean monitoring program in the NE Atlantic, ongoing since 2012, that counts on international participation of biologists to collect data from platforms of opportunity. CETUS dataset is made available open access at OBIS and EmodNET portals. We aim to optimize and allow the proper use of the dataset by applying verification and validation methods; creating criteria that quantifies and allow users to account for the quality of the data; and assessing the influence of bias parameters. Thus, here we present a discriminative framework that directly exploits dataset bias: (1) validation process, based on photographic confirmation of identified species using photographs obtained by marine mammal observers (MMOs), who have integrated the project in the period of 2012–2019, and (2) correlate the number of sightings and validated identifications with the observers experience (through *curricula vitae*), to reach data reliability. Future research to determine data bias will include modelling of sightings using explanatory variables accounting for weather conditions, height of observation platform, experience and reliability of observers (CV experience and detection rate / species identification success) and distance of sighting to the vessels. Ultimately, the work will contribute to an adequate and informed use of the CETUS dataset, expanding its application in science and support in marine management and conservation.

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It will also create validation and verification methodologies applicable to identical monitoring programs.

Keywords – cetacean occurrence; biodiversity monitoring; long-term dataset; data verification; data validation; methodological bias

Single and combined impact of microplastics and cadmium on antioxidant defence and innate immunity in European seabass (*Dicentrarchus labrax*)

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Abstract

Microplastics (MPs) exposure can cause different toxic effects in fish organs and tissues, however this toxicity can depend on the chemicals that are adhered to its surface. In fact, the MPs can absorb heavy metals into their surface and later release them into the environment. Considering this information, the present *in vivo* study describes the effects of MPs and waterborne cadmium (Cd) exposure (in a single and combined way) on the haematological profile and antioxidant defence in European seabass (*Dicentrarchus labrax*) at 5 and 10 days. For that, four groups were designated: i) control group (unexposed); ii) Cd group [exposed to Cd (0.1 mg L⁻¹); iii) MPs group [exposed to MPs (0.25 mg L⁻¹); iv) MPs-Cd mixture group [exposed to Cd and MPs (0.1 mg L⁻¹ and 0.25 mg L⁻¹, respectively)]. Our results revealed variations in the haematological profile with an increase in the number of red blood cells (RBCs) and white blood cells (WBCs) in fish exposed to Cd compared to the unexposed group at 5 days of exposure. In fish from MPs-Cd mixture group, a decrease and an increase of RBCs and WBCs numbers, respectively, was observed over-time. Similarly, the values of haematocrit decreased in fish exposed to Cd and MPs-Cd after 10 days of exposure regarding the values found at 5 days. The differential counting of the WBC revealed a decrease of the numbers of monocytes in fish exposed to MPs and MPs-Cd after 10 days of exposure compared to the control group. Regarding the organo-somatic indexes in liver and spleen, only the spleen index registered an increase in fish exposed to MPs after 5 days of exposure compared to the unexposed group. On the other hand, the oxidative stress-related parameters measured in the liver exhibited low variations. Lipid peroxidation, catalase and glutathione-s-transferase activities did not suffer alterations in the liver of exposed and unexposed fish. However, the total glutathione activity increased over-time in fish exposed to Cd. Having these results in consideration, the combination of MPs with Cd does not seem to increase the toxicity of this heavy metal in European seabass.

Keywords – Microplastics; Cadmium; Haematological profile; Oxidative stress

Microbial community monitoring in a sole (*Solea senegalensis*) hatchery recirculating aquaculture system

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Abstract

Recirculating aquaculture systems (RAS) allow to reduce water usage and to improve waste management and nutrient recycling in fish production, making intensive aquaculture compatible with environmental sustainability. The main challenge of this approach is to manage disease outbreaks, since in these systems a healthy microbial community contributes to water purification and water quality. The interaction of the different factors that can influence the fish and the various treatment steps, is still poorly understood. The main objective of this study is to monitor and model the water quality and microbial communities of a seawater-based RAS, taking a closer look at the biofilter, enabling the control of the water quality and bacterial infections throughout the production cycle. The work is being developed in partnership with a sole hatchery where a comprehensive characterization of the microbial communities from all RAS compartments, including the prokaryotic community of the biofilters, water, biofilms and the physicochemical parameters was conducted. Total DNA was isolated from the different matrices and the V4-V5 region of the 16S rRNA gene was sequenced using Illumina MiSeq® platform and the output analysed in the DADA2 pipeline and annotated using SILVA reference database. This study showed a highly dynamic prokaryotic community in the RAS, separate compartments developed different taxonomic profiles, even with the same water supply. The prokaryotic community shifts were found to be modelled by water parameters such as salinity, temperature, pH and nutrient load. Presently we are examining the microbiome data sets to extract knowledge on microbial interactions within this RAS unit.

Keywords – Recirculating aquaculture systems; hatchery; microbial community

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Genetic and Chemical Diversity of a Novel Halogenase Class

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Abstract

A novel di-metal carboxylate halogenase, named CylC, was recently reported. This enzyme participates in cylindrocyclophane biosynthesis in the cyanobacterium *Cylindrospermum licheniforme* ATCC 29412. CylC catalyses a cryptic chlorination that activates a carbon-hydrogen bond, allowing for posterior dimerization. Although this halogenation is not present in the final product, homologous genes were found in biosynthetic gene clusters (BGCs) that encode for chlorinated secondary metabolites, such as the bartolosides and columbamides.

Previously, our lab identified CylC homologs in several cyanobacterial BGCs. In the current study, we carried out a phylogenetic analysis based on 16S rRNA gene sequence to evaluate the distribution of CylC homologs among the different cyanobacterial orders. Overall, we show that CylC homologs are widespread in cyanobacteria and they should provide interesting starting points for natural products (NPs) discovery.

Furthermore, we selected CylC-harboursing BGCs to discover their encoded NPs. Here, we present the approaches we are using to uncover and characterize the compound encoded in the BGC from *Microcystis aeruginosa* LEGE 91341 that contains a CylC homolog. These involved attempts at knockout generation to abolish compound production in mutant strains. We were unable to transform cyanobacteria with this approach. In the future we will experiment heterologous expression of the entire BGC in *E. coli* using DiPaC-SLIC.

Keywords – CylC; Heterologous Expression; Natural Products; Natural Transformation; *Microcystis aeruginosa* LEGE 91341; DiPaC-SLIC

Maërl/rhodoliths beds as essential biohabitats for associated invertebrate communities in impacted environments

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Abstract

Rhodolith beds (non-geniculated aggregates of coralline red algae) are extremely common and abundant in the Brazilian coast, especially in the Northeastern region. These algae are usually considered biodiversity hotspots for marine invertebrates. Nonetheless, ecological characterization studies are still scarce in the region. This study evaluated the rhodoliths and invertebrates associated at three beaches in Northeastern Brazil, with different levels of environmental impacts, Seixas, Miramar and Maceió. The highest densities of rhodoliths, as well as, the diversity and density of invertebrates, were registered in Seixas Beach, despite the higher touristic pressure. This result was associated with the high availability of calcium carbonate in nearby locations. On the opposite, higher mortality of rhodoliths and lower diversity and density of invertebrates were recorded in the most polluted beach, Maceió, which had high concentrations of thermotolerant coliforms (>1000 units/100mL). In respect to the functional composition of the invertebrate community, a similar variation trend was found for the less polluted beaches, with polychaete and echinoderm dominance, with these taxa being mostly biodiffusors, slow free movement and predators. These traits appear advantageous for the interaction rhodolith-habitat and invertebrate, due to the food and structural protection provided by the rhodoliths, but also due to oxygenation and remobilization of the sediment by the resident fauna. The most polluted beach showed distinct characteristics marked by the absence of the taxa and traits described for the other beaches, with the dominance of bivalves, mostly suspension feeders with limited movement. This result can be associated with a higher suspended material more common in organic polluted environments.

Keywords – Coralline red algae; Biodiversity; Environmental drivers; Traits; Northeast Brazil

Cyano-EPS: An environmental based screening on the Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC)

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Abstract

Microbial exopolysaccharides (EPS) have industrial interest due to their chemodiversity and cellular location. The Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC) is a biobank that comprises more than 600 cyanobacterial strains mostly isolated from Portuguese ecosystems. The cyanobacterial EPS can be classified in two main groups: cell-bound polysaccharides (CPS) comprising slime, sheaths, and “capsular”; (2) released polysaccharides (RPS). These light-powered organisms consume inorganic carbon to produce oxygen, which can promote carbon neutrality. However, the sustainability of the whole bioprocess is highly dependent on the combination of strain selection, production and downstream processing steps. The EPS cyanobacterial bioprocess (Cyano-EPS) commonly bioprospects the strain by the EPS productivity under optimal growth conditions. However, this criterion can hide the real potential of a strain to a predefined environment in which the integration of a natural resource can impact in the techno-economic balance of the bioprocess. Additionally, the RPS are characterised for being soluble in culture medium and imply simplified unitary processes for polysaccharide extraction. In order to assess the outdoor potential of Cyano-EPS we propose an environmental based cultivation screening. The RPS productivity will correspond to the ability of that one strain to be grown outdoors in a determined location at one specific period of the year. For that 30 cyanobacterial strains (Synechococcales, Oscillatoriales, Chroococcales and Nostocales orders) from LEGE-CC were selected to be grown under a simulated Portuguese summer (light and temperature profile) in Algem ® labscale photobioreactor for a period of 15 days. Cellular physiology, biomass production, and culture medium composition (carbohydrates and proteins) were tracked. The RPS produced will be evaluated in terms of monosaccharidic composition and molecular weight. Biomass and RPS productivities will be compared to the polysaccharide uniqueness and a maximum of 6 strains will be selected for further process optimization.

Keywords – Cyanobacteria; Bioprocess; Exopolysaccharides; Screening; Strain Selection

Prokaryotic Dynamics across the North Pacific Subtropical Front

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Abstract

Ocean fronts are very dynamic areas of intensified horizontal gradients of water characteristics. Microbial communities are crucial for ecosystem functions occurring in these areas, such as primary production and nutrient cycling. However, few studies have addressed the distribution of microplankton communities in ocean fronts. This study addresses this gap by investigating the patterns of prokaryotic communities distribution in the North Pacific Subtropical Front (NPSF) located in the deep oligotrophic region of the Pacific Ocean.

Water column samples were collected 1000 nautical miles off the coast of Southern California on board of the Schmidt Ocean Institute research vessel along the NPSF. To study the depth effect on prokaryotic communities distribution, samples were collected at five different depths (Surface [5m], Above DCM [80m], DCM (Deep Chlorophyll Maximum) [108-130m], Below DCM [175-200m], Mesopelagic [500m]). Samples were also collected across the ocean front, targeting the different water masses (Polar, Front, Subtropical). Prokaryotic community composition and diversity were studied by a metabarcoding approach of the 16S rRNA gene using next-generation sequencing (NGS).

Results showed that the dynamics of the prokaryotic communities were highly influenced by depth. Specifically, it was possible to observe distinct microbiomes across the five different depth categories, which shared only 10 amplicon sequence variants (ASVs) from a total of 6445 ASVs identified. Furthermore, species richness and alpha diversity increased sharply with depth, until 200m, leading to believe that there is a diversity hotspot below the DCM. On the other hand, the type of water mass was not a significant influence on the prokaryotic community structure. A similar microbiome was observed in the three different water masses. At both depths, the number of shared ASVs between the water masses was relatively high, 411 at Surface, and 937 at DCM from a total of 6445 ASVs retrieved from all samples. Subsequently, the horizontal distribution of the prokaryotic communities did not demonstrate a visible habitat preference. Ultimately, this research demonstrates a strong influence of depth on the microbial community structure within the depth of 500 m and a weak influence of the horizontal gradients in the NPSF.

Keywords – Ocean Fronts; North Pacific Subtropical Front; Metabarcoding; Prokaryotes

Towards the optimization of upstream process of *Cyanobium* sp. as source of phycobiliproteins

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Abstract

Phycobiliproteins are water-soluble pigments responsible for a great part of light-harvesting in cyanobacteria. These pigments have been described as potential bioactive compounds and has become highly attractive for food and cosmeceutical industries. Thus, in order to produce phycobiliproteins from cyanobacteria for industrial application, it is necessary to optimize upstream processing parameters (such as light, nitrogen source, pH, temperature and salinity), that affects the growth and phycobiliprotein accumulation.

In this work, the optimization of phycobiliproteins production from *Cyanobium* sp. was performed using photobiology, biotechnology and factorial modulization as tools. The optimization was performed in several steps: i) light intensity and source, and culture medium; ii) light quality supplementation, iii) temperature, pH and salinity; and iv) two-light-stages cultivation process. In all experimental trials, growth, photosynthetic activity, antioxidant capacity and phycobiliproteins content were evaluated.

Optimal condition for phycobiliproteins production was set as: a two-phase process (10 days of white LED + 4 days of red LED) with a light intensity of $200 \mu\text{mol}_{\text{photons}}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; a modified blue green medium (BG11) with addition of NaCl ($10 \text{ g}\cdot\text{L}^{-1}$), NaNO_3 ($3 \text{ g}\cdot\text{L}^{-1}$), NaHCO_3 ($0.1 \text{ g}\cdot\text{L}^{-1}$) and K_2HPO_4 ($0.1 \text{ g}\cdot\text{L}^{-1}$), as culture medium; and a pH set at 9.0.

Major findings include: a) the use of monochromatic red light, which increased, by itself, the content of phycobiliproteins by 1.5-fold; b) the antioxidant capacity of intracellular extracts of *Cyanobium*, that had a positive correlation with the phycobiliproteins content, suggesting a potential bioactivity of these pigments; and c) the versatility of the strain in several different conditions, as no inhibition of growth was found in the tested range of: light intensity (50 to $300 \mu\text{mol}_{\text{photons}}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), light source (fluorescent, low-pressure sodium and light-emitting diode), temperature (20 to $30 \text{ }^{\circ}\text{C}$), pH (6 to 9) and salinity (0 to $30 \text{ g}\cdot\text{L}^{-1}$ of $[\text{NaCl}]$).

Overall, in *Cyanobium* sp., the followed optimization, was successful and led to an increase of phycobiliproteins of 3.7-fold in the content (from 3.3 to $12.4 \text{ \%}_{\text{DW}}$) and 6.8-fold on the productivity (from 2.5 to $17.0 \text{ mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$). This study provides guidelines to a possible application of this strain for industrial level, as it seems promising for the production of phycobiliproteins.

Keywords – light; abiotic factors; cultivation; pigments; antioxidant capacity; cyanobacteria

Green Roofs application in Water Reservoirs

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Abstract

Green roofs are examples of Nature-based solutions and are considered systems with vegetation built on a structure, which may be settle at ground level or on top of the buildings. Vegetation in green roofs poses a major importance since it acts as a natural cooling mechanism through the promotion of evapotranspiration and heat dissipation, besides other intrinsic benefits. Modern green roofs are engineered systems that comprise several layers and follow standard guidelines. They may be part of a retrofit strategy in existing buildings or planned to be incorporated in new ones. They can contribute to the city's actual challenges, concerning resilience towards climate change, increasing of impermeabilization, territorial fragmentation and consecutively loss of green spaces. A key benefit of green roofs in urban areas is the ecosystem multifunctionality. They provide several ecosystem services including: mitigation of the urban heat island, regulation of building temperatures leading to energy savings, reduction of air pollution, stormwater management, biodiversity promotion and wildlife habitat. Green roofs can be classified as intensive, semi-intensive and extensive, depending on their water requirements, maintenance and structure of layers. Within this classification green roofs can have different purposes of use. They can be implemented for example on residential, commercial and industrial buildings, as covering parks and other structures. The use of green roofs in water reservoirs has a long history although is poorly explored. Potable water tank reservoirs are of great importance to store water that has already been treated and is waiting to be delivered to the final consumers. The use of green roof in these structures is explored in the present work. This study aims to carry out a survey of existant water reservoirs with green roofs in the city of Porto, and to assess the main benefits that green roofs can bring to them. 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.

Keywords – ecosystem services; water management; water cycle; green infrastructures; biodiversity

Preliminary results on isolation, identification and characterization of cyanobacteria from Morocco

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Abstract

Cyanobacteria are microorganisms that have remarkable adaptability and can inhabit various types of aquatic and terrestrial ecosystems worldwide including extreme environments (e.g., freshwater, marine, terrestrial, hot springs, deserts). This group of organisms are considered a rich source of secondary metabolites with potential biotechnological applications and have the capability to produce some potent toxins that can induce consequences to human health. The current number of cyanobacterial species is subject to debate. It is believed that there are more than 5.000 species described but the expected total number can exceed 8000 which means that there exists a huge diversity of new species of cyanobacteria to discover and explore. The Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC) is a biological resource centre located at Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), comprising more than 1200 different cyanobacterial and microalgae strains. LEGE-CC strains were mainly isolated from Portuguese ecosystems (including Madeira and Azores Islands) but also from other countries worldwide (e.g. Australia, Brazil, Colombia, Morocco, Mexico, Dominican Republic, Cape Verde). In this preliminary work, we will highlight the preliminary screening efforts that have been doing regarding to sampling, isolation and characterization of cyanobacteria isolates from Morocco ecosystems with the aim to found a new cyanobacterial diversity with biotechnological and pharmaceutical potential (e.g. antibacterial, antiviral, antifungal, anti-inflammatory, anticancer, antifouling, ant-obesity or antioxidant).

Keywords – LEGE-CC; culture collection; cyanobacteria; cyanotoxins; marine sources

Virtual Screening of Synthetic Compounds Potential Inhibitors of Envelope Protein of Zika Virus

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Abstract

Zika virus (ZIKV) infection is a “neglected tropical disease” associated with brain development abnormalities and fetal death [1]. ZIKV was ignored since its first isolation in 1947, in Africa [2], until after its major outbreak in Americas in 2015, when WHO declared Public Health Emergency of International Concern [1]. There is still no specific medicine or vaccine to treat ZIKV infection, leading to the urgent need to improve our understanding of ZIKV pathogenesis and therapeutic strategies. ZIKV is a member of the family Flaviviridae transmitted mainly by Aedes mosquitoes [1]. Commonly to enveloped RNA-composed virus, glycosaminoglycans displayed at the surface of host cells must form a first-moment complex with proteins embedded on the envelope viral particle to trigger viral infection [3]. In our group several small molecules have been obtained in the last decade and promising hits were discovery particularly with anticoagulant [4-6], antiviral [7], and antifouling activity [8,9]. In this work, a docking study of new compounds synthesized in Grupo de Produtos Naturais e Química Medicinal (CIIMAR/FFUP) on ZIKV E protein (PDB code 5JHM) was carried out using AutoDock Vina. Virtual screening revealed interaction of different functional groups with ZIKV-E protein in the same binding pocket as Suramin. Therefore, this new series of compounds deserves further exploration as potential inhibitors of ZIKV infection. Molecular dynamics simulations will give relevant biological insight of the bound-state conformation.

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Keywords – Zika virus; RNA-enveloped viruses; synthetic compounds; docking; virtual screening

Local Scour Near Detached Coastal Protection Structures

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Abstract

Over the past decades, coastal erosion has gained significant importance, particularly in some stretches along the Portuguese coast that are in a more fragile situation and exposed to the severe conditions of the North Atlantic. This phenomenon makes coastal areas more exposed and vulnerable to extreme events, putting people and assets of high patrimonial value at risk. Furthermore, these problems tend to get worse in the future due to climate change effects, thus justifying interventions to reduce coastal risks (*e.g.*, overtopping and flooding).

The main objective of this presentation is to study and characterize the scour phenomena near detached coastal protection interventions. For that purpose, Carneiro's beach is used as case study. This beach is located at the seafront of Porto, north of Felgueiras' breakwater, and is relatively exposed to the marine environmental conditions. Therefore, during storms, waves may overtop the seawall of D. Carlos' Avenue and cause the removal of sand from the beach, with its deposition offshore and on land. To mitigate existing problems and risks, Consulmar Lda. designed a coastal protection intervention consisting of five detached breakwaters. Since the hydraulic and the structural performance of the first designed intervention was considered unsatisfactory, it has been reformulated and reassessed in a new physical model study.

The experimental study of the new coastal protection intervention was carried out in the wave tank of the Hydraulic Laboratory of the Hydraulics, Water Resources and Environment Division of the Faculty of Engineering of the University of Porto. The study was performed at a 1/60 geometric scale for four water levels, three peak wave periods and various significant wave heights. The analysis of results was focused on the variation of the sea bottom morphology in the vicinity of the five detached breakwaters in order to characterise the scour and deposition patterns, but also included the study of the evolution of damage in the three rubble-mound breakwaters (by zone, *i.e.*, head, trunk and toe berm) and the overall effectiveness of the intervention in reducing the lee side's levels of agitation, *i.e.* in Carneiro's beach.

In order to complete the analysis, the experimental study was complemented with the application of the Delft3D software, in which a numerical model of the coastal stretch under study was implemented. Thus, with the use of the Delft3D-WAVE module to propagate waves to the coast, the Delft3D_FLOW module to simulate the hydrodynamic conditions and also the Delft3DMORPHOLOGY module to reproduce the morphodynamics of the case study, it was

possible to analyse the effectiveness of the five structures designed to shelter Carneiro's beach and compare the obtained results with those of the physical model. In addition, the numerical study allowed to establish important conclusions regarding the variation in the depth of the scour hole as a function of water level, peak wave period and significant wave height. The results obtained, both in the physical model and in the numerical model, made it possible to conclude that the five detached breakwaters effectively improved the tranquillity conditions on its lee side. On the other hand, it was found that the evolution of scour in the area surrounding the structures in the numerical model corresponds, in general, to what had occurred in the physical model. Finally, it was found that the rubble-mound breakwaters, either with the armour layer built with stones or materialised by Antifer cubic blocs, were not able to withstand the most energetic wave conditions, for the highest water levels, namely that resulting from the combination of the highest astronomical tide level, with a meteorological tide and considering also a slight increase in the mean sea level due to climate change effects.

Keywords – morphodynamics; physical modelling; numerical modelling; Delft3D

New cyanobacterial natural products from *Nodularia* sp. LEGE 06071 display anticancer and antimicrobial activity

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Abstract

Introduction: Natural products have been the basis for pharmacology for centuries and the search for new bioactive compounds has been increasing. Cyanobacteria are gram-negative bacteria with a high rate of discovery of new and diverse chemical entities. Three new compounds, 01, 02 and 03, were previously isolated in our group from the cyanobacterial strain *Nodularia* sp. LEGE 06071. The aim of this study is to evaluate these new compounds regarding their anticancer and antimicrobial activity.

Methodologies: The anticancer activity of compounds 02 and 03 was evaluated through the commonly used IC₅₀ and the alternative GR₅₀, in three tumoral cell lines (HCT-116, MCF7 and MG-63) and one non-tumor cell line (hCMEC/D3). The cells were exposed to the compounds in concentrations up to 25 µM for 48 hours and the cell viability was evaluated by the MTT assay.

The antimicrobial activity was evaluated through the disk diffusion assay, in two gram-negative bacteria (*Escherichia coli* and *Salmonella*), two gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), and one fungus (*Candida albicans*). The compounds were used in 1 mg/mL concentration.

Results/Discussion: The IC₅₀ and GR₅₀ values for compound 02 and 03 fall in the micromolar range for the four cell lines used. The highest activity for compound 02 was achieved in the MCF7 cell line and compound 03 showed to have higher activity in HCT-116 (IC₅₀ assay) and MCF7 (GR₅₀ assay). Both compounds present less activity in the non-tumor cell line, hCMEC/D3. Compound 02 showed to have antimicrobial activity against *Bacillus subtilis*.

Conclusion: These are new natural products from a cyanobacteria that present cytotoxic and antimicrobial activity. These results are promising and in the future new bioactivity assays can be performed and the compounds can be used for drug discovery.

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Keywords – Natural products; Cyanobacteria; Anticancer; Antimicrobial

Floating wetland islands as ecotechnology to promote ecosystems establishment

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Abstract

Nature-based solutions (NBS) are a fulcrum part of action for climate and biodiversity challenges. They deliver several ecosystem services namely the contribution to the management of water resources. They can be functional in several environments including, fresh water, coastal and urban settings. Floating Wetland Islands (FWI) are an example of NBS that can provide enhancement of water quality, promotion of biodiversity and habitat conservation and ecosystems rehabilitation. This ecotechnology has been applied in several water bodies, such as ponds, lakes and rivers, promoting water depuration through phytoremediation processes; although in saline environment, such as port marinas, they are poorly studied. In this context, FWI were implemented in two marinas of a seaport (the two FWI in Marina of Leça and one at Marina Terminal) in order to evaluate their establishment and potential for water quality enhancement and biodiversity promotion. They were vegetated with selected halophytes (*Sarcocornia perennis*, *Halimione portulacoides*, *Spartina maritima* and *Inula crithmoides*). A preliminary assessment was carried out focusing on the monitoring of the water physicochemical parameters and on the development of the plant species and associated biodiversity (macroinvertebrates and microorganisms). Results obtained so far indicate that this system can be successfully established in this harsh environment. Future studies will now evaluate if FWI can be implemented as a support in the sustainable management of water resources in marinas.

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Keywords – Floating Wetland Islands; nature-based solutions; ecotechnology; marina environment

Synthetic studies towards fumiquinazoline derived alkaloids

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Abstract

Introduction: The search for new compounds with simplified structures displaying enhanced biological activities is a current challenge in organic and medicinal chemistry. Marine-derived fungi exhibit a variety of unique molecules with a broad spectrum of biological activities, which make them promising candidates for drug development ¹. Specifically, the fumiquinazoline family of alkaloids, containing a pyrazino[3,1-*b*]quinazoline-3,6-dione core linked to an indole moiety, have been key players for further chemical modifications to improve their chemotherapeutic properties ². Gyantrypine is a glycine derived alkaloid, isolated from the marine fungus *Aspergillus clavatus* and it is described as an antibacterial agent active against *Vibrio harveyi* (MIC = 32 µg/mL)³. Fumiquinazolines F and G are alanine derived pyrazinoquinazolinones reported as metabolites of the marine fungus *Aspergillus fumigatus*. Synthetic fumiquinazoline G was recently discovered by our group to be an antitumor agent with GI₅₀ values lower than 20 µM⁴.

Methodologies: Herein, we report the synthetic steps of the multistep approach ⁵ towards marine products gyantrypine, fumiquinazoline G and fumiquinazoline F, through conjugation of anthranilic acid, D-tryptophan methyl ester hydrochloride and the correspondent third amino acid. Synthetic details and structure characterization (by infrared spectroscopy and 1D NMR studies) of marine products and intermediary compounds, will be presented and discussed.

Results: Following a multistep methodology, the common dipeptide intermediate was synthesized in 82% yield and characterized by IR and NMR spectroscopy, alongside with respectively tripeptide linear intermediates of gyantrypine, fumiquinazoline G and fumiquinazoline F.

Conclusion: Future work will consist on screening these intermediates and final products for chemotherapeutic properties and to develop a library of fumiquinazoline-related analogues.

Acknowledgments – To national funds through FCT within the scope of UIDB/04423/2020 and UIDP/04423/2020 and Project No. POCI-01-0145-FEDER-028736, COMPETE 2020, Portugal 2020, European Union ERDF, QREN, FEDER, and BYT CIIMAR for a scholarship to H. Ramos.

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Keywords – Marine-derived fungi; fumiquinazolines; medicinal chemistry; bioactivity; organic synthesis

***Tenacibaculum maritimum* pathogenesis: crosstalk between host and pathogen and beyond**

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Abstract

One of the most devastating bacterial diseases of wild and farmed marine fish is tenacibaculosis, which causative agent is *Tenacibaculum maritimum*. This pathogen has a wide range of host species and worldwide distribution, inducing ulcerative lesions mainly on host skin and fins. Due to the high mortality and economic losses associated with this bacterium it is relevant to gather more knowledge for more effective methods to avoid the spread of tenacibaculosis. This pathogen has the ability of producing extracellular polymers and other extracellular products (ECPs) containing exotoxins, which allows it to persist in aquatic environments and provoke cellular necrosis in internal organs with highly toxic effects. Although it is known that ECPs are important to a successful invasion of the host, the identity of the ECPs' components remain unknown. To address this issue, we are developing a work plan that includes several tasks. To detect putative protein toxins secreted by *T. maritimum*, concentrated culture supernatants from a virulent strain will be resolved by native-PAGE and protein fractions tested *in vivo* for the capacity to induce harmful/toxic effects in European seabass and Senegalese sole. ECPs from virulent and non-virulent strains will be compared by SDS-PAGE and proteins exclusively present in the ECPs from virulent strains will be identified LC-MS/MS. To evaluate *T. maritimum* evading mechanisms, immune-related parameters of European seabass and Senegalese sole will be assessed with virulent and non-virulent strains, through *ex vivo* method and live pieces of skin tissue with evaluation of gene expression profile, cellular response parameters and apoptosis. *In vivo* time-course challenges of European seabass and Senegalese sole with *T. maritimum* non-pathogenic and pathogenic strains will be performed. To identify genes/gene pathways possibly related with virulence, the *in vivo* induced antigen technology will be used. Briefly, the serum collected from infected fish will be utilized to identify *T. maritimum* components that are immunogenic and expressed *in vivo*. By identifying important virulence factors of the pathogen and defining the host immune response against infection, this work, will increase the knowledge on *T. maritimum*

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pathogenesis and will contribute to the development of future strategies to prevent or treat tenacibaculosis.

Keywords – Host defence; Virulence factors; Immunology; Aquaculture

Monitoring of physico-chemical parameters and their influence on the cyanobacteria occurrence: a case study in Alqueva reservoirs

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Abstract

Alqueva complex is constituted by 17 reservoirs/dams interconnected by waterways, being built to provide water for agricultural irrigation, as well as for human consumption and recreational activities in Alentejo region. Most of these reservoirs are eutrophic and show contamination by cyanobacteria. In this respect, it is important to characterize the quality of the water considering the concentration of cyanobacteria with toxic potential and cyanotoxins and its association with the commonly monitored physico-chemical parameters. Understanding the dynamics of cyanobacteria in water and its causes can help to predict future blooms and then to take measures in order to prevent water contamination and the human exposure to this contaminant. In this work, the relationship between the environmental physico-chemical variables and the abundance of each group of cyanobacteria was performed through the analysis of canonical correspondences (ACC). This analysis was done in two ways with monitoring data acquired periodically between 2012-2018, one with "standard ACC" and the other with "partial ACC", in which the effect of seasonal variation was eliminated. With this technique it was possible to verify that certain physico-chemical parameters, in relation to others, contribute significantly to the density of some genera/species of cyanobacteria, such as *Microcystis* and *Cylindrospermopsis* (among others cyanotoxin producers). Temperature was the most important variable in the gradients that define the abundance of, cyanobacteria, as it is a variable with a strong seasonal character and is strongly correlated with solar radiation, which is determinant in the increase of cyanobacteria production (reflected in chlorophyll a). Furthermore, chlorophyll was then another parameter with a great correlation with cyanobacteria, as well as nutrients such as nitrate, in which the greater the concentration of these parameters the greater the density of cyanobacteria. These results emphasize the potential threats of climate change and agricultural pollution, which favours an overall increase in temperature and the water eutrophication, contributing to an increase in the proliferation of toxic cyanobacteria. In conclusion, the mentioned physico-chemical parameters can serve as a useful tool for predicting the occurrence of toxic cyanobacteria allowing decision

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makers to prioritize reservoirs where it is necessary to maintain recurrent monitoring of cyanobacteria.

Keywords – Cyanobacteria; cyanotoxins; climate change; eutrophication; canonical correspondence analysis; physico-chemical parameters

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Optimization of the extraction protocol for *Planctomycetes* metabolites

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Abstract

Planctomycetes is an unexplored group with unique features among bacteria that keeps challenging the scientific community. Although secondary metabolism studies of these bacteria have only recently appeared, there is no doubt about their biotechnological potential. Already, based on genomic and HTS screenings, bioactivities including antimicrobial and anti-cancer have been previously identified by our group. However, extraction of planctomycetal metabolites is a difficult endeavor, as they have unique cellular structures, strains have slow growth and low biomass production and consecutively very low production of metabolites, evidence which was also reinforced by dereplication of bioactive extracts and observation of none to few and small peaks in the UV/MS spectra. Furthermore, as only few studies addressed this thematic, very different protocols of extraction of metabolites were applied, displaying distinct outcomes. Considering the presented challenges, in this work, we aimed to optimize the protocol to use in the extraction of planctomycetes metabolites for further application in compound isolation of up-scaled cultures of previously identified bioactive strains. In this study, representative strains from various taxa were chosen and fermented for both 7 and 14 days. Aliquots of the cultures were collected, biomass and supernatant separated and freeze-dried. Extracts were prepared using 3 organic solvents (acetone, methanol and ethyl acetate) and 2 incubation times (1 and 24h). The efficiency of extraction was calculated for each individual combination of conditions and results compared. Extracts obtained were then dissolved in DMSO 20% at the same concentration and screened for antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. We observed that, although biomass production and efficiency of extraction were very low for most conditions, overall, 7-day incubation of strains was the most effective. Biomass extraction was also much more efficient than the whole broth extraction. Regarding the solvents, methanol appears the more efficient one. Solvent incubation time showed no major differences. Regarding the antimicrobial screening, mild activities were observed against both targets. However, those activities were almost all concentrated into methanol extracts from different conditions, which suggests that indeed methanol is the most efficient solvent of the three tested regarding both quantity and presence of metabolites with antimicrobial activity.

Keywords – *Planctomycetes*; Biotechnology; Secondary metabolites; Bioactivities.

***Chroococcidiopsis* sp. bioactive potential: an interesting marine source of pigments and antioxidant compounds**

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Abstract

Chroococcidiopsis genus belongs to a group of extremophile cyanobacteria, which exceptionally survives in the harshest conditions (i.e. osmotic stress, high temperature, strong radiation). This ability has recently disclosed its interest to go to Mars. Yet, scarce studies have focused on this marine genus as a potential source of value-added compounds (i.e. pigments, phenolic compounds), meant for application in food and nutraceutical fields. The aim of this study was to explore the biotechnological potential of the strain *Chroococcidiopsis* LEGE 06174. For that, the biochemical characterization of several *Chroococcidiopsis* sp. extracts was studied, in particular the pigment content and profile (chlorophylls, carotenoids and phycobiliproteins), total phenolic compounds, antioxidant capacity and its cytotoxicity. Furthermore, extracts were obtained via series of successive extractions: phosphate buffer-saline (PBS) (serie A), Ethanol-PBS (serie B), Acetone-PBS (serie C), Methanol-PBS (serie D) and Acetone-Methanol-PBS (serie E); and due its mucilaginous sheath, a preliminary treatment with sulfuric acid, NaOH, PBS, DMSO 20%, DMSO 100% and acetone was also tested, to enhance the effectiveness of pigments extraction. The PBS pre-treatment allowed an efficient extraction of pigments and was selected to proceed to *Chroococcidiopsis* compound characterization. The methanol and PBS successive extractions (serie D) showed to be the best serie of solvents to extract the total pigment content; with the highest content in carotenoids; in particular for echinenone, β -carotene and α -carotene derivative. On the other hand, the total phenolic compounds presented higher concentration in the ethanolic successive extraction (serie B). The extract of the pre-treatment (PBS) has shown the highest antioxidant capacity both for ABTS^{•+} and NO^{•-}. The organic extracts did not present any signs of cytotoxicity, whilst the aqueous extracts presented a loss of cell viability for up to 48% (concentration of 200 $\mu\text{g}_{\text{extract}}\cdot\text{mL}^{-1}$, 48h). Not only, *Chroococcidiopsis* sp. has revealed to be a

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potential biotechnological resource to be explored in terms of high-value compounds, but also was found preliminary a green and multiple extraction methodology.

Keywords – *Chroococcidiopsis* sp.; pigments; phenolic compounds; antioxidant capacity; biotechnological potential

Ascidians reported in Portugal and their genetic profiling

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Abstract

Ascidians are marine invertebrates (Chordata: Tunicata) vastly studied regarding their ecological, evolutionary, and biotechnological relevance, due to their close phylogenetic association with vertebrates, invasive potential and for being part of distinct ecological niches for several organisms. Particularly, secondary metabolites with varied bioactivities have been isolated and studied from these symbiotic associations. However, the ascidians' biodiversity in Portugal is still poorly known.

Therefore, in this study the diversity of ascidians reported in Portugal along with an assessment of their available genetic information, resorting to literature and online databases (*e.g.*, NCBI nucleotide database) were reviewed. Furthermore, sampling of 2 species, *Ciona* sp. and *Molgula* sp. was performed at Porto de Leixões (Matosinhos, Portugal), aiming their molecular identification as a *case study*. DNA extraction, amplification, and sequencing of a cytochrome *c* oxidase subunit I (*COI*) gene fragment and further bioinformatic analyses (*e.g.*, BLAST and phylogenetic analyses) were accomplished.

The compiled information regarding ascidians representativity in Portugal is mostly reported in public portals. In Portugal, there are 30 species described in these portals and in the reduced literature that was found. Despite the diversity of ascidians documented, the genetic studies are scarce. Moreover, despite being the most diverse order in Ascidiacea, Aplousobranchia is not the most studied one. In fact, most of the ascidians' species with a high number of sequences reported in NCBI database are model organisms. Notwithstanding the studies on ascidians being important and relevant, such as, for bioactive compounds prospecting, the available genetic data are mostly limited to the *COI* gene. Overall, this work revealed that the most suitable organ to extract DNA for ascidian barcoding is the gonads. The morphological data suggested that the collected samples were *Ciona intestinalis* and *Molgula socialis*, which were later confirmed by the bioinformatic analyses conducted. The collected samples were grouped in a phylogenetic clade with their respective ascidian families, in accordance with the morphological data; supporting a correct identification of the samples. Thus, this study provided the first steps to execute a genetic characterization of *Ciona intestinalis* and *Molgula socialis* collected in Portugal.

Keywords – Ascidians; *COI* gene; genetic characterization; ascidian diversity

Antimicrobials from the sea: inspiring models to revert multidrug resistance

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Abstract

Introduction: The Sea constitutes a reservoir of novel and diversified compounds displaying a wide array of biological and pharmacological activities. The fact that pathogenic microorganisms are finding ways to evade all known antimicrobials is a pressing issue of our days, and efforts have been put into finding new antimicrobial compounds that can overcome resistance.

Methodologies: The antimicrobial activity investigation of several libraries of marine-inspired derivatives was performed, and minimum inhibitory concentrations (MIC) were determined by broth microdilution methods, following CLSI protocols. Antibacterial activity was accessed for reference strains (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) and multidrug-resistant isolates (ESBL *E. coli* SA/2, MRSA *S. aureus* 66/1, VRE *E. faecalis* B3/101). Potential synergies with clinically relevant antibiotics were evaluated by the checkerboard method, using cefotaxime for *E. coli*, oxacillin for *S. aureus* and vancomycin for *E. faecalis*, and prevention of biofilm formation was assessed through the crystal violet method. Antifungal activity was evaluated for reference strains *Candida albicans* ATCC 10231, *Aspergillus fumigatus* ATCC 46645, and clinical isolate *Trichophyton rubrum* FF5. Inhibition of dimorphic transition in *C. albicans* was also assessed.

Results/Discussion: Antibacterial activity was found on Gram-positive bacteria (MICs ranging between 32 and 64 µg/mL for *S. aureus* and *E. faecalis* reference strains). Potential synergies were observed between several compounds and cefotaxime for *E. coli* SA/2, and vancomycin for *E. faecalis* B3/101. Inhibition of biofilm formation of *S. aureus*, *E. faecalis* and *E. coli* reference strains was detected. Antifungal activity was found for *T. rubrum* FF5 (MICs ranging between 2 and

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256 µg/mL. Slight activity against *A. fumigatus* ATCC was detected, with a MIC of 256 µg/mL. MICs for *C. albicans* ATCC ranged between 64 and 256 µg/mL, and inhibition of dimorphic transition, which can reduce its virulence and biofilm formation, was also detected.

Conclusion: Despite the fact that the majority of compounds did not present antimicrobial activity, promising results were found, particularly in combination with antibacterial drugs in resistant strains, suggesting activity in resistance mechanisms. Future studies will involve insights into these mechanisms and testing of new compounds.

Keywords – Marine-inspired derivatives; antifungal activity; antibacterial activity; antimicrobial resistance

Chiral stationary phases based on xanthonic derivatives for liquid chromatography: synthesis, enantioresolution performance and mechanism of chiral recognition

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Abstract

Introduction: Nowadays, direct liquid chromatography (LC) method using chiral stationary phases (CSPs) proved to be one of the most important approach for preparative applications as well as for analytical purposes [1]. The progresses established in chiral chromatography allowed the improvement of the chromatographic performance of CSPs for the enantioseparation of a variety of analytes and the extension of application areas, being this technique applied since the first steps of the drug discovery to a full-scale process [2]. Nevertheless, the development of new CSPs continues to be an important field of research [3]. This work focus on the preparation and enantioresolution evaluation of new CSPs.

Methodologies: In this study, two CSPs comprising two chiral moieties were successfully prepared by multi-step reaction pathways based on the most promising selectors previously reported [4]. We aimed to obtain versatile and efficient CSPs by exploring the effect of the number, chemical nature and position of the substituents in the central aromatic core of the chemical framework and, consequently, their effect on the enantioselective performance of the CSPs. The chiral selectors were covalently bonded to a chromatographic support, and further packed into LC stainless-steel columns (150 x 2.1 mm I.D.). Their enantioselective performance was evaluated using different classes of chiral compounds. Besides, assessment of chiral recognition mechanisms was performed by molecular docking approach.

Results/Discussion: The CSPs demonstrated that they have enantiomeric specificity for some chiral compounds under normal-phase elution conditions. It was also possible to conclude that the number and position of the chiral units in the xanthone scaffold of the chiral selectors can interfere with the chiral recognition mechanism by steric hindrance. Concerning the results obtained through docking studies, the main conclusions were that *R*-enantiomers presented a superior

affinity to the selector than the *S*-enantiomers, being these data in agreement with the obtained chromatographic parameters [5].

Conclusion: The development of new CSPs for LC is a continuous and challenging issue. This work is an important contribution for the improvement of the design of new selectors and preparation of more efficient CSPs based on xanthone derivatives.

Keywords – Liquid chromatography; Chiral stationary phase; Xanthone; Chiral recognition; Docking

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A novel O-alkylating enzyme that esterifies bartolosides with free fatty acids

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Abstract

In biological chemistry, carboxyester formation usually involves the nucleophilic attack of an alcohol onto an activated carbonyl group. Still, carboxylates can act as the nucleophiles in esterifications, for example in SAM-dependent methylations. On the other hand, carboxyester formation involving a carboxylate nucleophile and alkyl halide electrophile – a reaction well-known in synthetic chemistry – has only been transiently observed in enzymatic catalysis.

Here, we show that a novel enzyme – BrtB – is responsible for the O-alkylation of free fatty acids with the secondary alkyl halide moieties found in the bartolosides, a series of cyanobacterial chlorinated dialkylresorcinols. This discovery emerged from our observation of a series of esterified bartolosides, formed both naturally and under supplementation of fatty acids. BrtB, encoded in the bartolosides biosynthetic gene cluster, is capable of generating bartoloside esters using fatty carboxylates as nucleophiles and shows high promiscuity as to fatty acid length and saturation levels, which might be relevant for its application in biocatalysis.

Keywords – natural product; biosynthesis; esterification; enzymatic catalysis

Diketopiperazines and other bioactive compounds from bacterial symbionts of marine sponges

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Abstract

Humanity faces great challenges, such as the rise of bacterial antibiotic resistance and cancer incidence. Thus, the discovery of novel therapeutics from underexplored environments, such as marine habitats, is fundamental. In this study, twelve strains from the phylum Firmicutes and thirty-four strains from the phylum Proteobacteria, isolated from marine sponges of the *Erylus* genus collected in Portuguese waters, were tested for bioactivities and the secondary metabolites were characterised. Bioactivity screenings included antimicrobial, anti-fungal, anti-parasitic and anti-cancer assays. Selected bioactive extracts were further analysed for already described molecules through high performance liquid chromatography and mass spectrometry. Several bioactivities were observed against the fungus *Aspergillus fumigatus*, bacteria (methicillin-resistant *Staphylococcus aureus* and *Escherichia coli*), the human liver cancer cell line HepG2 and the parasite *Trypanosoma cruzi*. Medium scale-up volume extracts confirmed anti-fungal activity by strains *Proteus mirabilis* #118_13 and *Proteus* sp. strain #118_20 (JX006497). Anti-parasitic activity was also confirmed in *Enterococcus faecalis* strain #118_3. Moreover, *P. mirabilis* #118_13 showed bioactivity in human melanoma cell line A2058 and the cell line HepG2. The dereplication of bioactive extracts showed the existence of a variety of secondary metabolites, with some unidentifiable molecules. This work shows that bacterial communities of sponges are indeed good candidates for drug discovery and, as far as we know, we describe anti-parasitic activity of a strain of *E. faecalis* and the presence of diketopiperazines in *Proteus* genus for the first time.

Keywords – Firmicutes; Proteobacteria; Bioactivities; Secondary metabolites; cyclo([iso]leucylprolyl); caerulomycin G; cyclo(phenylalanylprolyl)

Integrating green roofs into the concept of circular cities-

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Abstract

Nature-based solutions are discussed to manage urban waters, to increase biodiversity or to support the regulation of urban climate, to mitigate urban heat islands and thus to counteract urban heat stress. As such, green roofs are extremely important within the dialogue of sustainable development, especially for Southern Europe.

When green infrastructure is discussed the focus is mainly on their ecosystem services while the ecological burdens of their construction, use and demolition are not considered with the same weight. Looking at a conventional green roof the majority of the CO₂ emissions are due to the production of the energy intensive substrate materials and the synthetic layers. Because the emissions cannot be amortized during their expected lifetime of 45 years, we propose to use different materials in the substrate and in the drainage layer that reflect the concept of a circular city. The conventional green roof is compared to alternative green roof that contains recycled bricks instead of expanded clay and insulation cork board as an alternative for the synthetic drainage layer. A comparative live cycle assessment (LCA), including all life cycle stages, was conducted to calculate the global warming potential.

The results confirm that the conventional green roof cannot amortize the CO₂ emissions over its life cycle, whereas the alternative green roof has the potential of saving CO₂ emissions. Furthermore, the results show that especially concerning the demolition available data and infrastructure is missing. It is particularly this information and infrastructure gap that needs to be closed to sufficiently integrate the concept of nature-based solutions into the circular city concept and to appropriately assess the performance of nature-based solutions, therefore gaining the maximal benefit.

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Keywords – circular city, nature based solution, green roof, LCA, carbon footprint, cork

Benefits of green roofs in densely urban settlements - Macao SAR as a case-study

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Abstract

According to the United Nations (UN) World Urbanization Prospects 2018, cities are growing in both size and number, posing challenges for sustainable development. With urbanization, the increase of impermeabilization and landscape fragmentation, urges for territorial planning and resources management. In this context, Macao is the second densely populated city in the world, according to the UN Department of Economic and Social Affairs Population Division (World Population Prospects 2019), where the demand for a sustainable *economic, social and environmental development is high*.

In order to promote resilient and resourceful cities, strategic planning has to be taken in consideration, where nature-based solutions can be included into the built environment, counteracting the negative effects of urbanization through the provision of ecosystem services. Accordingly, to The United Nations World Water Development Report 2018, "Nature-based solutions (NBS) are inspired and supported by nature and use, or mimic, natural processes to contribute to the improved management of water". Green roofs are examples of NBS that provide a panoply of ecosystem services at the level of the building and city, besides contributing for climate change mitigation and adaptation. This NBS can be included in the urban planning agenda in the new building or as a retrofit solution in the existing built environment.

The present study aims to assess the inclusion of green roofs in Macao and assess the ecosystem services that they may provide in a holistic approach aligned with a sustainable development. For that, a green roof has been implemented in Taipa Island Macao and is being monitored in terms of biodiversity present (through Winkler sampling methodology), carbon cycle analysis (through Leaf Litter decomposing methodology) and fungi and bacteria dynamics (through molecular biology tools). Further on, is being carry out a comparison between this green roof and a conventional roof in terms of thermal performance. Although, this study is ongoing, results till now have shown to be very promising in terms of the applicability of green roofs in Macao and delivering several ecosystem services.

Keywords – nature-based solutions; biodiversity; urban; water management; circular economy; climate change

Acquisition of social behavior in mammalian lineages is related with duplication events of FPR genes

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Abstract

Formyl peptide receptors (FPRs) were firstly detected in immune cells where they act as key mediators of leukocyte chemotaxis, promoting the host defense against pathogens. Recently, three paralogs were reported in *Homo sapiens* (FPR1-3) and seven paralogs in *Mus musculus* (FPR1, FPRs1-4, FPRs6 and FPRs7), but information from other mammalian lineages is scarce, including ambiguities in the current nomenclature system (e.g. absence of an orthologous relation between human and mouse FPR3). Here, we explored the FPR gene repertoire across 175 mammalian genomes using integrative phylogenetic and synteny analyses to describe the evolutionary history of FPRs in all mammalian orders. FPRs present a well conserved synteny but showed dynamic episodes of duplication events specific to several mammalian orders (Chiroptera, Perissodactyla, Primates and Rodentia), with up to 11 paralogs in some cases. Despite FPRs could be expressed in a panoply of tissues, there is a suggestion that they maintain an exclusive immunological function. However, we observed that species with social behavior have higher repertoire of FPRs in contrast with species with solitary lifestyle. Such evidence suggests a strict relationship between the optimization of the immunological system (by FPR duplication patterns) and the mammalian social behavior.

Keywords – FPRs; Social behavior; Mammals

Playing hard to sequence: Challenges and developments in sex chromosome sequencing

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Abstract

Sexual reproduction is a vital process for most eukaryotic species, fundamental for increasing and preserving genetic diversity. In gonochoristic organisms, this process is dependent on the differentiation of distinct sexes. A variety of different mechanisms can be responsible for determining sex and, consequently, triggering the differentiation of the gonads, dependent mostly on environmental cues or genetic factors. However, within vertebrates, the majority of species rely on a special pair of chromosomes. The immense diversity of sex chromosome arrangements found throughout all vertebrate lineages, as well as the impact of these special genomic structures in the evolution of species, was previously overlooked due to several constraints in their study. Even resorting to state-of-the-art technology, sex chromosomes remain some of the most complex regions of the genome to sequence and assemble. In this work, we review the challenges and recent developments involved in sequencing these important elements of the genome. Despite being extremely variable among lineages, sex chromosomes share unique and peculiar morphological characteristics that hamper the sequencing process. Their morphology and genetic content, rich in heterochromatin and highly repetitive regions – including multicopy gene families, are still obstacles for the collection of genomic data. However, new methodologies that combine accurate short reads from Next-Generation Sequencing (NGS) and long reads from modern Third-Generation Sequencing (TGS) techniques are helping researchers close the missing gaps in sex chromosome sequences. Moreover, several ongoing large-scale sequencing projects aim to generate high-quality and gapless whole-genome assemblies for a massive number of species, and most are focused in including repetitive regions that are challenging to assemble – such as sex chromosomes. Given the prevalence of these chromosomes in vertebrates and their impact in the evolution of species, this data will unveil valuable information for further evolutionary and comparative genomic studies, particularly for understanding the evolution of sex chromosomes and sex determining mechanisms.

Keywords – Sex determining mechanisms; Sex chromosomes; Vertebrates; Next-Generation Sequencing; Third-Generation Sequencing; Comparative genomics

Comparison of culture-dependent and independent approaches for the bioremediation of oil spills

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Abstract

Oil spills are the most hazardous disasters to occur in marine environments and current mitigation techniques are expensive and have short-term effect. Bioremediation approaches can become a cost-effective treatment to recover contaminated environments, by increasing the activity of microorganisms with biodegradation capability.

Cultivation-dependent approaches only allow to recover 1% of the world's biodiversity. With the development of cultivation-independent approaches, such as next-generation sequencing, it is possible to observe if the strains isolated in the laboratory are relevant among the total microbial community, as well as, apply specific culture media to recover the most promising bacterial strains.

In this study, we compare cultivation-dependent and independent approaches to observe if it was possible to retrieve potential hydrocarbon-degrading prokaryotes that were also most abundant in the oil-enriched communities and which culture media was the most suitable for this task.

Seawater samples were collected along three delimited areas in the NW coast of the Iberian Peninsula, at both intertidal zones and offshore sites. The samples were kept in microcosms exposed to crude-oil and spiked with nutrients, for two weeks. Afterwards, the microcosms' samples were filtered through Sterivex™ filters, for 16S rRNA next-generation sequencing, and also plated in three medium cultures (Marine Agar, M1 and Bushnell-Hass). Then, the DNA of the isolates was extracted for Sanger sequencing. Taxonomic characterization of NGS samples was performed with DADA2.

A total of 37 different genera were isolated, however only 25 of those were found in the NGS analysis. The most abundant bacterial strains that were cultivated belonged to the genera *Pseudomonas* (composing on average ≈6% of all communities from the NGS analysis), *Thalassospira* (≈9%), *Acinetobacter* (≈4%), *Joostella* (≈1%), *Alcanivorax* (≈48%), *Thioclava* (≈0.47%), *Cobetia* (≈ 0.47%), *Maribacter* (≈ 0.18%), *Cerelibacter* and *Sulfitobacter* (≈ 0.02%).

The most promising culture media was M1 because it retrieved a higher number of genera known to degrade petroleum compounds. Regarding the areas and the geography (intertidal or offshore), it was possible to isolate 7 common genera, such as *Alcanivorax*, *Cobetia*, *Joostella*, *Marinobacter*, *Pseudomonas*, *Thalassospira* and *Thioclava*.

These results demonstrate that it was possible to isolate the most abundant hydrocarbon-degrading genera present in the communities. This study is a starting point for the implementation of a bioremediation technique to mitigate oil spills, by using native microorganisms.

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Keywords – Oil spills, bioremediation, next-generation sequencing, cultivation-dependent, native microorganisms

The feasibility of the planctomycete *Rhodopirellula rubra* and the crustaceans (*Daphnia magna* and *Artemia franciscana*) enriched with this bacterium as food sources in aquaculture

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Abstract

Aquaculture is a fast growing food production sector and provides half of the world fisheries production for human consumption. However, this sector is highly dependent on fishmeal and fish oil derived from capture fisheries to manufacture feeds for aquaculture species. To reduce this pressure on fish stocks, further research is needed to find alternative protein sources that maintain feed performance, promote health benefits and simultaneously be more economical and environmentally friendly. Our previous studies have shown the ability of the planctomycete *Rhodopirellula rubra*, used in association with *Raphidocelis subcapitata*, to improve daphnids life history parameters, to provide them with pink coloration and to increase the hemoglobin levels. In addition, protein and glycogen content were increased and oxidative stress reduction was observed after exposed to the mixed diet. Our goal is to continue this research, testing *R. rubra*, *R. rubra* enriched *Daphnia magna* and *Artemia franciscana*, as food for aquaculture purposes. These feeds will be used as food sources for the fish *Danio rerio* and *Oncorhynchus mykiss*. Biochemical analyses will be performed to assess the nutritional value of these foods. Furthermore, several fish endpoints such as biometric measures, health conditions, fertility, oxidative stress, histopathological changes and pigmentation, will be measured to evaluate the impact of these foods in fish growth, performance and development. At the end of the project we ought to identify an alternative nutritional method for fish feeding, which at the same time can improve fish performance and help reducing issues related to diseases, malformation and stress resistance in various species of fish.

Keywords – fish feed; fish standard species; pigmentation; biomarkers

Portoamides A and B: a superhero also need friends!

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Abstract

Cyanobacteria are recognized as producer of natural products that in some case may have biotechnological applications. Often, these compounds are isolated from symbiotic or filamentous cyanobacteria. Portoamides A and B (**PAB**) were isolated from the strain *Phormidium* sp. LEGE 05929 and have allelopathic effects on the microalgae *Chlorella vulgaris* and cytotoxic effects against several cell lines. **PAB** were tested against the cancer cell line HCT116 cultured both in monolayer (2D) and as multicellular system (3D), and its penetration on the structure and consequential effects were analysed. The 2D approach revealed that **PAB** enters into cells on energy-independent mechanism, while fluorescent analyses on spheroids showed only cytotoxic effects against the outer, proliferative cell layers. Mitochondrial hyperpolarization was observed, a decrease of ATP content and disruption of OXPHOS with a significant decrease of maximal mitochondrial respiration. The overexpression of PINK1 additionally indicated that disruption of mitochondrial function is the underlying mechanism of cytotoxicity. Targeting mitochondria was recently described as a particularly interesting strategy for cancer treatment, and **PAB** reveal specificity on this target, penetrating the outer layer of spheroids.

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Keywords – natural compounds; 3D culture; cytotoxicity; mitochondria; OXPHOS

Feeds with a micro- and macroalgae blend to increase fish robustness

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Abstract

European seabass (*Dicentrarchus labrax*) is an important species of the Mediterranean region, but intensification of its farming often increases disease outbreaks. Due to the growing antimicrobial resistance, a prudent use of antibiotics in animals is advised. Algae are valuable sources of bioactive compounds with antibacterial activity, being promising products to improve fish health. This work aims to unravel the antimicrobial potential of commercially produced algae and their impact on *D. labrax* immune status and health. The *in vitro* bactericidal activity of two microalgae (*Nannochloropsis oceanica*; *Chlorella vulgaris*), two macroalgae (*Gracilaria gracilis*; *Ulva rigida*), and a blend of these algae was tested against main pathogenic bacteria for farmed fish (*Vibrio anguillarum*, *Photobacterium damsela* subsp. *piscicida*, *Tenacibaculum maritimum*, *V. harveyi*, *V. parahaemolyticus*, *Aeromonas hydrophyla*, *Yersinia ruckeri* and *Edwardsiella tarda*). Four isoproteic and isolipidic diets were formulated with increasing amounts of algae blend (0, 2, 4, 6%) for an *in vivo* study. After feeding diets to triplicate groups of European seabass for 12-weeks, fish were subjected to a bacterial bath challenge (*T. maritimum*) and reallocated into 2 new systems: one to register mortalities (8 days, triplicates of 10 fish); and other to collect samples (48h, duplicates of 10 fish). For the 48h-challenge, a non-infected group with similar handling was also included. The haematological profile of both groups was analysed: total white (WBC) and red blood cells (RBC) counts, haematocrit, haemoglobin and mean corpuscular volume (MCV), haemoglobin and haemoglobin concentration. Results showed that algae displayed bactericidal activity against most groups of pathogenic bacteria, but were unable to kill *V. anguillarum*, *A. hydrophyla* and had limited effects against *V. harveyi*. The highest activity (40-45%) was observed with all algae and blend against *T. maritimum*. After the 48h-challenge, haematocrit (regardless of infection treatment) and WBC (only in non-infected group) were increased in fish fed 6% diet compared to those fed CTRL (0%). RBC and MCV were higher and lower, respectively, in infected group compared to non-infected.

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The cumulative mortality was lowest in fish fed 4% diet (33%)<6%<Ctrl<2%. Further studies are required to confirm the potential of algae to improve fish health and disease resistance.

Keywords – European seabass, algae, fish robustness, bacterial disease

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Biodegradation of Atorvastatin by Different Bacterial Consortia

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Abstract

The worldwide use of pharmaceuticals in human and veterinary medicine is of growing concern, as tons of pharmaceuticals are produced and consumed annually.

Atorvastatin (ATO), a cholesterol lowering agent mainly known by its trade name Lipitor®, is one of the top ten most sold drugs in the world. Therefore, this pharmaceutical has been detected as a contaminant of natural waters and wastewater effluents. Once in the environment, ATO is expected to be resistant to biodegradation, mainly due to the presence of a fluorine atom in the molecule that renders the molecule more stable and recalcitrant.

Bioremediation, the use of living organisms, mostly microorganisms, to degrade contaminants and convert them into less toxic or nontoxic forms, is considered a sustainable approach for environmental pollution control.

This study aimed to investigate the biodegradation of ATO by bacterial consortia capable of biodegrading other halogenated emerging contaminants. To do so, bacterial consortia previously enriched with the fluorinated drugs enrofloxacin (ENR) and paroxetine (PRX), the chlorinated drug bezafibrate (BZF) and a fluorinated fungicide epoxiconazole (AE) were used as inocula. Biodegradation of ATO (3 mgL⁻¹) was investigated in aerobic conditions, in static mode, and in co-metabolism with sodium acetate (500 mgL⁻¹) during cycles of 21 days. Biodegradation was followed by monitoring bacterial growth by spectrophotometry and the release of fluoride ion by potentiometry, on a weekly basis. The best performing consortia were characterized following a culture-dependent approach and the different bacterial isolates were identified through 16S rRNA gene sequence analysis.

Results obtained in the biodegradation experiments showed that all tested consortia have the capacity to biodegrade ATO, however, only the consortia BZF and AE were able to completely defluorinate the compound. The identification of the isolated microorganisms revealed that most of the bacteria present in the original consortia were also retrieved after the biodegradation of ATO, which may indicate that they also have a role in the biodegradation of this pharmaceutical.

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Bacterial consortia BZF and AE displayed promising results and should be considered in the future as a biotechnological tool for the removal of halogenated compounds from contaminated sites.

Keywords – Atorvastatin; bioremediation; bacterial consortia; halogenated compounds; defluorination

Functional and biochemical effects of suspended sediments in the model species *Mytilus galloprovincialis* under hyperbaric conditions

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Abstract

The deep-sea is characterized by extreme conditions like high pressure, low temperatures and partial to total darkness. Despite the harsh conditions, the deep-sea is highly biodiverse and also the biggest ecosystem on earth. Even with the inaccessibility of deep-sea environments, they are subjected to anthropogenic stressors like deep-sea mining and the sediment plumes released during mining practices.

The effects of suspended sediments (0, 1, 2 and 4g/L) were studied in the model species *Mytilus galloprovincialis* under hyperbaric conditions (1, 4 and 50 Bar). Filtration rate and biochemical markers of oxidative stress [catalase (CAT), lipid peroxidation (LPO), glutathione S-transferase (GST) and superoxide dismutase (SOD)] were analyzed in juvenile mussels. The filtration rate decreased for all tested concentrations and pressures. Significant alterations were also found for all biomarkers with sediment and pressure dependency.

These findings will help fill the existing knowledge gaps regarding hazard assessment of deep-sea mining and its related stressors. Understanding how mining affects deep-sea environments is of the utmost importance to the development of guidelines to protect sensitive habitats and enable responsible and sustainable exploitation of mineral resources in the ocean floor.

Keywords – Hyperbaric chamber; *Mytilus galloprovincialis*; hazard assessment; suspended sediments; biomarkers; filtration rate

Bioactivity screening of cyanobacteria for intestinal lipid uptake

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Abstract

Obesity is a multifactorial disorder characterized by a sustained positive energy balance leading to excessive fat accumulation in different body regions. Its incidence has nearly tripled since the 1970s, causing over 4 million deaths each year worldwide. This, added to the fact that obesity increases the risk of developing comorbidities such as type II diabetes, hypertension and even some forms of cancer, makes it urgent to mitigate this global health problem. However, the adverse secondary effects related to the currently approved drugs, makes it necessary to find safer treatments. As cyanobacteria are known to produce a wide variety of secondary metabolites, these could be considered as a promising resource of novel anti-obesity compounds.

The aim of this work was to identify novel strains of cyanobacteria that could affect intestinal lipid uptake, using zebrafish larvae as a relevant animal model. A PED6 fluorescent reporter was applied in a primary screening for lipase inhibition activities. Active fractions were validated for normal functioning of the digestive physiology by analysis of intestinal protease activity with the fluorescent reporter EnzChek. To decipher the potential effect of active fractions on various species of lipids, secondary assays were used with fluorescent lipid analogs for short-chain fatty acids (SCFA) and long-chain fatty acids (LCFA). Finally, the work was complemented by analysis of alterations of lipid classes, as well as incorporation of LCFA into different lipid classes by Thin Layer Chromatography (TLC). From a total of 263 screened fractions, 11 showed moderate (9) to strong (2) lipase inhibition without altering digestion *in vivo*. 6 of these fractions also reduced significantly the uptake of LCFA in zebrafish larvae. Regarding the analysis of main lipid classes incorporated in larvae, 2 fractions did significantly decrease the content of whole-body triglycerides (TG) in comparison to the solvent control, whereas modulation of LCFA processing was not significantly observed for any fraction.

Future work will be focused on isolating the responsible compounds from the most promising strains.

Keywords – Obesity; zebrafish; fluorescent; screening; lipase; intestinal lipid uptake

Constructed wetlands for wastewater treatment in densely urban settlements

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Abstract

Constructed Wetlands (CWs) are nature-based solutions that mimic the biogeochemical processes that occur in natural wetland systems. They are often applied to remove pollutants from wastewater. Besides water treatment, CWs can provide several ecosystem services and benefits, namely mitigate floods, recycle water for irrigation, provide biomass for energy production and enhance biodiversity. Based on life cycle assessments data, CWs have shown to be cost-effective and a low environmental impact technology. This study aims to review and evaluate environmental impact of the main materials that make up CWs, as well as combining the concept of water circularity into urban wastewater management. Intensified strategies to tackle rate-limiting factors for improving CWs performance have also been explored. A final optimized design with selected sustainable materials would be proposed for implementation in Macao SAR, a city in China with the second highest population density in the world. We attempt to calculate the area needed to treat the post-primary treated wastewater from the Macao Peninsula wastewater treatment plant, and further locate suitable areas for CW implementation. Our study would enable decision-makers to look at wastewater management in a different perspective other than traditional hard-engineering projects. They would provide an insight into coupling nature-based solutions with urban wastewater treatment, flood mitigation, bioenergy production and at the same time, achieve circular water management.

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.

Keywords – circular city; nature-based solution; water management; LCA; ecosystem services; water treatment

Towards new BACE1 inhibitors: *in silico* studies and synthesis

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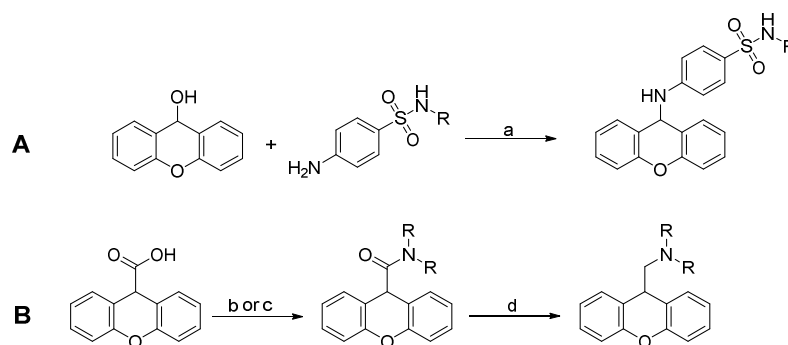
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Abstract

Beta-site APP-cleaving enzyme (BACE)1 is a type-1 membrane-anchored aspartyl protease playing an essential role in the release of A β peptides and Alzheimer's Disease (AD) progression. Hence, the development of potent BACE1 inhibitors represents a logical approach for AD therapy development and it have been widely explored by the pharmaceutical industry worldwide.¹

Herein, we report the design of a virtual library of 300 compounds and the synthesis of a first set of derivatives for *in vitro* BACE1 inhibition assessment. These compounds arise from the conjugation of tricyclic fragments with aliphatic and aromatic amines, motifs identified in the literature by their ability to establish essential interactions with the amino acids present in the catalytic pocket of BACE1. Affinity for BACE1 was measure through the binding energy estimation of the ligand-protein complex. Additionally, the compounds designed were assessed through the Lipinski's rule of 5 and additional attributes crucial for central nervous system (CNS) drugs were also considered.²

A first series of compounds arising from the conjugation of xanthidrol with several sulfonamides was obtained according with the conditions stated in **Figure 1, A**. Following, 9*H*-xanthene-9-carboxamides were synthesized through the conjugation of xanthene-9-carboxylic acid with several aromatic and aliphatic amines. Subsequent amide reduction led to the respective amines (**Figure 1, B**).



Reagents and conditions: (a) acetic acid, 25 °C, 2-24h; (b) anhydrous CH₂Cl₂, DIEA, COMU, amine, 0 °C to rt, 2-24h; (c) anhydrous THF, TEA, TBTU, amine, rt; (d) i) BH₃.THF, THF, 0 °C to reflux, 2h; ii) HCl 6N, 0 °C, 30 min;

Figure 1. (A) Synthesis of xanthydrolyl-sulfonamide derivatives ³. (B) Synthesis of 9H-xanthene-9-carboxamides and subsequent reduction.

Keywords – BACE1; ALZHEIMER'S DISEASE; XANTHENE DERIVATIVES

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Tropomyosin variations in the mussel *Mytilus galloprovincialis* exposed to Diarrhetic Shellfish Toxins

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Abstract

The consumption of seafood contaminated with diarrhetic shellfish toxins (DSTs) represents a food safety threat, being shellfish the main vector for human consumers. Moreover, DSTs can produce significant alterations at the protein level in shellfish and this can affect the content of allergens. Two assays have been developed to study the variations in the tropomyosin (TM) content in mussels (*Mytilus galloprovincialis*) during the accumulation/depuration of DSTs. In both assays, 6 aquaria were used, 3 of control, feed with non-toxic mixture of *Isochrysis* and *Tetraselmis* (5.0×10^7 cells/L (1st assay) and 1.0×10^7 cells/L (2nd assay)) and 3 of treatment, feed with *Prorocentrum lima* (2.6×10^8 cells/L (1st assay) and 1.0×10^6 cells/L (2nd assay)). These assays were established in two phases: intoxication/accumulation (5 days) and depuration (14 days). DSTs were determined by LC-MS and the TM content was determined by using quantitative Elisa KITs (Immunolab).

Regarding the dynamics of DSTs, the results of both assays showed that there was an exponential trend in the accumulation of toxins during the intoxication process, although in the first assay higher concentrations of both okadaic acid (OA) and dinophysistoxin-1 (DTX-1) were registered in mussels (a maximum of $10648 \mu\text{g OA/kg}$ and $3659 \mu\text{g DTX-1/kg}$), in comparison with the second assay (a maximum of $2819 \mu\text{g OA/kg}$ and $1107 \mu\text{g DTX-1/kg}$). Considering this pattern of accumulation, it was possible to conclude that 14 days of depuration are not enough to reduce the content of DSTs in mussels to levels below the legal limit ($160 \mu\text{g}$ of OA equivalent/kg of shellfish), which can be of concern in terms of public health. Concerning the dynamics of TM in mussels, although in the second assay the differences were slighter, there was in both assays a decrease in the TM content corresponding to the maximum content of DSTs in mussels, returning to basal values after the depuration phase. This effect is observed probably because OA acts as a serine/threonine protein phosphatase inhibitor, affecting in particular the phosphorylation patterns of proteins, which may be of relevance in the TM content, which is a fundamental component of cytoskeleton in muscular and non-muscular cells.

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Keywords – Diarrhetic Shellfish Toxins; *Mytilus galloprovincialis*; DSTs accumulation; DSTs depuration; allergens; tropomyosin

Acknowledgments – This research was supported by the Strategic Funding UID/Multi/04423/2013 and UID/Multi/04423/2019 and the research project MOREBIVALVES (PTDC/ASP-PES/31762/ 2017).

An evaluation of the ecotoxicity of surface waters of Pernambuco Rivers (Brazil)

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Abstract

The contamination of continental aquatic ecosystems has become a major concern for society, existing, presently, a vast literature on the impacts caused by different types of contaminants on these ecosystems. Human activities, resulting from land use and occupation, and industrial activities are the main factors responsible for water pollution. In Brazil, despite the importance that water resources play for the country's development, the quality and quantity of river waters have been increasingly affected by the disordered occupation of the hydrographic basins. In the present work, an holistic approach will be utilized to assess the quality of surface waters of two hydrographic basins (Botafoogo basin and Bitá/Utinga basin) of rivers in the state of Pernambuco, Brazil: i) assessment of the waters physico-chemical parameters, ii) evaluation of presence/concentration of ECs and heavy metals, and iii) biological effects assessment, through the application of zebrafish (*D. rerio*) embryo bioassays. Preliminary results of surface waters from Botafoogo basin, showed significant alterations in zebrafish (*D. rerio*) embryo development, hatch rate, and larvae length (sampling points P6 and P9). Overall, this study further supports the use of zebrafish embryo bioassays as a fast, high throughput approach for screening the toxicity of water samples, evidencing the toxic effects of some of the analyzed samples, which highlight the need for more detail evaluations combining biological and chemical analysis. In summary, we expect to contribute to the improvement of the ecotoxicological risk assessment of ECs and heavy metals, while at the same time support with scientific knowledge to the establishment and improvement of Pernambuco regulatory environmental measures.

Keywords – Ecotoxicity; surface waters; embryo bioassays; emerging contaminants; heavy metals

Assessment of the potential of oleate as functional ingredient on oxidative stress of European seabass (*Dicentrarchus labrax*) juveniles fed high-lipid diets

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Abstract

Nowadays, aquafeeds contain high lipid levels, which are a source of essential fatty acids and energy and constitutes an approach to spare protein utilization for plastic purposes. However, high lipid levels lead to an increase in tissue fat deposition and susceptibility to lipid peroxidation, jeopardizing flesh quality which is of major concern for the final consumer. Functional ingredients have been used as a nutritional strategy to improve animal health and to mitigate harmful effects related to diets, including those associated with the use of high-fat diets. Oleate (OA) was already proven to benefit the health and nutritional status of higher animals. For fish, the potential of OA as a functional ingredient was up to now not studied. Thus, this proposal aimed to evaluate the potential of OA as a functional ingredient in aquafeeds and its antioxidant effect in the liver and intestine of European seabass juveniles fed lipid-rich diets. For that purpose, six experimental diets with fishmeal and cod liver oil as protein and lipid sources were designed to contain two lipid levels (16 and 22%), supplemented or not with OA (0, 1, and 2%). Triplicate groups of European seabass juveniles (21.4g) were fed with these diets for 9 weeks. At the end of the trial liver and intestine were collected for oxidative stress assessment.

Regardless of dietary lipid levels, OA supplementation increased hepatic glucose-6-phosphate-dehydrogenase (G6PDH) activity but has no effect on superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPX) activities. Similarly, hepatic lipid peroxidation was not affected by dietary OA levels but increased with dietary lipid increase from 16 to 22%.

In the intestine, the increase of dietary lipid levels decreased SOD activity and decreased GPX activity, without affecting G6PDH and GR activities. Dietary oleate supplementation increased SOD and CAT activities. The results suggest that oleate modulated the liver and intestine antioxidant mechanisms without impacts on their lipid oxidative status. Further studies on the role of oleate in modulating fish oxidative status are necessary to confirm its potential as a functional ingredient in high-lipid diets for European seabass.

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Keywords – Functional ingredients; Lipid level; Oleate; European seabass; oxidative stress

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Sex determination and a phylogenetic analysis of *AMH*, the candidate sex-determining gene in platypus (Mammalia: Monotremata)

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Abstract

Sex determination and differentiation are crucial steps intrinsically linked to the development of a new individual, largely contributing to species' survival and reproductive success. This complex process can be controlled by either environmental or genetic factors, with genotypic sex determination (GSD) being the most widespread mechanism within vertebrates. Sex determination in vertebrates involves (i) sex chromosomes differing among the major taxonomic groups, with the two most studied systems being the avian ZW and the mammalian XY, and (ii) specialized genes, also different between these groups. In birds, marsupials and placental mammals, the sex-determining genes have already been unravelled. However, in platypus (*Ornithorhynchus anatinus*), one of the five extant species of the Monotremata order (Mammalia), these genes remain undetermined. This species possesses a complex sex chromosome system, with five XY pairs, which shares homology with the avian ZW sex chromosomes, but not with the marsupial and eutherian XY system. One of the candidates for the role of sex-determining gene in this species is the Anti-Müllerian Hormone (*AMH*), a gene involved in the initial steps of sex determination across vertebrates. Thus, in this study, a *Maximum-likelihood* phylogenetic analysis of the *AMH* gene was performed, aiming to explore the evolution of this gene across vertebrates. For this, 132 nucleotide sequences from Reptilia (Crocodylia), Aves and Mammalia classes were retrieved from the NCBI gene database (assessed May, 2020). This comparative genetic analysis revealed that the platypus *AMH* sequence is most closely related to other mammals than it is to birds or crocodiles. However, it was also possible to observe that the length of the platypus phylogenetic branch indicates a high number of nucleotide substitutions *per* sequence site in the *AMH* gene. This result shows a clear divergence between the Monotreme and the Therian *AMH* gene, which might add support to the hypothesis of a possible role of this gene as sex-determining in platypus. However, further studies are necessary to predict the impact of these substitutions on the *AMH* protein structure and function, and therefore, we suggest integrative omics analyses to enlighten its role in the Monotreme lineage.

Keywords – Sex determination; sex chromosomes; sex-determining genes; *AMH*; *Ornithorhynchus anatinus*; phylogenetic analysis

Undercovering Antibiotic Resistance in the phylum Planctomycetes: a genomic approach

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Abstract

Antibiotic resistance (AR) is primarily studied in pathogenic bacteria since they represent a major threat to human health, with a recent high-profile report estimating that, by 2050, 10 million people will die every year due to AR. Nevertheless, different studies have pointed out that AR determinants found in clinical relevant strains can arise from natural environments, hence the study of environmental bacteria and their associated resistance mechanisms might be useful. The *Planctomycetes* are a phylum of Gram-negative bacteria that are ubiquitous around the globe and possess many uncommon characteristics, such as large genomes with 40 to 50% of predicted proteins being determined as hypothetical. Recent studies have highlighted their broad range AR, yet the mechanisms underlying such resistances are still poorly studied.

This study tested the platforms Comprehensive Antibiotic Resistance Database Resistance Gene Identifier (CARD-RGI), Resfinder and PointFinder, using the genome of a strain from our collection, '*Bremerella alba*' strain FF15, which in a previous *in vitro* study showed to be resistant to a wide range of antibiotics. The search was then extended to all the currently available genomes from this phylum in the NCBI database using the CARD-RGI. All posterior analyses were performed using R software.

The Resfinder was unable to find matches within the genome of '*B. alba*' FF15, even with the lowest threshold for similarity, while PointFinder retrieve some point mutations on 16 rRNA genes, but this could be explained by the fact that the platform is optimized to match sequences within the same species and none of the available option is from *Planctomycetes*. CARD-RGI was able to find potential 322 matches on the '*B. alba*' FF15 genome. All except one of the matches are frail and could be partial hits that are not conferring the phenotypic resistances observed. When expanding the search to the full collection of planctomycetal genomes, all had matches, but most,

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if not all, were loose matches.

This study reinforces the lack of *in silico* tools to accurately detect AR genes in environmental isolates and identify potential targets for *in vitro* studies to further understand the underlying AR mechanisms in this phylum.

Keywords – Planctomycetes; Antibiotic; Resistance; Genomics

Comparative genomic analyses reveal the enrichment of Eukaryotic-like proteins and secretion systems in Sponge-Associated *Vibrio* spp.

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Abstract

Sponges (Phylum Porifera) are sessile, filter-feeding animals, which harbour abundant and diverse microbial communities. Microbes live in symbiotic association benefiting sponges by providing nutrients and metabolites. Host-microbe co-evolution enabled the symbionts to thrive in a host-specific niche, making them significantly different from free-living counterparts. Although the knowledge of the mechanisms responsible for a successful microbial relationship is limited, advances in genome sequencing provided an insight into some of the molecular adaptations that occur in these species.

Here, we sequenced the genome of six strains of *Vibrio* sp. isolated from a marine sponge sampled from the Atlantic coast of Portugal and performed comparative genomics analysis to identify the genomic features required for a prosperous symbiotic association.

We identified numerous genes encoding eukaryotic-like proteins, mainly, tetratricopeptide repeats and Sel1 repeats that enable the microbe to avoid the host's immune system response and encode genes that facilitate the attachment to the eukaryotic host. We further evaluated these genomes and classified multiple genomic islands (GI) that seem to be crucial to disseminate genes related to symbiosis. In these GIs, that varied in number between 28 and 43, we screened for secretion systems and determined the presence of type III/IV/VI secretion systems, which seem to contribute to the virulence of these bacteria. By identifying the clusters of orthologous groups of proteins, we discovered that our strains had multiple clusters represented in abundance, related to energy production and metabolism, signal transduction mechanisms, transcription and carbohydrate transport and metabolism. These clusters appear to be associated with specialized physiological functions, such as a rapid adaptation to changing environments. This discovery suggests an evolutionary strategy to increase these bacteria to colonize the sponge host.

In conclusion, the findings of this analysis revealed genomic repertoires that favour the establishment of symbiotics relationship with marine sponges.

Keywords – sponge-microbe symbiosis; *Vibrio* sp.; eukaryotic-like protein; secretion systems; COG analysis; adaptation

Innate immune response of gilthead seabream (*Sparus aurata*) juveniles to bacterial infection with *Photobacterium damsela* *piscicida*

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Abstract

Aquaculture is an emergent industry challenged by the occurrence of different pathogenic agents which lead to productive and monetary losses. In order to anticipate and protect fish from this threat, the present study was designed for a better comprehension of haematological and 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 randomly selected and intraperitoneally (i.p.) injected with 100 µl PBS (control group) or 100 µl of exponentially growing *Phdp* (10^6 CFU/ mL; infected group) and distributed as a complete randomized design in 6 recirculating seawater systems (i.e. triplicates per experimental condition). 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 such as total and differential counting of peripheral leucocytes and total circulating erythrocytes counts. The remaining blood was centrifuged and plasma was collected for innate humoral parameters determination (i.e. antiproteases, proteases and peroxidase activities).

Significant differences were found in several parameters analysed. Peripheral erythrocyte levels decreased in infected animals compared to sham injected groups regardless time, whereas haematocrit, haemoglobin and mean corpuscular haemoglobin concentration were found diminished in infected animals at 24 and 48 h post infection.

Even though total peripheral leucocytes did not change between both conditions, circulating neutrophil and monocyte populations showed augmented numbers, regardless sampling time, in infected animals since these constitute the first cell defence line when animals are infected.

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Further analyses are currently being performed, focusing on head-kidney's immune-related gene expression, in order to study how *Phdp* infection influences gilthead seabream immune machinery.

Keywords – Animal health; infection; immune response; red blood cells; leucocytes; neutrophils

Exploring cyanobacterial genomes for post-translationally modified peptides discovery

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Abstract

Microorganisms have been increasingly exploited for their remarkable ability to produce diverse natural products which display distinct bioactivities. Among them, Cyanobacteria have been pointed out as one of the most promising groups of bacteria for natural products discovery. Genome sequencing has revealed that cyanobacteria harbor many more biosynthetic gene clusters (BGCs) for natural products than are currently characterized. In fact, BGCs encoding Ribosomally synthesized and Post translationally modified Peptides (RiPPs) are one of the most abundant in cyanobacterial genomes. These biosynthetic pathways are highly flexible and can achieve an impressive chemical and functional diversity due to their extensive posttranslational modifications. However, such peptidic natural products comprise just a fraction of the chemical diversity reported from Cyanobacteria. In this work, we mine the genomes of 50 phylogenetically distinct cyanobacterial strains belonging to Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC) in order to identify biosynthetic gene clusters encoding RiPPs. Furthermore, combining genomics with chemical analysis we were able to identify new RiPPs from LEGE cyanobacterial strains. The next steps of this work will focus on the isolation of such compounds and evaluate their bioactivity.

Keywords – Cyanobacteria; natural products; Ribosomally synthesized and post-translationally modified peptides (RiPPs); genome mining

Food safety of fish – *Anisakis* occurrence in Iberian Peninsula

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Abstract

The world per capita fish consumption pattern has almost doubled in the last decades, which raises a concern about food security. Parasitosis affecting the human population has been more rigorously evaluated due to changes in eating habits, due to the increased intake of fish eaten raw or undercooked. Among the possible parasites that affect the human population, *Anisakis* sp. is a nematode that can trigger allergic processes or gastrointestinal infection. Within this perspective, the food safety of fish in Iberian Peninsula has been evaluated. The study was based on work referring to the presence of *Anisakis* spp. in fish in the Iberian Peninsula. A survey was carried out in the period 2000 to 2020, which made it possible to compile 30 fish species with an incidence of 6 species of *Anisakis*. Subsequently, a relationship was established between prevalence and intensity of *Anisakis* contamination in the hosts described. The data also show that in 99% of the species the parasite is encapsulated or free circulating in the muscle region, digestive cavity or viscera; such as stomach, liver and intestine. The infection data attract attention for a better control of fish quality in Portugal and Spain.

Keywords – Food Security; Fish; *Anisakis* and High Infection levels

Inclusion of antioxidants in European sea bass feeds – a comparison between natural and synthetic sources

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Abstract

The feed industry relies on antioxidants for feed conservation, fish health and fillet quality. Synthetic Vitamin E is one of the most widely used antioxidants in aquafeeds, but consumer trends and concerns regarding potential health hazards of synthetic antioxidants caused a shift towards natural sources. Annual plant production has passed 600 million tonnes, generating a surplus of byproducts rich in natural antioxidants, e.g. carotenoids. In this study, a commercial-based diet supplemented with a regular dose of synthetic Vitamin E (100 mg.kg⁻¹) was used as control (CTRL), and compared with a diet containing a higher dosage (500 mg.kg⁻¹; VITE). Three natural antioxidant sources (tomato, carrot and coriander) were added to the control diet at 2%, originating diets TO, CA and CO, respectively. Carotenoid content and antioxidant potential of byproducts and diets was analysed. All five isoproteic/isolipidic diets were fed *ad libitum* to *Dicentrarchus labrax* (IBW = 114g) during 85 days, after which growth performance, body composition, liver antioxidant enzyme activity, lipid peroxidation (LPO), immunomodulatory effects, flesh quality and consumer acceptance were evaluated. LPO, muscle and skin colour, and flesh texture were also evaluated in fillets stored on ice for 8 days. Tomato, carrot and coriander were rich in lycopene, α -Carotene and lutein, respectively.

DPPH radical scavenging potential of CA and CO more than tripled compared to CTRL. CO also had the highest ABTS value. Diets were well accepted by fish (FBW = 237-248g). Growth, nutrient utilization and whole body composition were similar among treatments. Immune status and plasma bactericidal activity remained unaffected. Muscle Vitamin E deposition reflected dietary levels, being significantly higher in fish fed VITE compared to remaining treatments.

Liver antioxidant enzyme activity and total antioxidant potential revealed no differences among treatments, as was the case of ABTS radical scavenging potential and fillet LPO after 8 days of storage. Despite byproduct inclusion inducing colour alterations in fillets, consumer acceptance was unaffected. Overall, results suggest that neither a high dose of Vitamin E (500 mg.kg⁻¹) nor a 2% inclusion of natural carotenoid sources resulted in antioxidant benefits in cultured sea bass compared to diets supplemented with 100 mg.kg⁻¹ of Vitamin E.

Keywords – natural antioxidants; Vitamin E; circular economy; functional feeds; carotenoids; *Dicentrarchus labrax*

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A molecular cell-based sensor to detect PPAR ligands – an optimized tool to drug discovery in cyanobacteria

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Abstract

Cyanobacteria are photosynthetic prokaryotes that produce a vast array of secondary metabolites, with an increasing biotechnological potential in pharmaceutical, cosmeceutical and agricultural industries. Peroxisome Proliferator-Activated Receptors (PPARs) are ligand-activated nuclear receptors (NR) involved in several metabolic and cellular processes, such as fatty acids oxidation, inflammation, and lipids storage. Three PPAR isoforms have been identified, responding to a spectrum of endogenous and exogenous compounds. Natural ligands include unsaturated fatty acids and their derivatives. Cell-based assays can be applied to measure specific biological responses to compounds in a pharmacological high throughput screening (HTS) approach for the discovery of new drugs. More specifically, such assays can be used to assess NR activity upon exposure ligands, by transfecting cells with a two-vector reporter system: expressing a target NR and two reporter genes. NR modulation by test compounds is thus measured, with high sensitivity and reliability, upon gene expression changes and subsequent reporter gene signal quantification. The main goal of this work is to optimize a cell-based luminescent sensor for HTS of cyanobacteria extracts from the Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC) to screen for PPAR ligands. The sensor constructed allowed assay miniaturisation, from 24- to 96-well plates, reducing reagents' volumes and costs, while increasing the number of test samples per plate. Moreover, by engineering the vector system, replacing a conventional luciferase with a last generation reporter (NanoLuc®), the luminescent signal was enhanced and stabilized. Screening three PPARs simultaneously (multiplexing) increased the information obtained from each well, further reducing time and costs. Biomass of cyanobacteria of different morphologies and degrees of complexity was freeze-dried and extracted with methanol. Eight fractions were obtained using an HPLC equipped with a fractions' collector, in an acetonitrile/water gradient, being each fractions analysed under the current assay's optimization status. Fractions causing expression repression (< 0.5) or induction (> 2.0) were later tested with individual PPARs, for confirmation and clarification. Results indicate that this approach is very efficient and can be

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used to bioprospect large libraries of compounds focus on drug discovery.

Keywords – Cell- based sensor; High throughput screening; Cyanobacteria; PPAR; Bioluminescent assays; Drug discovery

Discovery of new cyanobacterial antibiotics

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Abstract

Cyanobacteria are well-known producers of secondary metabolites with a large range of bioactivities. The cyanobacteria LEGE 10410 has a type II polyketide synthase (PKSII) biosynthetic gene cluster (BGC) that was never described for other cyanobacterial strains. PKSII are a powerful source of bioactive compounds with great pharmacological potential. The isolation of new natural products (NPs) from this cyanobacterial strain, LEGE 10410, appears to be an interesting pathway to follow.

The first approach taken for the discovery of a new NP was genome-guided based on the identification and annotation of the PKSII gene cluster. The strain LEGE 10410 was grown in large-scale to obtain enough biomass to proceed to a bioassay-guided approach. The transcriptional levels of LEGE 10410, growing in large-scale, were evaluated by RT-PCR in order to check the expression of important genes in the BGC. Then, after biomass organic extraction and fractionation, the bioactivity of the crude extracts was tested in antimicrobial assays. As an alternative to the bioassay-guided approach, it was attempted to create knockout mutants of the cyclase and subunits genes from the PKSII cluster of the cyanobacterial strain in study. The recombinant plasmids were constructed by cloning both the upstream and downstream fragments of each gene to be deleted, followed by the cloning of a kanamycin cassette into the vector. In order to transfer the recombinant plasmids for the knockouts in LEGE 10410, two different methods were attempted: natural transformation and electroporation.

The genome-guided approach allowed the identification and characterization of the best prediction border of the cluster and also the selection of the cyclase and subunits genes for the gene expression studies and for the knockout mutants. The transcriptional results, revealed that the PKSII genes were being expressed. The recombinant plasmids for the knockout mutants were successfully constructed, but the efficiency of the transformation methods remain to be determined. In case these methods work, the next step is to compare the production of the secondary metabolites between the LEGE 10410 knockout mutants and the WT strain, through a metabolic approach. The antimicrobial susceptibility assays revealed no activity against the bacterial strains in study. Nevertheless, for the upcoming work cytotoxicity assays against different cell lines will be performed, in order to test if the fractions obtained from the crude extract may present anticancer activity.

Keywords – natural products; cyanobacteria; type II polyketide; bioactivity; knockout

Environmental control of ichthyoplankton assemblages in a coastal Marine Protected Area “Parque Natural Litoral Norte (PNLN)”

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Abstract

Planktonic communities, including ichthyoplankton (eggs and larval stages of fishes) play a critical role in marine ecosystems and are responsible for fundamental processes that support marine communities. The present study investigated the environmental control of both abiotic and biotic parameters in the ichthyoplankton dynamics of a coastal Marine Protect Area in the NW Portuguese coast, the Parque Natural do Litoral Norte (PNLN). Seasonal surveys were conducted from Autumn 2017 to Summer 2019 at 9 sampling stations distributed along the PNLN. At each sampling station planktonic tows were carried out using a 500 µm mesh planktonic net for ichthyoplankton; and a 150 µm mesh conic net for zooplankton collections, planktonic samples were then fixed and preserved for laboratorial identification. At each sampling station, vertical profiles of water physical-chemical parameters were measured, and surface and bottom water samples were collected for further laboratory quantification of nutrients and chlorophyll a, total particulate matter and particulate organic matter. CCA analysis were performed to investigate the effects of environmental factors on ichthyoplankton. Water column stratification varied seasonally, being almost absent during winter and increasing until summer, when a clear thermocline was evident. A total of 4170 fish larvae were collected, belonging to 39 different taxa, mostly marine species and few estuarine species. Overall, the diversity was low, since the larval fish assemblage was dominated by three taxa, responsible for 79.1% of the total larvae collected namely *Parablennius gattorugine* (54.6%), *Ammodytes tobianus* (15.7%) and *Clupeidae* spp. (8.8%). In general, ichthyoplankton abundance had a clear seasonal trend, with higher values during spring seasons, coincident with peaks of chlorophyll a and zooplankton abundance, supporting the match-mismatch hypothesis. Ichthyoplankton did not vary spatially, indicating an homogenous distribution along the study area. This study highlighted the importance of abiotic and biotic factors as drivers of ichthyoplankton dynamics.

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Keywords – Ichthyoplankton; MPA; Environmental Control; Coastal nursery

Cyanobacterial bioprospecting for natural marine antifouling agents – the story so far

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Abstract

The natural phenomenon of marine biofouling consists in the colonization of a range of micro- and macroorganisms in all submerged surfaces, but the processes in use for its mitigation present a detrimental effect on the environment, economics and human health. Therefore, new effective and environmentally safe antifouling technologies are under research, with focus on natural products as bioactive agents. Cyanobacteria are known to produce a variety of bioactive secondary metabolites with a wide range of biotechnological applications, including antifouling potential. However very few studies were conducted considering a global approach for antifouling potential from bioactivity to ecotoxicity with mode of action unravelling. This research intends to focus on the discovery of new environmentally friendly bioactive compounds with antifouling properties, by exploring the metabolic diversity of cyanobacterial strains existing in LEGE Culture Collection and identifying new effective natural compounds with a specific antifouling mode of action towards selected biofouling species. In a preliminary screening, the antifouling potential of cyanobacterial methanolic fractions was tested at different levels of biological organization, to assess their bioactivity against macrofouling (*Mytilus galloprovincialis* larvae) and microfouling organisms (marine bacteria and microalgal biofilms). Results from the 320 fractions screened, suggested antifouling potential of some strains. These promising fractions are now under process of sub-fractioning and chemical elucidation to be further tested for antifouling effectiveness versus toxicity and identification of antifouling molecular targets.

Keywords – Natural products; Cyanobacteria; Biofouling; Antifouling

Defatted Black soldier fly (*Hermetia illucens*) pre-pupae larvae meal as fishmeal replacement in diets for gilthead seabream (*Sparus aurata*) juveniles: effects on growth performance

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Abstract

Fishmeal (FM) has been the preferred protein source for marine carnivorous fish feeds but its shortage in supply and environmental impacts has prompted the search for alternative protein ingredients. Insect meals have had an increasing interest in aquafeeds due to its nutritional quality, fast growth, and more sustainable production. Black soldier fly (*Hermetia illucens*) is of particular interest since it possesses a high protein content (~60%) with a balanced amino acid profile similar to that of FM, and is also a good source of lipids, vitamins, and minerals, being a solid candidate to be used in feeds for marine carnivorous fish species, such as gilthead seabream (*Sparus aurata*).

In this study, a growth trial was conducted to evaluate dietary FM replacement with defatted *Hermetia illucens* pre-pupae larvae meal (HM) on the growth performance of gilthead seabream juveniles. A FM-based diet was used as control and three other experimental diets were formulated to include increasing levels of HM: 15%, 30%, and 45%, replacing total dietary FM protein at 22, 60, and 100%, respectively. Diets were randomly assigned to triplicate groups of gilthead seabream juveniles (initial weight of 32g) which were fed the experimental diets for 67 days. Fish were kept in a recirculating seawater system and at the end of the trial, feed consumption was recorded, and fish were bulk-weighted to determine growth performance parameters.

At the end of the trial, the inclusion of HM did not compromise diet's palatability as all diets were well accepted and fish had similar feed intake. While feed efficiency was also unaffected, the inclusion of HM led to a significant decrease in fish growth when compared to the control diet. Yet, growth performance was similar amongst the HM-containing diets, even at total FM substitution. This decreased growth could be attributed to the presence of chitin, which has been linked to compromised nutrient digestibility and uptake and, consequently, growth. Therefore, further studies are required to fully understand the effects of HM inclusion in nutrient digestibility and digestive enzymes activity of gilthead seabream and to evaluate new approaches to improve HM utilization by fish.

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Keywords – Insect meal; *Hermetia illucens*; alternative protein sources; gilthead seabream

Larval fish connectivity between estuaries and coastal areas

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Abstract

Although many advances have been made on the study of larval fish connectivity in coral reefs and open water ecosystems, there seems to be a considerable lack of information on the topic of larval dispersal for estuarine environments. Estuaries are well known as nursery grounds for numerous fish species, many of which are marine during most of their life history. This highlights a gap in our understanding of larval dispersal processes between populations and across the estuarine-coastal gradient. Here, we undertake a systematic review of the literature concerning larval fish connectivity between estuaries and coastal areas in order to: (1) describe the most recent advances and methodologies for studying larval estuarine fish connectivity; and (2) suggest potential research-lines to help further scientific understanding of larval fish connectivity across estuarine-coastal gradient. For this purpose, we used 4 major scientific literature databases and searched peer-reviewed publications containing the words “fish larvae”, “connectivity”, “estuarine” or “coastal” (as well as their synonyms). Amongst the 93 publications found with these criteria, 51 were discarded after being thoroughly read, as they were not found relevant. Our synthesis was performed based on a subset of 42 papers. Only 23,8% of these papers focused on a specific species, whereas the others concerned larvae assemblages. Consequently, behaviour peculiarities are rarely taken into account, neither in field samplings nor in numerical models. Salinity along with temperature appear as the most influencing parameters of spatial distribution of larvae for 48% of the papers, followed by turbidity (14%) and as secondary influence, wind and tide. Concerning numerical modelling, despite the increasingly popular use of Individual-Based Models (IBMs), 71% are two-dimensional instead of three-dimensional, often with few to none field sampling validating modelling results. Also, only few studies consider local bottom geomorphology, although it has been shown to influence larval behaviour as well as passive or active transport, which are dependent on estuarine hydrodynamics. Overall, we suggest that more multi-disciplinary approaches are necessary to insure accurate depictions of larval connectivity in estuarine-coastal systems, namely more multidisciplinary field studies considering different topics as geomorphology, physical oceanography, modelling and bio-ecology.

Keywords – Connectivity; fish larvae; coastal; estuarine; recruitment; nurseries

Using citizen science to raise awareness about Natura 2000 – a case study

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Abstract

Nowadays, 13 otter species are recognized worldwide, but only one is found in Europe: the European otter (*Lutra lutra*). Although this species is listed as least concerning both globally and in Portugal, this mammal faces some threats in the country, such as the pollution of water bodies, the destruction of riverine vegetation, among others. Partially due to its charismatic character, this species was chosen as the focal point of a citizen science activity, designed for the general public, with the aim of raising awareness about Natura 2000 – a European network of protected areas. During this activity, participants were expected to look for otters' tracks and faeces along a transect in the banks of water bodies. Overall, five field sessions were held inside and outside Natura 2000 sites, involving over 30 volunteers. Whenever possible, pre- and post-tests were implemented. Pre-tests were fulfilled by 18 participants, aged 19-62 years; 39% (n = 7) of them held a bachelor's degree and 61% (n = 11) worked/studied in fields related to biology or nature conservation. The questionnaires revealed that only 33% (n = 6) of volunteers knew what Natura 2000 was beforehand, while 44% (n = 8) had never heard of that term previously. On average, the participants who knew what Natura 2000 was were able to correctly name 2.2 ± 1.5 (out of 10) sites in Portugal; which is a modest number considering this country currently has 167 protected areas integrated into Natura 2000. So, although this population sample was expected to be more literate about environmental issues, the level of awareness and knowledge regarding Natura 2000 was still low. Post-tests, which were fulfilled by 16 volunteers, showed an improvement in the literacy about this network for all respondents. For instance, in post-tests, all participants self-reported that they knew what Natura 2000 was. Moreover, after the activity, on average, volunteers correctly mentioned 5.1 ± 2.4 Portuguese sites that belonged to Natura 2000. Despite more questionnaires being needed to properly evaluate the educational potential of this citizen science activity, these early results suggest that it might be successful in raising awareness about Natura 2000 among the general public.

Keywords – Environmental education; science communication

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A review of the use of next generation sequencing in marine aquaculture

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Abstract

This work aimed to evaluate the state of the art regarding microbial community studies in marine recirculating aquaculture systems (RAS) and if recent studies regard what different parameters influence the water quality and community and how, whether Next Generation Sequencing (NGS) techniques are employed to achieve this.

It was found that several parameters were already studied, such as salinity, nitrogenous compounds and others. Salinity was the most frequent one with 9 studies, followed by nitrogenous compounds with 2. The 10 other articles demonstrated a wide diversity of parameters to be tested. Of these 21 studies, 16 were published in 2019 and 2020. Different salinity values demonstrated a significant impact on the microbial community structure and diversity. Many of these recent studies resort to NGS techniques to find what parameters influence the microbial community.

The community taxa described were also analyzed at the phylum and genus level. Out of 37 articles, 24 were from 2019 and 2020 and 21 (57%) used Illumina, demonstrating a raise on the usage of NGS, becoming a more common and used technique. Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria and Planctomycetes were the most frequently found phyla. The most frequent genera were *Pseudomonas*, *Vibrio*, *Acinetobacter* and *Pseudoalteromonas*.

In recent years, more and more aquaculture studies are resorting to NGS, Illumina being the most common one, challenging the status of a lack of NGS studies in aquaculture. A total of 37 articles were analyzed so far. It is expected more will be added for the presentation.

Keywords – Recirculating aquaculture systems; next generation sequencing; microbial community; water parameters

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Long term exposure to simvastatin disrupts zebrafish (*Danio rerio*) lipid and energy metabolism

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Abstract

Simvastatin (SIM), a hypocholesterolaemic compound, is among the most prescribed pharmaceuticals for cardiovascular disease prevention worldwide. Several studies have shown that acute exposure to SIM causes multiple adverse effects in aquatic organisms. However, uncertainties still remain regarding the chronic effects of SIM in aquatic ecosystems. Therefore, the present study aimed to investigate the effects of SIM in the freshwater teleost *Danio rerio* following 90 days of exposure to environmentally relevant concentrations ranging from 8 ng/L to 1000 ng/L. This study used a multi-parameter approach integrating distinct ecologically-relevant endpoints, i.e. survival, growth, reproduction and embryonic development, with biochemical markers (cholesterol and triglycerides). Real Time PCR was used to analyze the transcription levels of key genes involved in the cholesterol biosynthetic pathway and energy metabolism in liver and brain tissues, respectively. Globally, SIM induced several effects that did not follow a dose-response relationship; embryonic development, biochemical and molecular markers, were significantly impacted in the lower concentrations, 8 ng/L, 40 ng/L and/or 200 ng/L. Taken together, these findings expand our understanding of statin effects in teleosts, demonstrating significant impacts at environmentally relevant concentrations and highlight the importance of addressing the effects of chemicals under chronic low-level concentrations.

Keywords – Zebrafish, Simvastatin; Chronic exposure; Low-level exposures; Liver; Brain

Syllabus and Educational Resources to Teach Geoethics in Higher Education

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Abstract

The Earth system, as we know, is dramatically changing. This fact is evidenced by the effects of climate change, having impact for instance at the lever of water availability and quality, the increasing number of natural disasters, and the artificialization of natural landscapes, among other aspects. In this scenario, human behavior towards planet Earth has particular relevance. It is necessary to change behaviors in a contextualized and holistic direction, supported by ethical values in favor of planetary sustainability. Geoethics performs an essential function in this shift. The ethics related to Earth's sciences is a new and developing scientific branch that seeks to reflect on the impact of geosciences knowledge and practice on the planet. Knowing the power of education to reach and transform society, experts of six countries developed the Erasmus+ project GOAL – Geoethics Outcomes and Awareness Learning (ref. 2017-1-PTO1-KA203-035790). Grounded on the belief that improvement of future citizens' training, in specific geoscientists, requires an approach based on Geoethics in an attempt to minimize Earth-human problems, the GOAL Project developed educational tools for Higher Education. These latter made it possible to combine knowledge from multiple areas, mobilized for the creation of a syllabus and more than a dozen educational resources within the scope of Geoethics, compiled in the eBook *“Teaching Geoethics: Resources for Higher Education”* (available in <https://goal-erasmus.eu/ehandbook/>). Towards its ten chapters, this eBook presents, in a clear but even so profound manner, the theoretical support for the entire application of the syllabus and its educational resources on the Higher Education worldwide. By covering a range of topics (such as the theoretical aspects of geoethics, geological resources exploitation, geoheritage, water and georisks management), these educational tools provide the possibility to teach and learn geoethics in an interdisciplinary and meaningful way, from the theory to practice. Geoscientists endowed with an ethical understanding of their actions on the planet are professionals more conscious (and capable of aware society) about the importance of the Earth for the human species prosperity and survival.

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Keywords – Educational resources; Geoethics; Geoethical syllabus; Geosciences education; Higher Education

Bioactivity Screening of Marine and Freshwater Cyanobacteria for the Isolation of Novel Compounds for Diabetes Type II using *Danio rerio*

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Abstract

Diabetes, and in particular diabetes type II (DMT2), shows an increase of incidence worldwide and different therapeutics have been applied. Cyanobacteria, known as blue-green algae, are recognized to produce many secondary metabolites with applications to the human health. The aim of this study is to investigate the effect of cyanobacteria from the CIIMAR in-house cyanobacterial culture collection (LEGEcc) on the increase of glucose uptake using *Danio rerio* larvae. 3DPF zebrafish larvae were exposed to the cyanobacterial fractions and glucose uptake was quantified by the fluorescent dye 2-NBDG. Extraction and production of fractions of cyanobacterial biomass was performed with hexane, ethyl acetate and methanol, either in a polarity gradient extraction or in a sequential extraction. The screening of cyanobacteria resulted in the identification of cyanobacterial strains with promising bioactivities. Evaluation of proteins involved in the glucose uptake, like glucose transporters (GLUT's) was analysed by phylogeny to see the similarities between human, mouse and zebrafish. Western blot technique was applied to access the effect of promising fractions on the GLUT levels. Next step will be the isolation of the responsible bioactive compounds and elucidate their structures.

Keywords – Diabetes; Cyanobacteria; Zebrafish; GLUT

Medusozoans in Portugal: compiling genetic information, assessing ecological and economic impacts

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Abstract

Cnidarians of the Medusozoa clade, generally named *jellyfish* in their free-swimming medusa phase, can produce *blooms* with several impacts in many industries *e.g.*, fisheries, aquaculture and tourism. This phenomenon is increasing worldwide mostly motivated by anthropogenic and natural factors, such as, global warming, wind and water tides, eutrophication and widening of invasive species habitats. Given its relevance in a climate changing world, the medusozoans reported in Portugal, their publicly available genetic data, and the local works concerning *jellyfish* species with more economic and ecological relevance were reviewed, resorting to literature and several online databases.

The information gathered revealed a total of 272 medusozoans species comprising 254 hydrozoans, 15 scyphozoans, 2 staurozoans, and 1 cubozoan occurring in Portugal. The data compiled showed that 26% of the reported species, did not present any genetic information in the GenBank Nucleotide database (May 2019). Most of the sequences belonged to the mitochondrial markers 16S rRNA, COX1, and to the nuclear markers 18S rRNA, 28S rRNA. Moreover, our work exposes the hydrozoans *Blackfordia virginica*, *Physalia physalis*, *Velella velella*, and the scyphozoans *Aurelia aurita*, *Catostylus tagi*, *Pelagia noctiluca*, *Rhizostoma luteum* as being the medusozoans with higher economic and ecological relevance; due to their ability to occur *in mass* in the Portuguese coast and affect the ecosystems dynamic.

Despite of being already reported in the Portuguese coast, medusozoans mass occurrence events and associated impact information is still scarce, possibly due to underestimation of its importance, diversity and abundance. Despite the increasing number of medusozoan studies in recent years, the number of species described in Portugal, assessed in this review, may be undervalued due to the limited number of local studies. Thus, more studies and monitoring programs throughout the entire country should be implemented for a better Medusozoa diversity evaluation; helping to improve their still very scarce genetic representativeness in public databases and further genomic studies aiming the development of mass occurrences early detection tools.

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This review serves as a “wake-up” call to the authorities about the importance of monitoring medusozoans, as well as, to create strategies to face putative ecological and economic losses caused by them.

Keywords – Cnidaria; Medusozoa; *jellyfish*; *blooms*; mass occurrences; molecular markers

Assessment of water runoff quality from a green roof in a coastal area

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Abstract

Green roofs have been widely used due to their vast environmental, economic and social benefits. However, the influence of green roofs in water runoff remains a controversial topic and a research priority as they can act as a source or a sink of nutrients. This study investigated how different plant-substrate selections influence water runoff quality under coastal conditions.

In a rooftop located in the coastal area of Matosinhos (Portugal), green roof systems were designed from the bottom up with: an expanded cork board (ICB) working as a water retention and drainage layer, a filter layer of thermoset propylene, a 12 cm commercial substrate and vegetation. The selected plants were *Ammophila arenaria*, *Corema album* (L.) D. Don and *Helichrysum italicum*, which are perennial and autochthonous of the Portuguese coast. They were implemented as mono and polyculture in parallel with a control without plants. The study period was from October 2018 to May 2019 and there was no artificial watering, maintenance or removal of naturally colonizing plants. Water quality from the different treatments in study and from rainfall was evaluated through various parameters, namely pH, electrical conductivity (EC) and nutrients (NO_3^- , NH_4^+ and PO_4^{3-}). Water samples were collected in eight random rainfall events within the experimental period.

Among the tested plants *A. arenaria* showed the best survival capacity. *C. album* showed low development and *H.italicum* did not prosperate. We observed that water runoff from both vegetated and non-vegetated systems contained significant higher levels of EC and leached phosphates when comparing to rainwater. This was probably associated to substrate composition. Furthermore, we observed lower phosphates values (by 40%) in the *A. Arenaria* systems when comparing to the control - probably as a result of its uptake. The presented results highlight the potential of vegetation in relation to nutrient dynamics in green roofs.

A long-term study of water quality from green roofs in coastal areas is recommended not only to understand the impact of green roof components and its dynamics, but also to contribute in developing more sustainable buildings.

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Keywords – Green roofs; cork; coastal plants; water quality; water management; nature-based solutions

Mangroves as nature-based solution for coastal water eutrophication in Macao, SAR China

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Abstract

Mangrove forests are one of the most important coastal ecosystems in the world, providing a wide variety of ecosystems services. They are also branded as natural wastewater treatment plants because of their ability to filter and/or uptake pollutants in the water. Macao, a coastal city situated on the west shore of the Pearl River is home to several species of mangroves. The Pearl River Delta (PRD) Economic Zone is one of the most industrialized regions in the world and several studies indicate significant levels of aquatic pollutants in the area. Eutrophication is one of the most serious water pollution problems in the PRD mainly due to anthropogenic causes. Eutrophication index in Macao, especially in the Inner Harbor area, indicated a significant increasing trend in the last 5 years. This is mainly due to the direction of water flow, topographical conditions and inputs of pollutants from upstream.

Although regular monitoring of nitrogen and phosphorus pollution are being carried out in Macao, no studies have been reported so far on the use of nature-based solutions to address this issue. This study, therefore, aimed to evaluate the ability of mangroves to remediate nitrogen and phosphorus pollution in Macao's coastal waters. This work will present preliminary data on nutrient pollution (nitrogen and phosphorus enrichment) along various locations (with and without mangroves) in Macao's coastal waters as well as results of mesocosm experiments to evaluate specific nutrient uptake capabilities of local mangroves species. Our preliminary data suggest that concentration of nutrients in water is lower in mangroves area compared to areas without mangroves. It is therefore important to protect the existing mangroves in the area as well as to plant more mangroves along the coastline of Macao. Mangroves will not only help in coastal water pollution remediation but may also generate co-benefits in the city such as the reduction of water-related risks, ecosystems rehabilitation and maintenance as well as enhancement and protection of biodiversity in the local coastal wetland ecosystems.

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Keywords – water pollution; water treatment; coastal management; biodiversity; phosphorus; nitrogen

FINAL REMARKS

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The idea of a students conference at CIIMAR started last year. At first, we thought about a small and closed event, and within the covid-19 pandemic we had to restructure all the ideas and the way we were doing that, risking to have no conference at all. Now, after 104 abstracts submissions, 10 sponsors (including ISME and 3 high impact journals from MDPI) and more than 200 registrations from across the globe, we are glad this event is now official.

We leave this year full of hope in the middle of a chaos and willing to do better. That was a start, but more will come...

Thank you for attending,
CIIMAR's PhD Students Committee

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