

# BLUE THINK CONFERENCE

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SHARE SCIENCE, SPREAD KNOWLEDGE



## BOOK OF ABSTRACTS

Matosinhos, Portugal

27<sup>th</sup> of September 2022

# BLUE THINK CONFERENCE

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SHARE SCIENCE, SPREAD KNOWLEDGE

## **BLUE THINK CONFERENCE 2022**

Share Science, Spread Knowledge

## **BOOK OF ABSTRACTS**

ISBN 978-989-54965-6-3

## **DISCLAIMER**

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

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## THE CONFERENCE

The third - and our very first in-person - edition of the Blue Think Conference (BTC) is organized by the Interdisciplinary Centre of Marine and Environmental Research (CIIMAR) PhD Students' Committee. Just like in the previous years, our aim is to create an engaging environment for exchange and collaboration within the community of CIIMAR-affiliated young researchers.

Leaning on CIIMAR's main research lines, we divided the presentations into four sessions entitled **Genomics and Evolution, Ecology and Ecosystem Services, Biochemistry and Biotechnology and Aquaculture and Aquatic Resources**. At the start of each session, participants will hear a keynote lecture by a renowned scientist about parts of their research career and most important scientific achievements. These lectures are followed by selected oral presentations, flash talks and posters.

We hope the conference expands our knowledge and unites researchers from different research groups and faculties. Everyone is welcome to join the discussion of the latest science that CIIMAR's youngest minds are involved in.

## 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 third Blue Think Conference organized by the PhD students committee of CIIMAR is here. On the 27<sup>th</sup> of September 2022, students will have the opportunity to show their works, discuss their ideas, and plan their future and to hear and interact with distinguished renowned international scientists. It will be a great serendipity environment, which certainly will make you all grow from scientific and personal points of view.

This is the first conference after the covid-19 pandemic that showed clearly the importance of science and technology in the wellbeing and welfare of our population and the relevance of a sustainable exploitation of our resources.

I 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 you careers.



Professor Vitor Vasconcelos  
Director of CIIMAR

## ORGANIZING COMMITTEE

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## **SESSION 1**

### **GENOMICS AND EVOLUTION**

*MODERATION*

*TOMÁS RODRIGUES AND JEAN-BAPTISTE LEDOUX*



## KEYNOTE SESSION – JOSEFIN STILLER



### **Using genomic data to understand the origins of biodiversity**

Josefin Stiller is an evolutionary biologist interested in understanding the factors that generate biodiversity. She is currently an Assistant Professor at the University of Copenhagen, where she studies the evolutionary history of various animal groups. After obtaining a B.Sc. in Biology from Free University Berlin and a M.Sc. in Organismic Biology and Evolution from Humboldt University Berlin, she received a Ph.D. in Marine Biology from Scripps Institution of Oceanography in San Diego, studying population structure of the amazing seadragons of southern Australia. During her postdoc on the Bird 10,000 Genome Project (B10K) in Copenhagen, she used whole genome to reconstruct a new phylogeny of birds. She has embarked on adventures collecting invertebrates aboard a ship in Antarctica, visiting methane seeps using remotely operated vehicles and scuba diving to collect samples of seadragons. Josefin is honored to have a marine worm named after her.

# BLUE THINK CONFERENCE

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**ABSTRACTS**

**ORAL COMMUNICATIONS**

## Filling Gaps: The genome assembly of two Holocephali species

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Chondrichthyans (sharks, rays and chimeras) are an early branch of vertebrates with peculiar traits and diversified/unique phenotypes. The chimeriformes order, the only extant order of the Subclass Holocephali and the closest living relatives of Subclass Elasmobranchii family, is composed of three families (Callorhynchidae Garman, 1901, Rhinochimaeridae Garman, 1901, and Chimaeridae Bonaparte, 1831). Despite, being one of the most enigmatic lineages of vertebrates, the chimeriformes order remains unexplored and understudied, in part due the lack of genomic resources available.

To fill this gap of knowledge we sequenced and annotated the genome of two chimaeras species (Rhinochimaeridae and Chimaeridae families). Using Illumina Paired-end and long insert size Mate-pair reads, as well as Pacific Biosciences (PacBio) Single Molecule High-Fidelity (HiFi) reads, we obtained genomes with 1-1.1gb of size, N50 length values ranging 0,044 to 1,4Mb at contig level and 1,44-4,8Mb at scaffold level. The BUSCO analyses indicated high level of completeness, with more than 94% of the single-copy orthologs in the Vertebrata library profile found in both genome assemblies. Additionally, we found repetitive elements in 40 - 45% of genome content and predicted more than 20,000 proteins in both species. Both genomes will be essential to understand and scrutinize the evolutionary relationship of specific gene families in Chondrichthyans, as well as to establish links between phenotypes and genome features in Holocephali lineages.

**Acknowledgments:** This work is a result of the project ATLANTIDA (ref. NORTE-01-0145-FEDER-000040), supported by NORTE 2020, under the PORTUGAL 2020 Partnership Agreement and through the European Regional Development Fund (ERDF). FCT - Foundation for Science and Technology supported A.M.M. (DFA/BD/8069/2020).

## Massive gene loss and the making of xenobiotic-induced responses in cetacea

Diogo Oliveira<sup>1,2,3</sup>, Raul Valente<sup>2,3,4</sup>, Daniela Soares<sup>2</sup>, Susana Barbosa<sup>2</sup>, Miguel Santos<sup>1,3</sup>, Filipe Castro<sup>2,3</sup> and Raquel Ruivo<sup>1</sup>

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How animal species cope with chemical insults is a key question of the Anthropocene Era. This research avenue is specifically relevant for marine mammals (e.g. whales and dolphins). In fact, Cetacea hold record levels of environmental contaminant accumulation. Such chemical exposure was shown to disrupt cetacean immune responses. To fight these external contaminants, or xenobiotics, animals rely on an integrated network of genes and pathways for their oxidation (cytochrome P450 (CYPs)), conjugation (UDP-glucuronosyltransferases (UGTs), glutathione S-transferases (GSTs) and sulfotransferases (SULTs)) and transport (organic anion transporters, multidrug resistance transporters, multidrug resistance associated proteins). The Pregnane-X-Receptor (PXR) and the Constitutive Androstane Receptor (CAR) are nuclear receptors that, among other functions, are considered the master regulators of xenobiotic metabolism. Cetacea have been shown to lack functional PXR and CAR receptors and to possess a lower number of cytosolic GSTs when compared to terrestrial mammals. Thus, using knockout mice and comparative genomics, the conservation status of the Cetacea xenobiotic metabolism genes regulated by PXR and CAR was examined, and a drastic reduction in the number of functional genes for CYPs, UGTs and SULTs was verified. These results bring us some insight into how the evolution of genomes has shaped the chemical defense of whales and dolphins, which will benefit the preservation of such iconic species.

**Acknowledgments:** Work funded by FCT projects PTDC/CTA-AMB/31342/2017, PTDC/CTA-AMB/31544/2017 and PTDC/CTA-AMB/7948/2020 and a PhD fellowship to Diogo Oliveira (2021.06293.BD) and Raul Valente (SFRH/BD/144786/2019).

## **Evolutionary History of vertebrate genes involved in mitochondrial biogenesis: insights into the origins of endothermy**

Hugo Moreno<sup>1,2</sup>, Tito Mendes<sup>2</sup>, Liliana Silva<sup>1,2</sup>, Luana Ramos<sup>1,2</sup> and Agostinho Antunes<sup>1,2</sup>

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Homeothermic endothermy is a physiological state characterized by an increased and stable body temperature, that has evolved independently in mammals and birds. It plays a crucial role in their ecological success by allowing them to expand their geographical distribution, increase their reproductive rates, and sustain an incremented aerobic metabolism. During the transition to endothermy, increased mitochondrial activity, content and density were attained, resulting in the higher metabolic rates and body temperatures found in endotherms. Despite the general assumption that the mitochondria were crucial in the emergence of homeothermic endothermy in modern vertebrates, the genomic changes that promoted the shift to this physiological state are still unclear. Therefore, this study aims to assess the evolution of genes that regulate the mitochondrial functioning and compare it in the Sauropsid lineage, thus shedding some light onto the ectothermy-endothermy transition from reptiles to birds.

Sequences of eighty genes, were collected from one hundred avian and reptile species' genomes, and used in positive selection analyses, codon usage bias analyses and correlations of genotype with endothermy related phenotype characters. Additionally, we will assess genomic acceleration enrichment of the genes' flanking regions. The analyses revealed that several genes are subjected to significant selective pressures in birds, and display codon usage bias, which can be used as a proxy of the gene expression levels. Besides that, we were able to detect significant positive correlations between genotype and phenotype traits, such as body temperature or the basal metabolic rate, thus further supporting the role of these genes in the transition to endothermy in Sauropsids. The signatures of positive selection and the putative high expression levels of the mitochondrial regulatory genes of endotherms, as well as their correlation with phenotypic traits, represent an important step for the comprehension of the genomic changes that promoted the acquisition of this physiological state, supporting their crucial role in the transition to endothermy, which ultimately allowed the great diversification and geographical expansion of the birds and mammals.

## Are birds what they eat? Comparative genomics of avian dietary adaptations

Rui Pinto<sup>1,2,3</sup>, Filipe Castro<sup>1,2</sup>, Raquel Ruivo<sup>1</sup>, Miguel Fonseca<sup>1</sup>, Josefin Stiller<sup>4</sup>, Rute Fonseca<sup>3</sup>

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Aves, the most species-rich tetrapod class, display an astonishing biological diversity. Yet, the molecular foundations of this multitude of forms are still elusive. The variety of feeding habits across lineages is one of the best documented examples of birds' ability to change and adapt, at evolutionary scales. However, the genomic determinants of these eco-phenotypes are mostly unknown. Currently, hundreds of genomes have been sequenced within this class, covering more than 90% of the families, mostly under the scope of the Bird10k project. We aim to leverage this wealth of resources to determine genomic events, such as gene loss, family expansion and signals of selection, using a comparative and functional genomics approach. This will allow us to comprehend the association of genetic and phenotypic traits associated with dietary behaviors.

Here, we address the extreme cases of dietary adaptations, namely groups with a very restricted diet, such as penguins and eagles. In these groups, we investigated the existence of disruptive mutations in genes which belong to pathways that are relevant to dietary adaptations, such as processes of digestion and absorption of specific nutrients or their regulation and storing. Additionally, we expanded an automatic pipeline to allow for the fast identification of coding disruptive mutations - pseudogenes - in large-scale datasets, using a previously developed web-tool<sup>1</sup>. By applying these approaches, we aim to pave the way to clarify the evolutionary history of the genetic pathways in the avian tree of life and how it explains the complexity of feeding behaviors in this tetrapod class.

[1] Luís Q Alves, Raquel Ruivo, Miguel M Fonseca, Mónica Lopes-Marques, Pedro Ribeiro, L Filipe C Castro, PseudoChecker: an integrated online platform for gene inactivation inference, *Nucleic Acids Research*, Volume 48, Issue W1, 02 July 2020, Pages W321–W331.

**Acknowledgments:** This work was supported by Fundação para a Ciência e a Tecnologia under the PhD scholarship 2020.08608.BD.

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**ABSTRACTS**

**FLASH TALKS**



## Modelling Mammalian behavioural traits: Inactivation of several Neuropeptides and Receptors in Cetacea and other mammals

Raul Valente<sup>1,2,3</sup>, Bernardo Pinto<sup>1,3</sup>, Filipe Salvador-Caramelo<sup>1,3</sup>, André Machado<sup>1,3</sup>, Filipe Alves<sup>4,5</sup>, Isabel Sousa-Pinto<sup>2,3</sup>, João Miguel Cordeiro<sup>1</sup>, Raquel Ruivo<sup>1</sup> and Luís Filipe Costa de Castro<sup>1,3</sup>

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Understanding the molecular and genetic mechanisms underlying phenotypic diversity is a fundamental goal of evolutionary biology. Between the molecular drivers of phenotypic novelty, gene loss has been associated with the development of phenotypic adaptations to specific ecological niches. A remarkable example of radical phenotypic transitions might be found within the Cetacea, a lineage represented by whales, dolphins, and porpoises. Within this taxon, the unique features of the brain have attracted considerable attention, as they were suggested to shape behavioural traits.

Among the panoply of signalling molecules that play a key role in behavioural regulation, neuropeptides are known to participate in the physiological alterations which the brain undergoes in response to external signals. Within this group of bioactive molecules, several neuropeptides have a well-known role in regulating sleep/arousal, appetite and energy expenditure – including, among others, *NPS*, *NPFF* and *QRFP*.

The aim of this study was to investigate the coding status and subsequent patterns of molecular convergence among 202 mammal species in 12 neuropeptides/receptors. By using a well-established sequence analysis pipeline, we were able to reveal a unique mutational landscape of gene erosion among mammals – with special emphasis on Cetacea and specific clades within Chiroptera and Carnivora. Additionally, comparison of cetacean RNA-seq data from different tissues, confirmed differences in gene expression for the brain. Our findings strongly suggest that inactivation of neuropeptides parallels the emergence of distinctive behaviours with powerful implications in shaping life-history traits.

**Acknowledgments:** This work is a result of the project ATLANTIDA (ref. NORTE-01-0145-FEDER-000040), supported by NORTE 2020, under the PORTUGAL 2020 Partnership Agreement and through the European Regional Development Fund (ERDF).



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**ABSTRACTS**

**POSTER PRESENTATIONS**

## Diversity of corals on the mainland shelf of Portugal: a DNA barcoding approach

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Corals play a crucial role in the structuring of marine benthic communities, particularly in deep-sea areas where they form vulnerable marine ecosystems (VMEs), i.e. coral reefs and gardens. These represent biodiversity hotspots and offer a wide variety of habitats for many fish and invertebrates. Despite their importance to the ecosystems, it is still unclear the extent of deep-sea corals' diversity and distribution on the northern coast of Portugal. The aim of this study was to undertake preliminary identifications of deep-sea corals collected as bycatch of bottom fisheries on the shelf and slope of northern Portugal, using a DNA barcoding approach. A total of 32 samples, representing 8 morphospecies had their DNA extracted, amplified and sequenced for the mitochondrial COI gene (Folmer region). High quality COI barcodes were obtained for 1/3 of the samples, and comparisons with sequences available in GenBank and the Barcode of Life Database, allowed their assignment to various taxa within the orders Zoantharias, Alcyonacea and Scleractinia. Most species constitute new records for the region, and some (e.g. *Dendrophyllia ramea* and *Dendrophyllia cornigera*) are currently classified as indicators of vulnerable marine ecosystems, which are of great conservation priority. This study represents a first step to assess the potential of a DNA barcoding approach for the identification of deep-sea corals in the region. Morphological analyses and sequencing of additional markers will allow to shed light on the diversity of corals on the shelf and slope of Northern Portugal, which will be essential to inform the management and conservation of these habitats in the area.

**Acknowledgments:** This study was supported by the DEEPbaseline project ([www.deepbaseline.org](http://www.deepbaseline.org)), an awardee of the Ocean Conservation Found funded by Oceanário de Lisboa and Oceano Azul Foundation (Ref. OLD/2019/044). Support of fishermen on the collection of bycatch samples is also gratefully acknowledged. It also constitutes a result of the project ATLANTIDA (ref. NORTE-01-0145- FEDER-000040), supported by the Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement and through the European Regional Development Fund (ERDF).



## SESSION 2 ECOLOGY AND ECOSYSTEM SERVICES

*MODERATION*

*RÚBEN PEREIRA AND MARINA DOLBETH*

## KEYNOTE SESSION – FLORA JULIE VINCENT



### **Viral impact in the ocean: from single cells to planktonic ecosystems**

Flora Vincent is a group leader at the EMBL Heidelberg in marine ecology and environmental microbiology. Trained as an agronomy engineer, she conducted her PhD at the ENS on the Tara Oceans project, followed by a post doc at the Weizmann Institute. Her research focuses on how marine microbes interact with each other, which led her to study symbiosis in plankton but also to assess the impact of viral infection on marine ecosystems. She is a strong advocate for more diversity in science and has co-founded the WAX Science non profit.



**ABSTRACTS**

**ORAL COMMUNICATIONS**

## Unravelling the composition of vulnerable marine ecosystems in the deep Eastern Atlantic Ocean through eDNA tools

Alejandra Mejía<sup>1</sup> and Joana R. Xavier<sup>1</sup>

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Vulnerable marine ecosystems (VMEs) are rare or unique ecosystems characterised by high functional significance, fragility, and structural complexity, together with low resilience to disturbance. In the deep sea, the vastest component of the ocean, VMEs encompass cold water coral- and sponge-dominated communities. Slow recovery rates make them extremely susceptible to human activity and are therefore of high conservation priority. However, conservation efforts are hampered by large information gaps on the distribution of these ecosystems, as seafloor exploration entails intricate logistics and vast economic means. Benthic studies in the deep sea typically rely on camera footage to detect and characterise communities. In addition to high acquisition costs, visual survey methods inherently have a limited taxonomic resolution. Metabarcoding of environmental DNA (eDNA) i.e. shed genetic material, is emerging as a powerful, fast and more affordable tool to aid biodiversity assessment and monitoring of aquatic habitats. In a single water sample, several species can be detected simultaneously by targeting short but highly variable portions of a gene. Reads are contrasted with genetic libraries to identify potentially occurring species and infer the community composition of the study area. eDNA metabarcoding has been tested in a range of marine environments where it generally shows a good correlation with visual approaches. In the deep sea, this method has mostly targeted fish and meiobenthic species. Although it shows great potential in the detection of cold-water scleractinian corals, it is yet to be tested on a wider taxonomic range of habitat-forming species. This project aims to evaluate the potential of eDNA tools to identify VMEs in the E Atlantic deep-sea benthos. We will develop an eDNA metabarcoding protocol to detect VME-indicator species from water samples and test its performance against camera-based surveys in well-studied areas. After calibration, we will apply this protocol to water samples from the Portuguese continental shelf and slope, which have not been visually explored but where VME-indicator species are frequently collected as bycatch from fisheries. We hope to provide a fast, low-cost detection tool that facilitates the conservation and management of these crucial deep-sea habitats.

**Acknowledgments:** This project fits within DEEPbaseline, a project supported by the Oceanario de Lisboa and Fundação Oceano Azul. We also acknowledge laCaixa Foundation for financing AM.

## **Sediment plumes cause detrimental effects at multiple levels in *Mytilus galloprovincialis* under hyperbaric conditions: a case study for deep-sea mining**

Marlene Pinheiro<sup>1,2</sup>, Adriana Oliveira<sup>2</sup>, Susana Barros<sup>1</sup>, Nélon Alves<sup>1</sup>, Joana Raimundo<sup>3,4</sup>, Miguel Caetano<sup>3,4</sup>, João Coimbra<sup>5</sup>, Teresa Neuparth<sup>1</sup> and Miguel M. Santos<sup>1,2</sup>

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The growing interest in deep-sea mining is being driven by an increased need for minerals/rare earth elements and the declining land-based deposits of these elements. Sediment plumes, released as a by-product of mining, are commonly considered one of the most immediate and extensive effects of deep-sea mining. To assess the potential effects of suspended sediments, juveniles of *Mytilus galloprovincialis* were used as a proxy species. We evaluated the effects of mixtures of different size classes of sediments with increasing concentrations and pressures in a hyperbaric chamber and evaluated the filtration rate and oxidative stress biomarkers in the digestive gland. As significant changes were seen for all tested endpoints, we then evaluated the effects of the different size classes individually by exposing mussels to different size classes of sediment individually. Expression of stress related genes, biochemical and functional endpoints were analyzed. Significant changes were observed for all endpoints. Our results show that sediments cause adverse effects and particular attention should be given to their release as to contribute to the establishment of guidelines to mitigate the impacts of deep-sea mining.

**Acknowledgments:** Marlene Pinheiro acknowledges the Portuguese Foundation for Science and Technology (FCT) under the PhD Grant SFRH/BD/147834/2019. This work was supported by the projects “DeepRisk – Deep-sea mining and climate change: new modeling tools in the support of environmental risk assessment” [PTDC/CTA-AMB/7948/2020], “HIPERSea – Collection and Life Support in a Hyperbaric system for Deep-sea Organisms” [POCI-01-0247-FEDER-033889 (AAC/03/SI/2017)] and “Atlantida – Platform for the monitoring of the North Atlantic Ocean and tools for the sustainable exploitation of the marine resources” [NORTE-01-0145-FEDER-000040].

# BLUE THINK CONFERENCE

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**ABSTRACTS**

**FLASH TALKS**



## Eco-Technologies for Treatment of Water Contaminated with Cyanotoxins

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Clean freshwater has become a scarce commodity, globally, which has led to the usage of eutrophic or low-quality water contaminated with cyanobacterial masses and cyanotoxins, especially for irrigation which in turn contaminates crops [1]. This causes a threat to food security and public health. Conventional solutions of water treatment are expensive, requiring specific infrastructures, specialized personnel, equipment and energy to operate. In this regard a challenge arises to treat surface waters contaminated with toxic cyanobacteria blooms from lakes, ponds, reservoirs, and irrigation systems. Constructed wetlands (CWs) are eco-friendly technologies of water treatment, which are also economically feasible.

The principle of CWs operation rely on its complex ecosystem structure consisting of water, plants, and microorganisms. These components function synergistically with physical, chemical and biological processes; including adsorption, sedimentation, precipitation, volatilization, plant uptake and microbial degradation [2]. These actions enable this technology to remove several persisting water pollutants including cyanotoxins [3].

The aim of this study is to optimize the design of CWs to treat eutrophic water contaminated with different cyanotoxins by testing different substrates, plants and different combinations of them. Initially, adsorption studies with different substrates will be done to determine the removal efficiencies for different cyanotoxins. Then, at least three species of plants known for phytoremediation will be compared, along with their polycultures, to determine which has higher removal efficiency. Later, CW microcosms with the best performing plants and substrates combinations will be tested.

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**Acknowledgments:** This project is being supported by a fellowship from “La Caixa” Foundation (ID 100010434; fellowship code “LCF/BQ/DI21/11860058). This project is also being supported by the CIIMAR Strategic Funding UIDB/04423/2020 and UIDP/04423/2020 funded through FCT and European Regional Development Fund (ERDF) and TOXICROP an EU research project (MSCA-RISE nr 823860).

## The effect of cadmium on the N<sub>2</sub>O net production by *Shewanella loihica* PV-4, a deep-sea isolate

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Deep-sea bacteria have high environmental importance due to their active role on nutrient cycling in a place where time appears to run slower. Some of these bacteria are responsible for maintaining low natural levels of nitrous oxide (N<sub>2</sub>O), a powerful greenhouse gas, by reducing it to dinitrogen gas (N<sub>2</sub>) with the action of N<sub>2</sub>O reductase, encoded by the *nosZ* gene. The growth of deep-sea mining activities expected for the next decades may increase the exposure of marine bacteria to toxic concentrations of metals, such as cadmium, copper, zinc, and lead. However, the susceptibility of the N<sub>2</sub>O-reducing pathway to metal exposure, is relatively unknown, especially in deep-sea conditions.

The aim of this study is to understand potential impacts of cadmium exposure on N<sub>2</sub>O net production by *Shewanella loihica* PV-4, a piezotolerant deep-sea strain. The potential inhibitory effect of cadmium in N<sub>2</sub>O net production is being investigated in controlled experiments by adding dissolved cadmium to growing cultures. In these experiments, N<sub>2</sub>O net production activities are being quantified by gas chromatography coupled to electron capture detection. We are also evaluating *nosZ* and *nirK* relative expression through real-time quantitative PCR. To get closer to deep-sea conditions, some of these experiments are being performed in high-pressure bioreactors.

We will present results regarding the effect of cadmium in N<sub>2</sub>O net production and *nosZ* and *nirK* expression. Also, we look forward to exploring and understanding the role of high hydrostatic pressure in these N<sub>2</sub>O activities.

**Acknowledgements:** This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 101038095.

## Developing Kelp Reforestation Methods

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During the past decades, the decline of kelp forests has been consistently reported along the European coastline. This results in important losses in biodiversity and ecosystem services. Currently, conservation actions, although important, are insufficient to maintain our ocean's health, and need to be complemented by restorative efforts. To achieve this, efficient methods need to be developed. The main purpose of this work is thus to develop reforestation methods for the Iberian kelp *Laminaria ochroleuca*.

To develop reforestation methods, two field experiments were carried out in Viana do Castelo and in Carreço. The first was set up to determine at which phase of *L. ochroleuca* life cycle reforesting would be more efficient and how the absence of canopy affected these efforts. The second one, based on results from the first, aimed to test the ability of microscopic life stage to recruit in protected (with canopy) and exposed areas and to validate methods of reforestation in areas affected by strong hydrodynamics.

We observed that the main obstacle to reforestation efforts is the competition by fast growing, opportunistic turf species, followed by the strong hydrodynamics and sedimentation which need to be considered when choosing the reforestation method.

This study provided useful information that is essential to develop efficient reforestation efforts that can be used for other perennial kelps species and along other coastal regions.

**Acknowledgments:** This work was supported by OCEAN3R [Norte2020: NORTE-01-0145-FEDER-00006]; and SeaForest [Fundo Azul: FA\_06\_2017\_067].

# BLUE THINK CONFERENCE

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**ABSTRACTS**

**POSTER PRESENTATIONS**

## Investigation of respiratory prokaryotic communities of free-ranging short-finned pilot whales using non-invasive sampling.

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Respiratory disease is one of the main causes of death in cetaceans, and characterizing the microbiomes of their respiratory tracts may constitute a suitable indication of health. For this purpose, it is necessary to first assess the baseline data on microorganisms usually related to cetacean's exhaled breath condensate (EBC), also known as blow. In this study, we aimed to validate the most suitable primer set to explore the microbial diversity of the respiratory tract in short-finned pilot whales (*Globicephala macrorhynchus*).

Blow samples were collected during at-sea campaign in Madeira Island in 2018. Metabarcoding analysis targeting V3–V4 and V4–V5- 16S rRNA gene regions, were performed. Preliminary results showed differences in richness and diversity between the two primers sets, with higher taxa coverage recorded for V3-V4 primer pair. In-depth taxonomic description of the blow microbiomes will allow for a detailed description of the blow microbiome, highlighting the presence of possible potential pathogens. This study will provide a network of consistent core taxa from the blow of short-finned pilot whale microbiome to ultimately develop an innovative non-invasive methodology for cetacean health monitoring.

**Acknowledgments:** This work is a result of the project ATLANTIDA (ref. NORTE-01-0145-FEDER-000040), (NORTE 2020).

## ***In vitro* and *in vivo* study of the effects of different nanoparticle coatings in trout cell lines and embryos of zebrafish**

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The properties of nanoparticles (NPs) captured the interest in the last decades. Titanium dioxide (TiO<sub>2</sub>) NPs are amongst the most produced NPs worldwide. To improve their properties and colloidal stability, NPs are coated with chemical compounds, such as silane-EDTA or sodium citrate. Thus, the aim of this work was to study the toxicity of TiO<sub>2</sub> NPs and their coatings, using rainbow trout cell lines and zebrafish embryos.

To evaluate the toxicological contribution of 45 nm TiO<sub>2</sub> NPs coatings, two *in vivo* embryo assays were performed: Nile Red assay and Development assay. Additionally, an *in vitro* MTT assay was carried out in rainbow trout cell lines.

Firstly, Nile Red assay allowed to tag, visualize and quantify the regional adiposity. This method consisted of exposing the zebrafish larvae at 25 and 500 mg/L to both TiO<sub>2</sub> NPs and each coating alone from 72 hours post fertilization (hpf) to 120 hpf. As a result, there were no significant effects of the coatings and NPs on the adipose tissue. The development assay consisted of measuring the mortality, hatching rate and malformations daily, while the length and heartbeat of the zebrafish embryos were measured at 96 hpf. Following the OECD 236 guideline, those embryos were exposed from 0 hpf to 96 hpf to TiO<sub>2</sub> NPs (5, 50 and 200 mg/L). Additionally, the embryos were also exposed to the coatings alone. Our results show that the coatings can be contributing on the toxic effects of TiO<sub>2</sub> NPs, especially silane treatments show some potential toxicity. At the end, larvae were stored in RNAlater to further quantify the expression of target genes, to understand the toxicological effects at the molecular level. Finally, MTT assay has been performed to measure the viability of rainbow trout cells (RTL-W1), by exposing the cells for 48h to 7 TiO<sub>2</sub> NPs dilutions (1:2) and the respective coatings alone. This assay is still ongoing. The results of this work will allow us to understand whether different coatings have additive, synergistic or antagonistic effects comparing to the toxicity of TiO<sub>2</sub> NPs in zebrafish and rainbow trout cell lines.

**Acknowledgments:** This work is funded by Acuinano Project - INTERREG VA Spain-Portugal cooperation programme (POCTEP) through the European Regional Development Fund (ERDF)-0712\_ACUINANO\_1\_E.



## Integrative taxonomy of marine sponges collected as bycatch of artisanal fisheries on the mainland shelf of Portugal

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Marine sponges (phylum Porifera) are important members of benthic communities, where they play numerous ecological roles, for instance in nutrient recycling and habitat provision. In the temperate Northeast Atlantic, knowledge of their diversity and distribution in shallow water is relatively comprehensive, yet little is known about the species inhabiting deeper mesophotic areas of continental shelves and slopes. The main aim of this study was to investigate the diversity of sponges collected as bycatch of bottom fisheries between 50-122 m depth in the northern Portuguese continental shelf, using an integrative taxonomic approach. This involved the characterization of external and internal morphological characters, alongside molecular identification, employing DNA barcoding and phylogenetic reconstructions. From a total of 29 specimens collected, 11 different species were identified from eight orders and nine families: *Axinella infundibuliformis*, *Calyx* sp., *Cliona celata*, *Desmacidon fruticosum*, *Axinella* sp., *Geodia* sp. 1, *Geodia* sp. 2, *Haliclona* (*Haliclona*) *oculata*, *Petrosia* (*Strongylophora*) *vansoesti*, *Raspailia* (*Clathriodendron*) *hispida*, and *Tethya aurantium*. Most of these constitute new records for the area and two species, *A. infundibuliformis* and *P. vansoesti* are considered indicators of vulnerable marine ecosystems, suggesting the potential presence of sponge aggregations in the region. This work shows the importance of the collaboration between local fishermen and the scientific community in advancing our knowledge of the biodiversity of mesophotic sponge fauna on the Portuguese shelf.

**Acknowledgments:** This work has been supported by the DEEPbaseline project, an awardee of the Ocean Conservation Fund funded by Oceanário de Lisboa and Oceano Azul Foundation (Ref. OLD/2019/044). Support of fishermen on the collection of bycatch samples is also gratefully acknowledged. It also constitutes a result of the project ATLANTIDA (ref. NORTE-01-0145- FEDER-000040), supported by the Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement and through the European Regional Development Fund (ERDF).

## Importance of Protection Service Against Erosion and Storm Events Provided by Coastal Ecosystems Under Climate Change Scenarios

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Increasing sea level rise (SLR) and frequency and intensity of storms, paired with degrading ecosystems, are exposing coastal areas to higher risks of damage by storm events. Coastal natural habitats, such as dunes or saltmarshes, can reduce exposure of coastlines to these events and help to reduce the impacts and the potential damage to coastal property. The goal of our study was to evaluate the current vulnerability of the Portuguese northern coast to erosion and flooding caused by extreme events and to assess the contribution of natural habitats in reducing both vulnerability and property damages considering SLR scenarios. The Integrated Valuation of Environmental Services and Trade-offs Coastal Vulnerability model was used to produce an Exposure Index (EI) for the northern Portuguese coastline, for the current situation, and for future International Panel for Climate Change (IPCC) scenarios Representative Concentration Pathway (RCP) 2.6, RCP4.5, and RCP8.5, considering the presence and absence of coastal habitats. Results showed an increase in exposure with rising sea levels and expansion of high-vulnerability areas. Coastal habitats contributed to a 28% reduction in highly exposed segments for the Current scenario, corresponding to a potential reduction of coastal property damage of 105 M€ during extreme events. For the SLR scenarios, coastal habitats could potentially reduce the amount of property damage by 190 M€ in 2050 and 285 M€ in 2100, considering RCP8.5 projections. This study highlights the importance of natural habitats in protecting vulnerable coastlines and reducing the potential damages to properties from flooding. Such results can be incorporated in management plans and support decision-making toward implementing an ecosystem-based approach to increase the resilience of coastal communities to cope with future environmental changes.

**Acknowledgments:** Ocean3R (NORTE-01-0145-FEDER-000064, NORTE 2020, PORTUGAL 2020 and ERDF), MarRisk (POCTEP 2014-2020 GALIZA-N, PORTUGAL, Ref: 0262\_MARRISK\_1\_E). FCT by the Strategic Funding UIDB/04423/2020 and UIDP/04423/2020 (FCT and ERDF), and a PhD fellowship to JC (ref. PD/BD/150359/2019).



## Performance assessment of a real scale constructed wetland for wastewater treatment

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Sustainable water management urges for the use and treatment of wastewater in an efficient way. Tourism units are often characterized by great variations in wastewater quantity and quality over the year. Constructed wetlands (CW) have been proposed as a nature-based solution to support the wastewater treatment from these units. In the present study, the potential wastewater treatment in a tourism unit through a real scale constructed wetland application is addressed. A CW was installed in a tourism unit in Ponte de Lima with a polyculture, delivering several ecosystem services.

The aim of the study is to monitor the efficiency of the biological system, after long term operation, based on phytoremediation processes, to treat the wastewater from the tourism unit. Wastewater quality will be assessed through the analysis of physic-chemical and microbiological parameters towards its reuse for irrigation purposes. CW substrate will also be analysed concerning the enzymatic activity associated to the rhizosphere of selected plants. The work to be developed is of outmost importance since it has a real implication on the water management of a tourism unit.

**Acknowledgments:** This research is supported by national funds through FCT - Foundation for Science and Technology within the scope of UIDB/04423/2020, UIDP/04423/2020, and UIDB/50016/2020 projects.

## Microplastics in Farmed Oysters (*Crassostrea gigas*) from Lima River Estuary

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Oysters are bivalve molluscs found in intertidal regions and are majorly exposed to microplastic contamination as a result of being filter feeders and due to their extensive seawater filtration capacity. Oysters are popular seafood and in order to respond to an increasing demand for human consumption, oyster aquaculture production has increased rapidly in recent decades. Typically cultivated in estuarine areas, oysters are exposed to microplastic contamination coming as marine litter from fishing and shipping, or from land sources<sup>1</sup>. In addition, oyster aquaculture often uses large amounts of plastic equipment (e.g. trays, baskets) and has been suggested as a potential source of microplastic contamination in the marine environment.

This study aimed to determine the microplastic contamination in oysters from a commercial farm operating in the Lima river estuary. On this farm, oysters are cultivated in suspended plastic baskets placed in intertidal areas of the estuary. A total of 15 adult oysters (mean length 78.5mm ± 21.9mm) were collected in February 2022 and analyzed to assess microplastic (MPs) contamination. MPs were extracted and quantified using specific methodologies in oysters as well as in water<sup>2</sup> and sediment samples from sites around the farm. Microplastics were then characterized in terms of size, color, and polymer type by microscopy and Fourier-transform infrared (FTIR) spectroscopy to identify the type and possible origin of the microplastic particles. Comparative analysis will allow us to determine the prevalence of oyster contamination and investigate the source of MPs found in shellfish and their environment.

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**Acknowledgments:** This research was partially funded by the Ocean3R (NORTE-01-0145-FEDER-000064) project supported by the Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement and through the European Regional Development Fund (ERDF). Additionally, FCT is acknowledged by the Strategic Funding UIDB/04423/2020 and UIDP/04423/2020 through national funds provided by FCT and ERDF, and a Ph.D. fellowship to S. M. Rodrigues (SFRH/BD/145736/2019), D. Silva (2020.06088.BD), and a research contract to S. Ramos (DL57/2016/CP1344/CT0020) and V. Freitas.

## Zooplankton and microplastics interferences in the Douro and Lima estuaries

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Microplastics (MPs) are described as plastic debris with a diameter below 5 mm. They are classified as primary MPs if manufactured in such small sizes, or as secondary MPs, that result from the breakdown of larger plastic pieces due to environmental factors. Besides the risk posed by their size and their suitability to be ingested by organisms, MPs are also able to adsorb and/or release other pollutants [1], becoming a growing global environmental concern. Smaller organisms, such as zooplankton, are particularly vulnerable to environmental threats, and so, demand increased attention regarding MP exposure. The present work aims to evaluate MP contamination, with focus on the interferences with zooplankton, in the Douro and Lima estuaries. Seasonal surveys were conducted in the Douro and Lima estuaries to collect water samples. Major zooplanktonic groups were quantified using a Bogorov chamber, and MP contamination was quantified through an analytical protocol previously developed by the team [2]. MPs were characterized by size, shape, color and plastic polymers are being characterized by FTIR analysis. Overall, results will be discussed in terms of ratio between zooplankton abundances and contaminations levels.

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**Acknowledgments:** This research was partially funded by Ocean3R (NORTE-01-0145-FEDER-000064) project supported by the Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement and through the European Regional Development Fund (ERDF). Additionally, FCT is acknowledged by the Strategic Funding UIDB/04423/2020 and UIDP/04423/2020 through national funds provided by FCT and ERDF, and a PhD fellowship to R. Pereira (2021.04850.BD) S. M. Rodrigues (SFRH/BD/145736/2019), D. Silva (2020.06088.BD), and a research contract to SRamos (DL57/2016/CP1344/CT0020).

## Evaluation of phycotoxin biodegradation by bivalve-associated bacteria

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Phycotoxins are potent organic compounds produced by marine phytoplankton which are known to accumulate in filter-feeding shellfish, causing enormous economic losses in aquaculture production and severe human health problems. This is a major problem in Portuguese shellfish harvesting and production sites which are frequently affected by harmful algal blooms. The main episodes of contamination in the last years have been due to diarrhetic (DSTs) and/or paralytic shellfish toxins (PSTs) [1]. In this context, the development of phycotoxins mitigation strategies is one of the top challenges in aquaculture and human health sectors.

The goal of this study was to explore the capacity of cultivable bacteria living in symbiosis with the cockle *Cerastoderma edule* to biodegrade marine phycotoxins. The microbiota associated with the intestinal tract of cockles was used as bacterial inoculum for enrichment experiments with okadaic acid. High-throughput 16S rRNA amplicon gene sequencing on the Illumina platform (NGS) was applied to assess the bacterial diversity and guide the screening process. Once an enriched culture was obtained, traditional plating methods were used to isolate single colonies. The selected bacterial strains were further identified by PCR amplification of 16S rRNA gene using universal bacterial primers followed by sequencing. Finally, a consortium composed of the microorganisms previously isolated was assembled according to their original relative abundances in the enriched culture and its potential to biodegrade 0.1 mgL<sup>-1</sup> of okadaic acid or saxitoxin was evaluated. The removal of the target compounds was analyzed by liquid chromatography–mass spectrometry (LC–MS) before and after an incubation period of 21 days.

The outcomes of this investigation will allow understanding if bivalves-associated bacteria can have an active role in the detoxification of phycotoxins. This finding may have a great scientific and economic impact at improving shellfish aquaculture management by the development of microbial-based solutions for the mitigation of phycotoxins.

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## Nature-based solutions for urban climate regulation and air quality

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The increase of population in cities associated with climate change impacts can create innumerable environmental, economic, and social problems, around the world. Urban areas have become more impermeable, with an increase fragmentation of habitats, ecosystem degradation, intensification of the heat island effect, water, air, and land pollution and consequently leading to deterioration of human wellbeing and disconnection to nature. The strategy for changing territories dynamics involves the use of Nature-Based Solutions (NBS) to promote multifunctional areas, operating at different scales and relying on nature to generate environmental (e.g., biodiversity conservation or adaptation to climate change), economic (e.g., job creation and property valuation) and social (e.g., water drainage) advantages.

The aim of the present study is to identify and characterize the main NBS that are being applied to mitigate two problems that many of Asian cities, including Macau, face: poor air quality and heat urban effect. The intention is to gather quantitative data to support a proposal towards a more sustainable city. The methodology will proceed with a state-of-the-art assessment followed by a systematic review focused on selected NBS. Statical analysis will be carried out for data assessment.

**Acknowledgments:** This research is supported by national funds through FCT - Foundation for Science and Technology within the scope of UIDB/04423/2020, UIDP/04423/2020, and UIDB/50016/2020 projects.



## Life cycle assessment of constructed wetland systems for tourism units wastewater treatment

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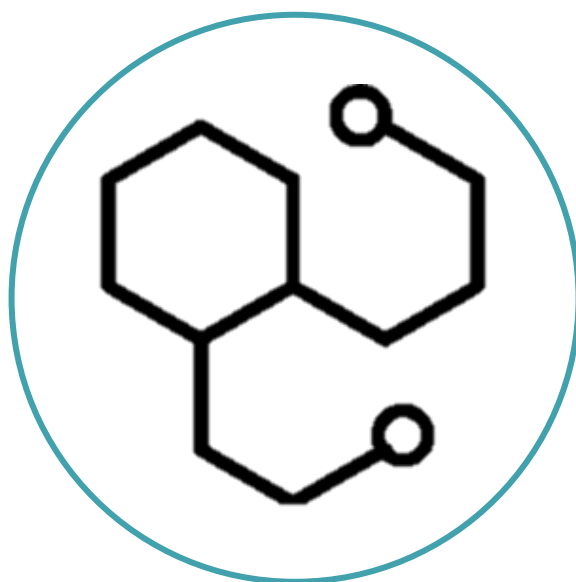
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Constructed wetlands (CW) are considered an environmentally friendly wastewater treatment technology which helps reducing environmental impacts and footprint of water management. Life Cycle Assessment (LCA) can support the design of wastewater treatment systems. Such tools allow decision makers to make informed choices about where and how action is needed and the selection of materials and design. LCA is a comprehensive tool for estimating potential environmental impacts of products and processes and can also be used to identify which life stage carries the most significant environmental impacts for particular system<sup>1</sup>. LCA is based on the following phases: goal and scope definition; inventory analysis; impacts assessment and interpretation of the results.

The purpose of the present study is to assess the environmental profile of a CW wastewater treatment based on performance, operation and maintenance. A real scale CW with a polyculture, in operation in a tourism unit, will be the focus of the LCA. The functional unit considers 1 m<sup>3</sup> of treated wastewater through a horizontal subsurface continuous flow. We expect to be able to contribute to spread the benefits of using such nature-based solutions as sustainable wastewater management strategies.

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**Acknowledgments:** This research is supported by national funds through FCT - Foundation for Science and Technology within the scope of UIDB/04423/2020, UIDP/04423/2020 projects.



## SESSION 3 BIOCHEMISTRY AND BIOTECHNOLOGY

*MODERATION*

*ANNE LIONG AND MARIA PAOLA TOMASINO*

## KEYNOTE – ELKE DITTMANN



### **Understanding the bottlenecks in cyanobacterial natural product research**

Prof. Dr. Elke Dittmann is a Full Professor of Microbiology and Head of the Microbiology Department at the University of Potsdam, Germany. Prof. Dittmann is also a member of the Advisory Board of the Cluster of Excellence: “Controlling microbes to fight infections”. Her research is focused on the biosynthesis, evolution and function of secondary metabolites in cyanobacteria. Prof. Dittmann has an established track record of high-standard research, with 87 publications in scientific journals and is a member of the editorial board of Environmental Microbiology.





**ABSTRACTS**

**ORAL COMMUNICATIONS**

## Biosynthetic diversity of Cyanobacteria biofilms using metagenomics

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Cyanobacteria are recognized as one of the richest bacterial phyla regarding the production of natural products (NPs). Recent (meta)genomic studies have shown that cyanobacteria genomes englobe a large diversity of biosynthetic gene clusters (BGCs). Many such BGCs are not associated with known NPs, highlighting the small fraction of currently known cyanobacterial metabolites. This knowledge gap occurs in part due to the difficulty to grow the cyanobacteria in laboratory or the difficulty to collect large amounts of biomass directly from the environment in non-tropical regions, which would be necessary to obtain biomass for the isolation of sufficient amounts of NPs for structural characterization. An alternative and promising route to NP isolation, involves capture and expression of BGCs in a heterologous host. In this study, a metagenomics approach was employed to study the diversity of biosynthetic gene clusters (BGCs) from six cyanobacteria-enriched environmental samples, including marine, freshwater and a subaerial sample. In total, 29 cyanobacterial metagenome-assembled genomes belonging mainly to Oscillatoriales and Chroococcioidiales orders, were recovered. Anti-SMASH analysis allowed for the identification of over 180 complete or near-complete BGCs. From these, only a small fraction could be clearly assigned to a known NP. Over 40 distinct BGCs encoding for non-ribosomal peptides, polyketides, microviridins, siderophores and an indole alkaloid were selected, and we are currently attempting their heterologous expression using the DiPaC-SLIC method.

**Acknowledgments:** This study was partially funded through the grant ATLANTIDA (ref. NORTE-01–0145-FEDER-000040), supported by the Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement and through the European Regional Development Fund (ERDF) and by Fundação para a Ciência e a Tecnologia through grants UIDB/04423/2020, UIDP/04423/2020 and a PhD scholarship (SFRH/BD/140567/2018). This project has also received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 95237.

## Chemical isolation of novel compounds from *Spirulina* with anti-obesity activity as future nutraceuticals

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One public health problem of utmost concern is the complex metabolic disease of obesity. Other high-risk diseases associated with obesity development include cardiovascular diseases, diabetes (T2DM), among others. New strategies besides lifestyle changes are necessary for treating this condition. Current solutions, both pharmaceutical and nutraceutical, either present poor effectiveness or pose great health risk and relevant toxicity. Due to societal values, search for new and improved treatments derived from natural products is being promoted. The Cyanobacteria phylum is regarded as a great source for natural bioactive compounds. Some species in *Arthrospira* genus (commercially, *Spirulina*) present potential for obesity treatment as recently proven by the discovery of the lipid-decreasing compound, 13<sup>2</sup>-hydroxypheophytin a (hpa).<sup>1</sup>

In this work, *Spirulina* was subjected to a bioassay-guided isolation strategy to discover anti-obesity compounds, to purify hpa and to determine its suitability as a new anti-obesity nutraceutical. *Arthrospira platensis* was successfully extracted using a mixture of methanol and dichloromethane. The extract was subjected to sequential steps of Vacuum Liquid Chromatography, manual and automated Flash Chromatography, and lastly normal phase High Performance Liquid Chromatography. The resulting fractions were gradually used in the Nile red zebrafish fat metabolism assay leading to the isolation of hpa and other compounds of interest. Metabolomics analyses were done for all fractions with Liquid Chromatography coupled to Mass Spectrometry data by GNPS and MZmine analysis. The chemical nature of the final active fractions was investigated by <sup>1</sup>H Nuclear Magnetic Resonance. Most isolated bioactive molecules resemble chlorophyll metabolites.

The hpa-containing fraction presented  $69.7 \pm 7.9\%$  neutral lipid reducing activity. Isolated hpa is currently being tested against commonly used obesity-fighting nutraceutical ingredients to compare its efficacy. With this work it was possible to confirm the generalized presence of hpa in *Spirulina* and validate its utility. To better assess its potential for the anti-obesity nutraceutical market, pre-clinical and clinical trials should be done in the future.

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## Anti-obesity activity of vitamin K family in zebrafish potentially involving different metabolic targets

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Obesity and its related metabolic dysfunctions are one of the main public health challenges and new therapeutic approaches are required to overcome this epidemic. Vitamins K are a fat-soluble family of compounds involved in a number of essential biological functions. Vitamin K1 and K2 are two naturally occurring compounds, while vitamin K3 is the synthetic form. In this work, a bioactivity-guided isolation method was used. The Nile red fat metabolism assay in zebrafish larvae led to the successful isolation of an analog of vitamin K1 for the first time from *Tychonema* sp. LEGE 07196. The structure confirmation was based on NMR spectroscopy and mass spectrometry. Although this analog has been firstly isolated for over 50 years from the cyanobacteria *Synechococcus elongatus*, little is known on its bioactivity<sup>1</sup>. The anti-obesity effects of all vitamin K forms, including the K1-analog, were studied. The compounds showed significant neutral lipid-reducing activity after 48 h of exposure with EC<sub>50</sub> values of 50, 20, 1, 31 µM, for K1, K2, K3 and K1-analog, respectively. This activity shows the importance of the naphthoquinone ring, as well as the impact of the saturation and polarity of the aliphatic side-chain for the structure-activity relationship. Furthermore, apart from the K3 vitamin for concentration higher than 7.6 µM, neither form cause any general toxicity or malformations. To further establish the distinct bioavailability and biotransformation of the different forms of vitamins in the organisms, metabolomics approaches were employed. The metabolite profiling was achieved using different databases and Metaboanalyst was used for associated analysis. The different vitamin supplementation clearly induced different biological effects and metabolic pathways indicating that different metabolic targets are involved. This work is ongoing, and the final aim is to understand the effects of vitamin K family on obesity and related co-morbidities, which could lay the ground to develop a future nutraceutical with lipid reduction activity.

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**Acknowledgments:** This research was funded by R&D&IATLANTIDA—Platform (reference NORTE-01-0145-FEDER-000040) and FCT PhD grant 2020.08437.BD.

## Discovery of desmamides A-C, new lipoglycopeptides from the endophytic cyanobacterium *desmonostoc muscorum* lege 12446

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Certain cyanobacteria of the secondary metabolite-rich order Nostocales can establish permanent symbioses with a large number of cycads, by accumulating in their coralloid roots and shifting their metabolism to dinitrogen fixation. Here, we report the discovery of two novel lipoglycopeptides, desmamides A and B, together with their aglycone desmamide C, from the cyanobacterium *Desmonostoc muscorum* LEGE 12446 isolated from a cycad coralloid root. The chemical structures of the compounds were elucidated using a combination of 1D and 2D NMR spectroscopy and mass spectrometry. The desmamides are decapeptides, featuring O- $\alpha$ -glycosylation of tyrosine (in A and B) and an unusual 3,5-dihydroxy-2-methyldecanoic acid residue. The biosynthesis of the desmamides was studied by substrate feeding experiments and bioinformatics. We describe herein the dsm biosynthetic gene cluster (BGC) and propose it to be associated with desmamide production. The discovery of this class of very abundant (>1.5% d.w.) bacterial lipoglycopeptides paves the way for exploration of their potential role in root endosymbiosis.

**Acknowledgments:** We thank support by Fundação para a Ciência e a Tecnologia (FCT) through grants UIDB/04423/2020 and UIDP/04423/2020 and scholarships SFRH/BD/116009/2016 to SF. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 952374.

# BLUE THINK CONFERENCE

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**ABSTRACTS**

**FLASH TALKS**

## Metals recovery from organic waste via anaerobic bioleaching and microbial induced carbonate precipitation

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Organic waste (OW) accounts for a significant share of global waste production. It has risen tenfold over the past century and it is predicted to reach 6 million ton/day by 2025 [1]. Anaerobic digestion (AD) is one of the most worldwide spread technologies to valorize OW allowing to produce a digestate with fertilizing properties while recovering energy in the form of biogas, increasing the interest towards this technology in the context of the envisaged circular economy [2]. Digestates can be used in agriculture replacing commercial fertilizers and soil amendments, however, AD can have relative high metal concentrations, leading to phytotoxicity problems and groundwater contamination [3]. This, together with a raising interest in metals extraction from secondary sources makes metals recovery from anaerobic digestates an attractive field to be investigated. Currently, the main factors influencing metals extraction and solubility during AD have been poorly explored. Therefore, the initial phase of this research focuses on evaluating the solubility of Cr, Cu, Al, Co, Ni and Zn at varying operational conditions in a mesophilic anaerobic semi-continuous reactor treating swine manure. Subsequently, solubilized metals are expected to be recovered through a microbial induced immobilization process, thus allowing to obtain a digestate with low metal concentration that can be used for agriculture. The combination of biological processes for OW treatment and metal recovery are expected to be a more sustainable and economically efficient approach for bio-resource recovery compared to conventional physico-chemical technologies.

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[3] C. Marcato, E. Pinelli, P. Pouech, P. Winterton and M. Guisresse, *Bioresource Technology*, **2008**, 99, 2340-2348

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



## Cyanobacteria as a source of valuable bioactive compounds for appetite suppression

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Currently, there are a small number of drugs approved for the treatment of obesity, a metabolic disorder that represents a serious health challenge worldwide that increased exponentially in the last years. Appetite suppressant drugs are a target for obesity treatment. In this context, this work aims the exploration of the bioactive potential of cyanobacterial strains belonging to The Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC) of CIIMAR (Interdisciplinary Centre of Marine and Environmental Research) for their capability to modify the food consumption behavior of zebrafish larvae - a whole small animal model. A library of 117 cyanobacterial fractions from marine and freshwater environments were assessed in two different bioassays for appetite-reducing activity, using fluorescent stained liposomes (passive food intake) or *Paramecia bursaria* (active food intake). Three cyanobacterial fractions from the order Nostocales, Chroococcidiopsidales and Pleurocapsales expressed appetite-suppression bioactivity in the liposomes assay, while three different fractions from the order Synechococcales, Oscillatoriales and Nostocales significantly decreased the appetite in the *Paramecium* assay. Dereplication by metabolomics approaches (LC-MS/MS) was accomplished as well as bioactivity-guided feature based molecular networking, and four masses were positively correlated and statistically significant with the bioactivity noted. No identification was found in any database (GNPS, Sirius, DNP and NPA) for these molecules, indicating putatively new compounds. Molecular studies are currently ongoing to uncover the involved genes that regulate the passive and active food intake in zebrafish larvae, and consequently find the mechanisms of action.

**Acknowledgments:** The authors thank the project ATLANTIDA (ref. NORTE-01-0145-FEDER-000040), supported by the Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement and through the European Regional Development Fund (ERDF), and by national funds through FCT (Fundação para a Ciência e Tecnologia, Portugal) scholarship grant UI/BD/150901/2021.

## Exploring the chemical diversity of a group of cyanobacterial chlorinated dialkylresorcinols – the bartolosides

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Cyanobacteria are rich in biosynthetic gene clusters encoding the production of a variety of chemically diverse metabolites. Exploring these biosynthetic pathways can also lead to the discovery of uncharacterized enzymes with biotechnological relevance. We have recently showed that a novel enzyme – BrtB – catalyzes the esterification of free fatty acid carboxylates into the chlorinated alkyl chains of bartolosides, a group of diverse cyanobacterial dialkylresorcinols. Besides BrtB, there is currently only one other characterized member from its family – CylK, involved in the biosynthesis of the cylindrocyclophanes.

Previously known bartolosides feature one or two glycosyl moieties and different levels of halogenation. With the discovery of BrtB we increased this metabolite library after revealing the presence of fatty acid branched bartolosides. Here, we show our most recent screening for bartoloside producing strains in the LEGE-CC, a culture collection with over 400 cyanobacterial strains. We coupled PCR screening, BLAST and the Global Natural Products Social Molecular Networking (GNPS)-based metabolomics to detect additional *brt* clusters and new bartoloside derivatives, respectively. Furthermore, we explore BrtB homolog diversity in the LEGE-CC and public databases, using *in silico* analysis with the Enzyme Function Initiative Tools. This study will inform future bartoloside and BrtB homolog discovery efforts.

# BLUE THINK CONFERENCE

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**ABSTRACTS**

**POSTER PRESENTATIONS**

## Eco-friendly synthesis of a new antifouling agent: one step towards the scale-up process

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Chemical protection is the most effective strategy against marine biofouling on underwater surfaces.<sup>1</sup> However, traditional coating approaches present severe environmental consequences on the marine ecosystems as they continuously release copper and other toxic biocides to the oceans.<sup>1</sup> Therefore, the development of new, more effective, and environmentally safe antifouling (AF) agents is highly desirable. In a previous research project of our group, a gallic acid (GA) synthetic derivative was obtained and revealed to be a highly promising eco-friendly AF agent.<sup>2</sup> However, its synthetic route wasn't environmentally safe, mainly due to the use of BBr<sub>3</sub>, a highly corrosive and toxic reagent, which also hindered the reaction scale-up.

In the present work, greener synthetic procedures were explored to significantly reduce the environmental footprint. GA was selected as the starting material as it can be extracted from grape wastes through green methodologies, making use of renewable feedstocks and allowing waste valorization. In the new eco-friendly synthetic pathway GA is chemically protected with benzyl groups (89% yield), followed by TBTU-mediated amine coupling (82% yield) and hereafter by respective deprotection (74% yield). This synthetic pathway proved to be scalable and was successfully applied for the synthesis of the targeted AF agent.

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**Acknowledgments:** This research was supported by national funds through FCT (Foundation for Science and Technology) within the scope of Base Funding UIDB/04423/2020 and UIDP/04423/2020 (CIIMAR) and as a result of the project PTDC/CTA-AMB/0853/2021. This research was also supported by the Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement and through the ERDF, as a result of the project ATLANTIDA (reference NORTE-01-0145-FEDER-000040).

## Identification of microalgae strains producing high-value compounds

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Marine-based products present high chemodiversity offering several advantages compared with conventional overexplored biological sources, and, therefore are progressively playing an important role in society and economy. Microalgae thus represent a valuable source of high-value compounds, HVC (e.g. polyunsaturated fatty acids (PUFA's); carotenoids, vitamins or even pharmaceutical compounds) with increasing biotechnology potential.

The target of this research is the identification of novel microalgae strains, producing HVC, that can impact the market landscape of nutraceuticals and cosmetics. After literature and market analysis, we selected twenty-one microalgae strains, from Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC), based on their genus and biotechnological potential. After laboratory cultivation, we assessed the levels of different HVCs in the selected strains: pigments (neoxanthin, astaxanthin, canthaxanthin, chlorophyll a,  $\beta$ -cryptoxanthin, lutein, echinenone,  $\epsilon$ -carotene, violaxanthin,  $\gamma$ -carotene,  $\beta$ -carotene, lycopene, zeaxanthin and fucoxanthin), phenols (total content), fatty acids (focus on PUFA's) and polysaccharides. In parallel to the HVC screening, growth curves were obtained for all microalgae coccoid strains, to understand the growth pattern and to allow for future optimization of HVC yields.

All microalgae strains were extracted with different organic solvents (cosmetic and food-grade, GRAS) for further characterization: HPLC-PDA pigments characterization; Total Phenols Content (TPC) determination by Folin-Ciocalteu method; GC-MS profiling of Fatty Acids (with the collaboration of the Lipidomics Laboratory of Mass Spectrometry Centre, Chemistry Department of University of Aveiro); and polysaccharides screening with qualitative analysis of neutral and negative polysaccharides by Alcian Blue Staining and quantitative analysis with Dubois assay.

Results demonstrate the potential of microalgae to produce HVC: we found six strains with promising phenols content; two strains showed above-average polysaccharide production. The remaining extracts are currently being quantified for different pigments and fatty acids. Preliminary results show interesting profiles, confirming a vast potential for nutraceutical and cosmetic applications.

## Semi-preparative enantioresolution, racemization and cytotoxicity studies with MDPV enantiomers

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New psychoactive substances represent a public health threat since they are sold as legal alternatives to illicit drugs, are easily accessible online and there is limited information about their properties. Among them, synthetic cathinones, such as 3,4-methylenedioxypyrovalerone (MDPV), are widely abused due to their psychostimulant effects<sup>1</sup>. Being chiral compounds, although scarce, the study of both enantiomers is of great importance, including the influence of stereochemistry in their biological/toxicological properties<sup>2,3</sup>, and the racemization phenomena since enantiomers can undergo racemization in certain temperature and acidic/basic environments<sup>4</sup>. Moreover, enantioresolution methods that allow the separation of the enantiomers of synthetic cathinones have been reported, being liquid chromatography (LC) using chiral stationary phases (CSPs) the main technique of choice<sup>5</sup>.

In this work, first, the semi-preparative enantioresolution of MDPV and a racemization study were performed by LC using polysaccharide-based CSPs. The enantiomers were separated with a good resolution and enantioselectivity and collected with high enantiomeric ratio and recovery rates. While no racemization occurred after 3 h for all tested conditions, signs of racemization started to appear after 48 h at 37°C and after 24 h at 70°C. Moreover, to evaluate the potential enantioselectivity of MDPV in cytotoxicity, MTT and NR assays were performed using the SH-SY5Y cell line. The cells were exposed to each MDPV enantiomer, in a concentration of 1.506 mM, for 24 h and no enantioselectivity was found.

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**Acknowledgements:** This research was supported by national funds by FCT (UIDB/04423/2020, UIDB/MULTI/04378/2020, UIDP/04423/2020 LA/P/0140/2020, PTDC/CTA-AMB/6686/2020 projects) and BYT-Plus 2021/22 scholarship from CIIMAR and Amadeu Dias Foundation.



## Screening for Novel Microcystin-Degrading Bacteria From Urban Lake Sources (Porto, Portugal)

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Cyanobacterial blooms are increasing in extension and frequency due to climate change and eutrophication associated to anthropogenic activities. This high growth has a lot of environmental consequences. Microcystins are one of the most common hepatotoxins produced by cyanobacteria in freshwaters [1]. They can affect a wide range of species, including humans. These toxins are very stable and not removed by conventional drinking water treatment processes. In this way, new innovative and sustainable solutions are needed for their removal. Biological treatments can be considered an option for effective removal of cyanotoxins.

The goal of this study was to evaluate the biodegradation capacity of environmental microorganisms native from a lake located in Parque da Cidade (Porto, Portugal) where toxic blooms of microcystin-producers frequently occur. Water samples collected from the lake were used as inoculum for a selective enrichment period, conducted in batch mode, with microcystin-LR (MC-LR, 0.25 mgL<sup>-1</sup>) supplied as a C and N source every 20 days. After two incubation cycles of 20 days, MC-LR levels and its degradation products were analysed at the beginning and end of the incubation period by HPLC-MS/MS. At the same sampling periods, the colorimetric quantification of PO<sub>4</sub><sup>3-</sup>, NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup> and NH<sub>3</sub> was also conducted. Furthermore, PCR-amplified genes encoding microcystin-degrading enzymes (*mlrA*, *mlrB*, *mlrC* and *mlrD*) were identified by gel electrophoresis through the whole enrichment process.

The results of the present study will open the way for the discovery of new bacterial strains with promising microcystin degrading properties, suitable for the development of innovative microbial-based solutions for cyanotoxins mitigation.

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## Direct Pathway Cloning (DiPaC) to discover new natural products from cyanobacteria

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Given the huge improvements observed in sequencing technologies, the number of sequenced genomes has increased dramatically over the last 10 years. For bacteria, this wealth of novel genomic data has revealed a large number of secondary metabolite biosynthetic gene clusters (BGCs), representing a potential source of unexplored compounds.

One group of chemically and genetically very rich bacteria, cyanobacteria, incorporate fatty acids (FAs) into a large fraction of their natural products (NPs). On average, these diverse photosynthetic bacteria devote 5-6% of their genomes to secondary metabolite BGCs. The percentage of orphan BGCs is estimated to be 80-90%. However, identifying the NP associated with an orphan BGC is still one of the major challenges in NPs studies.

In this project, we used a recently developed cloning strategy, Direct Pathway Cloning (DiPaC), to heterologously express a 26.9 kb fatty-acid incorporating BGC from the freshwater cyanobacterium *Nostoc* sp. in *Escherichia coli*.

By conducting organic extractions and LC-MS (liquid chromatography mass spectrometry) analysis, we could observe the production of novel compounds in *E. coli* BAP1 strain carrying the BGC, compared to an empty vector control.

In parallel, and due to the presence of a FA incorporating Fatty acyl-AMP ligase (FAAL) on our BGC of interest, we supplemented *Nostoc* sp. with isotope-labeled FAs to discover a group of related masses that we assign as novel NPs, using comparative metabolomics and MS<sup>2</sup> analysis.

The combination of these methods will enable us to isolate and structurally elucidate the corresponding NPs from the original *Nostoc* sp. host, as well as the related compounds from the heterologous host *E. coli*, which in the end will be tested for biological activity.

**Acknowledgments:** The authors thank to the European Research Council through a Starting Grant (759840), European Union's Horizon 2020 programme (WIDESPREAD, Grant Agreement 952374) and Fundação para a Ciência e a Tecnologia (FCT) through strategic funding grant UIDB/04423/2020 and Ph.D. grant 2020.08183.BD.

## Looking into beta-oxidation in cyanobacteria

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Cyanobacteria are an ancient group of oxygenic phototrophic prokaryotes from which chloroplasts have evolved. These organisms are gram-negative bacteria with an extensive morphological diversity, existing in forms that range from single-celled to differentiated multicellular forms. They are also present in various environments, such as marine, freshwater, or terrestrial habitats.

An interesting characteristic of the fatty acid metabolism of cyanobacteria is an apparent lack of a functional  $\beta$ -oxidation pathway. This property was taken advantage of for the development of a new strategy to uncover new cyanobacterial natural products. This new strategy led to further inquiries into the lack of a beta-oxidation process in cyanobacteria, which uncovered a possible alternative fatty-acid catabolic pathway.

We now intend to explore this observation and try to identify how the lipid metabolism of these ancient organisms works, which will not only bring understanding to this widespread question, but also take us a step further into cyanobacteria evolution.

For that, a comprehensive project was developed, that uses several tools, from various scientific fields, such as bioinformatics; chemistry and molecular biology, to clarify if cyanobacteria indeed lack a beta-oxidation pathway and/or if there is an alternative catabolic pathway.

## Nature-inspired flavonoids with antifouling activity

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The natural colonization of marine organisms on subsea surfaces, namely hulls of ships, is a huge issue for maritime industries, which causes enormous material and economic losses, as well as environmental problems due to the use of toxic antifouling agents and also the introduction of invasive species into ecosystems. Since the ban of tributyltin-based coatings, alternative antifouling agents, such as copper and booster biocides have been used, which have also been shown toxicity to the marine environment.<sup>1</sup> Natural prenylated flavonoids and their synthetic analogues have shown potential to prevent biofouling as a consequence of their effects against macro and microfouling species.<sup>2,3</sup> Moreover, the presence of 1,2,3-triazole ring in molecules of compounds incorporated in coatings has shown to contribute for antifouling and anticorrosive properties.<sup>4</sup> Considering this, a series of chemically related flavonoid analogues, having triazole and alkyl groups, was synthesized. The antifouling activity was then evaluated using the *in vivo* anti-settlement bioassay with *Mytilus galloprovincialis* larvae and growth inhibition of marine biofouling microorganisms was assessed. Flavonoids with antifouling activity against macro and microfouling species were identified. To evaluate their ecotoxicity against non-target marine organisms, complementary assays with *Artemia salina* were also performed.

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**Acknowledgments:** This research was supported by national funds through FCT (Foundation for Science and Technology) within the scope of UIDB/04423/2020, UIDP/04423/2020, and under the projects PTDC/CTA-AMB/0853/2021 and NASCEM-PTDC/BTA-BTA/31422/2017 (POCI-01-0145-FEDER-031422), co-financed by COMPETE 2020, Portugal 2020 and the European Union through the ERDF and by FCT through national funds and structured program of R&D&I ATLANTIDA (NORTE-01-0145-FEDER-000040), supported by NORTE2020, through ERDF. DP acknowledges FCT for the Ph.D. scholarship (grant number SFRH/BD/147207/2019).

## Metagenome Derived Discovery of New Cyanobacterial Natural Products

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Cyanobacteria is a diverse phylum that produces a variety of secondary metabolites with potentially beneficial bioactivities. Their slow growth rates and the difficulty in culturing some of these microorganisms in laboratory limit their chemical exploration. Genome-based discovery of new natural products has been driven by the reducing cost of genome sequencing and the advances of bioinformatic strategies. Metagenomics allows the study of biosynthetic gene clusters (BGCs) from non-cultured microbes through direct sequencing of DNA in environmental samples. Subsequently, it is possible to produce the encoded secondary metabolites in heterologous hosts. In this project we use such a strategy to uncover the biosynthetic potential of cyanobacteria that compose environmental biofilms. From a sample collected in Parque da Cidade, Porto, Portugal, we were able to recover three cyanobacterial metagenome-assembled genomes (MAGs) from the *Oscillatoriales* order: two belong to the already characterized genera *Planktothrix* and *Planktothricoides* and the other is unclassified at the genus level. Bioinformatic analysis revealed thirty-nine complete and near-complete BGCs from which we selected five of distinct biosynthetic classes for heterologous expression: two microviridins, a type I polyketide synthase (PKS)/non-ribosomal peptide synthase (NRPS), a cyanobactin and a NRPS. Currently, we are using Direct Pathway Cloning coupled with Sequence- and Ligation-Independent Cloning (DiPaC-SLIC) for the cloning and heterologous expression of the BGCs. We have succeeded at cloning two genes from one of the microviridin BGCs and one gene from the other microviridin BGC into *E.coli*. We will use a LC-MS-guided strategy to isolate the compounds. The isolated secondary metabolites will be tested to evaluate their bioactivity and their chemical structure will be elucidated through MS/MS and NMR.

## Horizontal distribution of marine microbial communities in the North Pacific Subtropical Front

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Microbial communities are crucial for important ecosystem functions in the open ocean, such as primary production and nutrient cycling. However, few studies have addressed the distribution of microplankton communities in the remote oligotrophic region of Pacific Ocean. Moreover, the biogeochemical and physical drivers of microbial community structure are not fully understood in these areas. This research aims to investigate the patterns of microbial prokaryotes and eukaryotes communities' distribution in the North Pacific Subtropical Front (NPSF), an area with limited biological surveys despite its intense trophic transferences and physicochemical gradients. We used a 16S and 18S rRNA gene sequencing approach to identify and characterize the communities at two depths, surface (3-5 m) and the deep chlorophyl maximum (DCM, 108-130 m) and to analyse how they are horizontally distributed and what influences these distributions. Results showed that the beta diversity of both communities were significantly influenced by the same parameters: depth, temperature and longitude. Regarding alpha diversity, both communities presented a higher diversity at surface in samples placed further east. The same happens with species richness. The prokaryotic, dominated by Proteobacteria and Cyanobacteria, and eukaryotic communities, dominated by Syndiniales, demonstrated a similar pattern at two depths. Nevertheless, for each depth, an outlier was found, possibly located next to an upwelling area. These samples demonstrated to be exceptions in the microorganisms distribution of both communities. Even though most studies do not consider this factor, These occurrences can be a prove that longitude is capable of having a strong impact and influence on the communities' structure. Especially, with the occurrence of oceanographic phenoms, limited to a horizontal area, such as an upwelling, which should be taken into consideration in future studies. This analysis advanced the understanding of the community structure and distribution patterns of the marine microbial communities in the under sampled north Pacific Ocean.

## Biotechnology and Ecotoxicology Culture Collection (LEGE-CC)- Cyanotoxins update

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Cyanobacteria are microorganisms that have remarkable adaptability and can inhabit various types of aquatic and terrestrial ecosystems worldwide including extreme environments. This group of organisms are considered a rich source of secondary metabolites with potential biotechnological applications and have the capability to produce some potent cyanotoxins that can induce consequences to human health. 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 than 1200 different cyanobacterial and microalgae strains. Until now, 36 strains were reported as producers of cyanotoxins distributed within different cyanobacterial orders. Cyanotoxins can be classified into five functional groups according to their primary target organ or effects being designated as hepatotoxins, neurotoxins, cytotoxins, dermatotoxins and irritant toxins. Recently LEGE-CC has increased in numbers due the isolation effort that has been made. In this work a screening of more than 200 cyanobacterial isolates from subaerial and freshwater environments targeting the genes involved in the cyanotoxins biosynthesis. As expected, genes involved in the production of the five toxins were detected by molecular biology tools. The strains where the genes were detected, were grown and sent to liquid chromatography–mass spectrometry (LC-MS) to confirm the production of cyanotoxins. As a culture collection, the screening of cyanotoxins is essential aspect of cyanobacterial research and provide a comprehensive idea of the production of these toxins for future works.

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## Potential of synthetic small molecules as inhibitors of SARS-CoV-2 cell entry

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Targeting viral attachment to the host cellular receptor is a therapeutic strategy to develop antivirals with broad-spectrum activity<sup>1</sup>, including for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) responsible for the current COVID-19 pandemic. The enormous potential of marine resources is a stimulus for the study of marine compounds as potential broad-spectrum antiviral agents targeting common cellular pathways<sup>2</sup>. For instance, griffithsin, a lectin isolated from a red alga, was shown to effectively inhibit SARS-CoV-2 infection, *in vitro*<sup>3</sup>. Thus, a library of approximately 300 small molecules, most of them inspired by compounds found in marine organisms, synthesized by “Grupo de Produtos Naturais e Química Medicinal (CIIMAR/FFUP)”, was studied *in silico* (AutoDock Vina) as competitors for two human host receptors of SARS-CoV-2: the receptor angiotensin-converting enzyme 2 (ACE2, PDB 6M17)<sup>4</sup> and the co-receptor cell surface glucose-regulated protein 78 (csGRP78, PDB 5E84)<sup>5</sup>. Approximately 40 compounds were found to bind to ACE2 and/or to csGRP78, with better or equal docking scores than the positive control used (ponatinib). From these promising compounds, 13 were selected for further evaluation of their effect on the expression levels of both cellular targets (ACE2 and csGRP78) in two human cell lines (MDA-MB-231 and A549). Four of these compounds reduced, and two of them increased, ACE2 expression by 20% (even though this was not statistically significant). Additional studies will be performed to verify if these compounds are competitors of SARS-CoV-2 for the ACE2 receptor, and to observe the effect of the compounds in the levels of SARS-CoV-2 subsequent cell infection.

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**Acknowledgments:** Foundation for Science and Technology within the scope of UIDB/04423/2020 and UIDP/04423/2020 (CIIMAR). FCT for Francisca Carvalhal PhD grant (Ref. 2020.07873.BD).



## Aminothioxanthone Derivatives as Potential Antifungal Agents

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Currently, fungal infections are one of the main causes of mortality and morbidity worldwide, and taking into consideration the increasing incidence of strains resistant to classical antifungal drugs, the development of new agents has become an urgent clinical need<sup>1,2</sup>. Considering that thioxanthenes are known for their anti-infective actions and bioisosteres of xanthenes, their scaffolds were selected for this work<sup>3,4</sup>. Using the broth microdilution method, three synthesized aminothioxanthenes were evaluated for *in vitro* antifungal activity against clinically relevant pathogenic fungi. The results showed that the tested compounds exhibited a broad-spectrum antifungal effect, including against fluconazole-resistant strains and (re)emerging species. Given the more significant antifungal potential presented, compound **1** was the subject of further investigations to study its anti-virulence activity and in an attempt to elucidate its mechanism of action. Compound **1** appears to act predominantly on the cell membrane of *C. albicans* ATCC 10231, altering its structural integrity, without interfering with ergosterol, while inhibiting significantly two crucial virulence factors - dimorphic transition and biofilm formation - frequently associated with *C. albicans* pathogenicity and resistance. In conclusion, the present work demonstrated the great potential of three aminothioxanthone derivatives as new models for antifungal agents.

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**Acknowledgements:** This research was supported by national funds through Foundation for Science and Technology (FCT) within the scope of UIDB/04423/2020, UIDP/04423/2020, and under the projects PTDC/SAU-PUB/28736/2017, EXPL/CTA-AMB/0810/2021, and PTDC/CTA-AMB/0853/2021, co-financed by COMPETE 2020, Portugal 2020 and the European Union through the ERDF and by FCT through national funds and by the structured program of R&D&I ATLANTIDA (reference NORTE-01-0145-FEDER-000040), supported by the North Portugal Regional Operational Programme (NORTE2020), through the ERDF.

## Optimization of BP-C4, a promising diarylpentanoid with antitumor activity

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*TP53* is one of the most relevant tumor suppressor genes, which is frequently mutated in human cancers. Therefore, in the last years, the search for small molecules able to reactivate the tumor suppressor p53 has received great attention as promising anticancer strategy.<sup>1</sup> Diarylpentanoids comprise a class of natural products possessing two aromatic rings linked by a five-carbon bridge, displaying promising antitumor activity.<sup>2</sup> Recently, our research group has shown that the antitumor activity of diarylpentanoid **BP-C4** can be related with activation of p53-dependent pathway.<sup>3</sup> The main purpose of this work was to explore molecular modifications of **BP-C4** to improve its potency and selectivity and to perform structure-activity relationship studies. Thus, a series of **BP-C4** analogs was design, synthesized, and tested for their growth inhibitory activity in colon cancer HCT116 cell lines expressing wild-type p53 (HCT116 p53<sup>+/+</sup>), and its p53-null isogenic derivative, in which p53 has been knocked out (HCT116 p53<sup>-/-</sup>). Results revealed that several of the tested compounds caused potent antiproliferative activity on HCT116 p53<sup>+/+</sup> cells (GI<sub>50</sub> < 10 µM). Compared to the hit compound, four compounds revealed higher antiproliferative activity than **BP-C4**, showing two of these compounds similar or increased selectivity for HCT116 p53<sup>+/+</sup> cells. The antiproliferative effect of compounds, whose results suggest a mechanism dependent of p53, was also tested in human fibroblasts HFF-1 cells in order to assess their cytotoxicity toward normal cells. Compounds **CTC-C4** and **BA-C4** proved to have similar or less cytotoxicity than **BP-C4**.

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**Acknowledgments:** This research was partially supported by the Strategic Funding UID/Multi/04423/2020, UID/QUI/50006/2020 (CIIMAR, Group of Natural Products and Medicinal Chemistry) and UIDB/50006/2020 (LAQV/REQUIMTE), through national funds provided by the FCT—Foundation for Science and Technology and European Regional Development Fund (ERDF), within the framework of the program PT2020. Joana Moreira and Joana Almeida acknowledges their grants (SFRH/BD/135852/2018 and 2020.05026.BD, respectively).

## Fischerazoles A-C, cyanobacterial polychlorinated lipids featuring fatty acyl chain rearrangement

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Cyanobacteria are rich in bioactive natural products with potential for biotechnological and pharmacological applications. A common feature of cyanobacterial secondary metabolites are fatty acid moieties. Our group has recently introduced a method for detection of novel fatty acid-containing compounds by supplementation of stable isotope-labeled fatty acids combined with comparative metabolomics. Here, this method revealed a new group of lipopeptides that we named fischerazoles A-C. These compounds had particularly interesting structural features such as extensive chlorination, a branched fatty acyl chain and two unusual termini. By mining the genome of the producing cyanobacterial strain PCC 9431 for halogenases, we detected a putative biosynthetic gene cluster (BGC). Bioinformatics and supplementation experiments with additional stable isotope-labeled precursors indicated a connection between the fischerazoles and the candidate BGC (*fsh*). Although the linear part of these compounds consists of only 15 carbons, to our surprise, all of the carbons in hexadecanoic acid (C16) were incorporated into the fischerazoles. This suggests that the carbon chain is rearranged to create the branched final structure. The enzymes involved in this unusual functionalization could be of interest for biocatalysis. These findings highlight how metabolomics-based methods can uncover highly novel chemical structures.

**Acknowledgments:** We acknowledge funding from the European Research Council through a Starting Grant (grant agreement 759840) to PNL. Parts of the work were supported by Fundação para a Ciência e a Tecnologia through grants PTDC/BIA-BQM/29710/2017, UIDB/04423/2021 and UIDP/04423/2021 and through scholarships to KA (SFRH/BD/146003/2019) and TPM (SFRH/BD/138308/2018). This project has also received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 952374 and by BLUEBIOLAB (0474\_BLUEBIOLAB\_1\_E), Programme INTERREG V A of Spain-Portugal (POCTEP).

## A cyanobacterial fractions library as the starting point for isolation of new bioactive compounds

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The process of isolation of new natural products from a biological collection is a very challenging and lengthy effort. The most traditional way of bioprospecting focuses on the screening of extracts followed by bioactivity-guided isolation which most often proves to be unsuccessful. To better explore the chemical variety of the cyanobacteria from the Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC), a new methodology was optimized for a swifter screening and selection of promising strains for the isolation of new bioactive compounds<sup>1</sup>.

This work will present the results of the creation of a cyanobacterial fractions library. Extracts from 64 strains of cyanobacteria were separated by HPLC, resulting in over 500 fractions for bioassay testing. This newly formed library was screened for its cytotoxicity using the HCT 116 human colon carcinoma cell line. Fractions that decreased cell viability were analyzed by LC-HRESI-MS/MS, followed by metabolomics studies using MZmine, GNPS and MetaboAnalyst. One strain with strong bioactivity and the other with a unique metabolomic profile presented masses with no dereplication and were selected for compound isolation.

Following mass spectrometry and bioactivity results, six novel compounds were isolated. Chemical structures were determined by NMR and MS/MS. Lusichelin A-E, five salicyl capped-thiazol(in)e siderophores, and phormidolide D, a new macrolide-type compound, presented bioactivity against the HCT 116 cell line as well as reversers of P-gp efflux activity. Using this library successfully led to the isolation of new bioactive compounds, highlighting its potential for natural products discovery.

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## Cosmetics for sensitive skin: presence of allergens

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According to epidemiological studies, individuals with sensitive skin are more prone to develop skin allergies.<sup>1</sup> Therefore, the presence of allergens in cosmetic products may induce skin sensitization more often in these individuals, even though skin allergy and sensitivity have a distinct pathophysiology. Herein, we report the assessment of the presence of allergens in facial-skin cosmetic products for sensitive skin.<sup>2</sup>

A pool of 88 cosmetic products from international brands commercially available in pharmacies and parapharmacies was analyzed regarding the occurrence of allergens, and those were compiled and grouped according to their function. The most common allergens were fragrances, being linalool, benzyl alcohol, geraniol, and citronellol the most prevalent, followed by skin-conditioning agents, surfactants, and preservatives. Fragrance allergens were present in 7% of the analyzed products and other allergens were found in almost all the cosmetics (95%). Overall, allergens were present mainly in combinations. In conclusion, most cosmetic formulations were absent of fragrance allergens. Regarding other allergens, they were present in most products, but the majority were considered rare skin sensitizers with few cases of skin sensitization reported in literature. This work highlights the importance of reading labels prior to using cosmetic products, especially by susceptible consumers, in order to avoid and recognize skin allergy.

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## Enantioresolution of promethazine and its metabolites for metabolic studies

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The hazards associated to an inappropriate use of pharmaceutical products at a global level are of great concern. The risks are considered even greater when these substances are chiral and marketed as racemate. Chirality implies that enantiomers may have different behaviors in terms of pharmacodynamics, pharmacokinetics and/or toxicity [1].

Promethazine (PMZ), a chiral antihistaminic drug marketed as racemate, has been an example of the inappropriate use of pharmaceuticals because in high doses it may causes severe toxicity effects [2]. The famous hallucinogenic drink "Purple Drank" that combines codeine and/or PMZ with soda, has been associated to serious health consequences and fatalities. However, the information about the enantioselectivity in toxicity of PMZ and its metabolites is scarce [2,3].

The aim of this work is to present a new enantioselective analytical method for monitoring the PMZ and its metabolites for further *in vitro* metabolic studies. For that, the enantioseparation was evaluated in five different chiral analytical columns with amylose and cellulose carbamates as chiral selectors in normal and reverse elution mode. Amylose derivative showed good enantioselective and resolution for all target compounds with the same chromatographic conditions.

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**Acknowledgments:** Foundation for Science and Technology through the projects UIDB/04423/2020 and UIDP/04423/2020 (Group of Natural Products and Medicinal Chemistry - CIIMAR) and European Regional Development Fund (ERDF), through the COMPETE –POFC program in the framework of the program PT2020. Maria Miguel Coelho acknowledges her PhD grant provided by FCT (SFRH/BD/146999/2019).

## Synthesis of fumiquinazoline derivatives, assessment of their antibacterial activity and potential as efflux pump inhibitors

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Drug resistance has reached alarming levels, posing a threat to public health worldwide.<sup>1</sup> For this reason, the development of novel antimicrobial agents is a priority in the current society, especially of compounds capable of overcoming common resistance mechanisms such as the overexpression of efflux pumps.<sup>2</sup> Previous work from our group has shown that fumiquinazoline derivatives can exhibit promising antibacterial activity.<sup>3</sup> Herein, we present the multi-step synthesis<sup>4</sup> and structure elucidation of a small library of fumiquinazoline alkaloids comprising several new compounds. To assess the antibacterial activity, the minimum inhibitory concentration of the compounds was determined against relevant bacterial species and a preliminary synergy study with standard antibiotics was performed; several compounds were active against the tested Gram-positive bacteria, and additionally, were also screened for their potential as efflux pump inhibitors as well as for their anti-biofilm capacity, with some of the alkaloids showing promising results.

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**Acknowledgments:** This research was supported by national funds through FCT (Foundation for Science and Technology) within the scope of Base Funding UIDB/04423/2020 and UIDP/04423/2020 (CIIMAR, Group of Natural Products and Medicinal Chemistry), projects EXPL/CTA-AMB/0810/2021, PTDC/CTA-AMB/0853/2021 and CHIRALBIO ACTIVE-PI-3RL-IINFACETS-2019 and by the Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement and through the ERDF, as a result of the project ATLANTIDA (reference NORTE-01-0145-FEDER-000040). Mariana Almeida acknowledges the BYT+ 2020/2021 scholarship to CIIMAR and the PhD grant (2021.05224.BD) to FCT.



## Biodiversity of Sponge-Associated Actinobacteria from Southern Mozambique and Northern Portugal

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Actinobacteria are commonly found on the rich microbiome of marine sponges (Phylum Porifera). Different species of these microorganisms have been shown to be prolific sources of molecules with a wide range of bioactivities. The objective of this work was to isolate and phylogenetically identify sponge-associated Actinobacteria, aiming at the future bioprospection of these isolates for novel bioactive compounds. Six marine sponges were collected in the island of Inhaca, Mozambique, and identified as *Haliclona* sp. (n=2), *Psammocinia* sp. (n=3) and *Callyspongia perforata* (n=1). Three sponges, collected off-coast Ovar, Portugal, were identified as *Cliona* cf. *celata* (n=1), *Axinella* sp. (n=1) and *Desmacidon fruticosum* (n=1). Over 100 strains of Actinobacteria were recovered from these sponges. Eighteen genera of Actinobacteria were identified, among which the most predominant were *Micromonospora* and *Streptomyces* that are known already for being prolific producers of bioactive metabolites. These two genera were also the most diverse regarding the number of species identified. In addition, several species of rare and particularly unexplored Actinobacteria genera were recovered, such as *Micrococcus*, *Brachybacterium*, *Kocuria*, *Dietzia*, *Williamsia*, *Rothia*, *Nesterenkonia* and *Kytococcus*, among others. Potential new species of *Mycolicibacterium* and *Microbacterium* have been isolated as well. In conclusion, a high number of isolates and a great variety of Actinobacteria were obtained from the marine sponges analyzed. These isolates constitute a promising source for novel bioactive secondary metabolites.

**Acknowledgments:** Mariana Oliveira would like to thank the Blue Young Talent program and the “Fundação para a Ciência e Tecnologia” (FCT) for their financial support through the doctoral grant reference UI/BD/150904/2021. This work was supported by the project “ATLANTIDA - Platform for the monitoring of the North Atlantic ocean and tools for the sustainable exploitation of the marine resources”, RL4- Marine biobanks as tools for marine biotechnology NORTE-01-0145-FEDER-000040, EP1 - Investigação, Desenvolvimento Tecnológico e Inovação), funded by Fundo Europeu de Desenvolvimento Regional (FEDER) through NORTE 2020, and FCT/MCTES by national funds through FCT within the scope of UIDB/04423/2020 and UIDP/04423/2020.

## Search for antiviral compounds from cyanobacteria with potential application in aquaculture

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Aquaculture suffers high economic losses due to pathogens, among which viruses are one of the most destructive. However, the reduced number of therapeutic substances and the high costs of chemotherapeutic agents lead to an urge to find solutions to this thread. The aim of this work is to find cyanobacterial bioactive compounds with antiviral potential that represent new sustainable antiviral solutions. Therefore, 10 cyanobacterial MeOH extracts were prepared and their antiviral potential was tested *in vitro* and *in vivo*. *In vitro* assays were performed with epithelial carp cells and viral hemorrhagic septicemia virus (VHSV), assessing extracts toxicity, virucidal activity and antiviral potential in different stages of the viral cycle. *In vivo* assays were performed with zebra fish embryo and larvae and spring viraemia of carp virus (SVCV), assessing extracts toxicity, antiviral potential and immune response in infected organisms. Five extracts that showed promising activity were fractionated using a reverse phase HPLC system, and tested for the same parameters. Only 4 fractions from 2 different strains retain the activity, and thus show potential for compound isolation with aquaculture applications. The isolation will be guided by bioactivity and mass spectroscopy data.

**Acknowledgments:** This research was developed under CIIMAR strategic funds UIDB/04423/2020 and UIDP/04423/2020, in the framework of the project BLUEBIOLAB (Proyecto cofinanciado por el Fondo Europeo de Desarrollo Regional FEDER en el marco del programa Interreg V A España – Portugal (POCTEP) 2014-2020).

## Microbial communities in fishing nets: can they harbor biotechnological potential?

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Plastic fishing gears represent a source of marine pollution once lost at sea. These gears serve as a surface for the development of biofilms with distinct microbial communities. To investigate the diversity and structure of these communities, a one-year *in situ* experiment was assembled within a recreational marine (Leixões), where 3 types of fishing nets (Net A: Braided Polyethylene (PE), Net B: Braided Nylon, Net C: Thin Nylon) were submersed in the water. Pieces of each net and surrounding seawater were sampled along time, for metabarcoding microbial community analysis by Next Generation Sequencing (NGS). Additional net samples were collected for bacterial strain isolation, after the first month of *in situ* experiment. Microbial communities attached to the nets (biofilms) were more diverse than those in seawater samples. These communities also varied in terms of structure, as seen by beta-diversity analysis. Proteobacteria, Bacteroidetes and Verrucomicrobiota were amongst the most abundant phyla within biofilms. Groups with plastic-degrading potential, such as the family *Sphingomonadaceae* and the genus *Erythrobacter* were also found (> 2% abundance). A total of 123 bacterial strains distributed by 46 bacterial genera were isolated from the 3 nets biofilms. Bacterial strains belonging to the genera *Acinetobacter*, *Bacillus*, *Rhodococcus*, *Shewanella*, *Streptomyces* and *Vibrio* were common to all nets. Furthermore, it was also possible to cultivate in the lab some bacterial genera associated with plastic degradation, such as *Erythrobacter*, *Exiguobacterium* and *Bacillus*. Future work should address the biotechnological potential of each strain for bioremediation of pollutants, such as hydrocarbons and plastics.

**Acknowledgments:** Rafaela Perdigão was supported by FCT (PhD grant 2020.04689.BD). This work was partially supported by the NETTAG project (EASME/EMFF/2017/1.2.1.12/S2/02/S12.789121) EASME-EMFF funding programme, and by projects Ocean3R (NORTE-01-0145-FEDER-000064) and ATLANTIDA (ref. NORTE-01-0145-FEDER-000040) supported by the North Portugal Regional Operational Programme (NORTE2020), under the PORTUGAL 2020 Partnership Agreement and through the European Regional Development Fund (ERDF).

## Investigation of the DGT fractionation technique to assess the impact of metals in bioreactors

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Anaerobic digestion is a process by which organic waste is broken down by microorganisms in the absence of oxygen, converting it to products such as biogas, a source of renewable energy, and digestate that can be used as fertilizer.

Some metals, such as cobalt and nickel, are considered essential for the production of biogas, since these are present in enzymes and coenzymes that are involved in the metabolism steps and biochemical reactions of anaerobic digestion. However, when present at high concentration in bioreactors, these elements and other metals have been shown to have inhibitory effects on biogas production.

In this present research project, the relationship between metals and anaerobic digestion is being studied. The aim is to determine chemical indicators that can help to anticipate metals impact on biogas production. The total metal in the reactor is not bioavailable to the microorganisms and metal fractionation is therefore needed. For this purpose, the diffusive gradients in thin films technique (DGT), a passive sampling technique is investigated. It allows to determine the labile metal fraction (free metal ions and weakly complexed metals), that could reflect the bioavailable metal.

To study biological activities, a biochemical methane potential (BMP) experiment was devised to have a lab-scale model of anaerobic digestion, inoculated with municipal sewage sludge, and using sucrose and peptone mixture as a substrate.

From this set-up we were able to determine biogas production, enzymatic activities, and their respective responses to the metal spiking of the BMPs. The correlation between these biological responses and the labile metal fraction estimated with DGT are investigated.

## Marine cyclic peptides with antimicrobial activity: synthesis and structure elucidation

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In recent years, marine cyclic peptides have aroused great interest due to their wide range of biological and pharmacological activities.<sup>1</sup> Among them, interesting antimicrobial activities were observed against numerous drug-resistant bacteria and fungi, making these compounds a very promising resource in the search for novel antimicrobial agents.<sup>2</sup> Unnarmicin A and C, isolated from the fermentation broth of a marine bacterium *Photobacterium* sp., are examples of cyclic depsipeptides with promising action as efflux pump inhibitors to reverse antimicrobial activity resistance against several pathogens including *Candida albicans*.<sup>3</sup> Marine cyclic peptides are also interesting models for molecular modifications and/or total synthesis to obtain more potent compounds, with improved properties and in high quantity.<sup>4</sup> One of the aims of our group is the total synthesis of marine-derived peptides and analogues for further characterization of their antimicrobial effects. Herein, we describe the synthetic strategy regarding the total synthesis of marine cyclic peptides. Coupling reactions and protection/deprotection BOC/methyl methodologies were applied. The structures of the synthesized compounds were established by IR and NMR spectral analysis. Further studies will include the investigation of the antimicrobial activities of the synthetic derivatives.

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**Acknowledgements:** Ricardo Ribeiro acknowledgment his PhD, BYTPhD program by CIIMAR, grant provided by FCT UI/BD/150912/2021.



## The effect of particles size in the cytotoxicity of titanium oxide and silver nanoparticles

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In the age of nanobiotechnology, silver (Ag) and titanium dioxide (TiO<sub>2</sub>) nanoparticles (NPs) are two of the most widely used engineered nanomaterials. Both nanoparticles being equally prized for their antimicrobial activity amongst other properties (photoactivity in TiO<sub>2</sub>NPs), have begun to be extensively used in the pharmaceutical, food, biomedical as well as cosmetic industries. AgNPs and TiO<sub>2</sub> NPs are generally presented to be non-toxic to human cells however new studies prove otherwise. Therefore, the application of these particles in daily life directly or indirectly raises concerns about their role in public health and safety. Several characteristics affect their potential toxicity namely size, concentration, coating, shape. In this work, we will be assessing specifically the effect of the size of these nanoparticles in exhibiting cytotoxicity.

HepG2 cells have been used in the study because after entering the human body, NPs accumulate in the liver and affect hepatocytes more than any other type of cells. The cells used were exposed to 0.8 to 100 ppm of AgNPs of sizes 15 nm and 100 nm and also to 0.8 to 100 ppm of TiO<sub>2</sub>NPs of sizes 5 nm, 25 nm and 45 nm as well as their respective coatings alone for 48 hours. After exposure, cell viability was measured with MTT assay where absorbance reading was taken at 570 nm wavelength of light.

The results show that AgNP of 100 nm have a concentration-dependent effect on HepG2 wherein AgNP of higher concentrations seem to cause increased cell death. AgNPs of 10 nm are expected to show the same results but with a resulting cell viability that is lesser than its larger counterpart. On the other hand, TiO<sub>2</sub> NPs of 45 nm seem to show no significant cytotoxicity but their smaller counter parts i.e 25 nm and 5 nm are expected to show comparatively higher cell death. The results of the study will allow to understand the difference in the degree of cytotoxicity induced by AgNPs and TiO<sub>2</sub>NPs of different sizes which will contribute to finding appropriate dimensions for applications in the relevant industries.

**Acknowledgments:** This work is funded by NANOCULTURE Project – INTERREG Atlantic Area Program (European Regional Development Fund, ERDF) – EAPA 590/2018.

## **Zebrafish as a screening tool to evaluate Cyanobacteria bioactivity on the Diabetes disease**

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Diabetes is a metabolic disease with a high incidence worldwide, already being considered a pandemic by the WHO. Diabetes is mainly characterized by the increase of blood glucose in consequence of insulin regulation failure. Even if some treatment options are available, new anti-diabetic molecules would be helpful to fight diabetes. Our group hosts a collection of more than 1000 strains of cyanobacteria and microalgae, being an important source of new compounds for bioactivity screenings.

In this work, 19 cyanobacteria strains were grown and then chemically fractionated to obtain a library of 182 fractions that were screened for their anti-diabetic potential. The 2-NBDG assay was used for bioactivity screening with 3DPF zebrafish embryos, which is based on the uptake of a fluorescent analogue of glucose, and searches for “insulin mimetic” compounds increasing the uptake of glucose by the embryos. The screening revealed two promising hits that were further analysed for its produced metabolites and effects on glucose transporters and target gene expression. Metabolite profiling was performed by LC-MS, using the GNPS platform, and several masses were identified that are either unique or at a higher concentration on the active fractions compared to non-active phylogenetically similar strains. The majority was still unknown, but some major clusters of masses related to polyprenols and hydroxycinnamic acids. A phylogenetic analysis demonstrated that glucose transporters (GLUT) had genetic similarities between Zebrafish and Human, and all isoforms clustered together. Zebrafish were exposed to positive fractions and GLUT1, GLUT3 and GLUT4 expression was analysed by western blot, and a statistically significant increase was identified on the expression of GLUT3. Currently, the mRNA expression of preproinsulin a, phosphoenolpyruvate carboxykinase, and glucose transporter 2 are being evaluated. In summary, our zebrafish model reveals promising anti-diabetic activity of still unknown cyanobacterial metabolites.

**Acknowledgments:** This work was supported by the FCT grant SFRH/BD/139131/2018 financed by national funds through FCT (Foundation for Science and Technology, Portugal), and by the project ATLANTIDA (ref. NORTE-01-0145-FEDER-000040), financed by the Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement and through the European Regional Development Fund (ERDF).





## SESSION 4 AQUACULTURE AND AQUATIC RESOURCES

*MODERATION*

*AIRES DUARTE AND SERGIO BOO*

## KEYNOTE SESSION – MANUEL MANCHADO



### **Recent advances and challenges in Senegalese sole aquaculture**

Dr Manuel Manchado is a staff tenure researcher at IFAPA (Junta de Andalucía, Spain) and leads the Genomic and Genetic group at center IFAPA El Toruño. Currently, he coordinates the scientific area for Aquaculture and Marine Resources at IFAPA and participates as advisor in the Andalusian committee for aquaculture. He is also representative in the Campus of Excellence in Marine Sciences (CEI·MAR) and participates in Atlantic and Mediterraneans alliances for blue bioeconomy. Scientific productivity includes more than 150 indexed papers in the fields of genetics, genomics and health in aquaculture. He keeps collaborations with several groups across Europe and has coordinated and participated several european, national and regional competitive R+D+i projects and international cooperation. He has supervised more than ten doctoral thesis and act as reviewer of projects for different agencies and journals. He maintains tight collaborations with European companies for genetic selection and development new marine byproducts. Recently, two products were recognized by UE as highly innovative and included in the European platform for novel highly innovative products.



**ABSTRACTS**

**ORAL COMMUNICATIONS**

## Can sardine cooking wastewaters extracts modulate European seabass feed intake?

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This work aimed to assess the efficacy of aromatic mixtures extracted from sardine cooking wastewaters, a by-product of the canning industry, as feed intake stimulants, in highly vegetable diets for European seabass (*D. labrax*), which have low palatability and intake. Sardine cooking waters were either used directly (CW) or after processing by vacuum condensation (VC) or liquid/liquid extraction with soybean oil (LLE). The chemical profile of the extracts differed, but the most abundant compound identified in all extracts was the 1-penten-3-ol, hence selected as marker and included at 2 µg/g in plant protein-based diets. Thus, four diets (3 aroma samples and a non-supplemented control) were produced and assigned to triplicate groups of fish (initial weight 95.7±13.5 g), hand-fed twice daily, until apparent satiation, in a recirculating saltwater system, at 21 °C. After 18 weeks, growth performance and nutrient utilization were evaluated. A sensory analysis with a consumer panel was also performed. Fish fed LLE had a significantly higher voluntary feed intake than those fed CW, although neither differed from the control. LLE also resulted in increased feed conversion ratio, but final weight, daily growth rate, whole-body composition, and nutrient gain remained similar among diets. During sensory analysis, the panel could not perceive any changes in the samples' taste or odor, characterizing all treatments as having a "characteristic fish" and "soft" odor/taste. Moreover, global liking of samples was similar among treatments, being all generally well accepted. Overall, aromas can modulate feed intake, but further optimization of either processing and/or incorporation levels is required to potentiate their effectiveness on fish growth, as well as an analysis of their impact on mechanisms involved in the central regulation of appetite in fish.

**Acknowledgments:** Work supported by Project MOBFOOD POCI-01-0247-FEDER-024524•LISBOA-01-0247-FEDER-024524, cofounded by PORTUGAL2020, Lisb@a2020, COMPETE 2020 and the EU. DR thanks FCT, SANFEED and Sense Test© for her PhD grant (PD/BDE/150524/2019). CIIMAR and GreenUPorto acknowledge FCT funds (UIDB/04423/2020, UIDP/04423/2020, UIDB/05748/2020 and UIDP/05748/2020).

## Including pineapple byproducts in aquafeeds for modulating feed antioxidant potential and European seabass stress-resistance

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The fruit industry annually discards tons of peels rich in natural antioxidants, i.e. polyphenols, vitamins and carotenoids. Including these byproducts in aquafeeds might help reduce fish oxidative stress and delaying feed/flesh oxidation during storage, while meeting consumer preferences for natural products. In a previous study, we addressed natural antioxidants in aquafeeds and how manufacturing temperature impacts the preservation of these compounds. Surprisingly, results revealed that conventionally hot extrusion (110°C) followed by quick drying at 60°C better retain antioxidant properties compared to lower processing temperatures (25°C and 35°C, respectively). Moreover, inclusion of 2% pineapple peel flour improved antioxidant potential of resulting diets. Thus, a new study was designed for evaluating the impact of pineapple flours on fish growth performance and stress response. Four isolipidic, isoproteic and isoenergetic diets were developed (110°C extrusion; 60°C drying): a commercial-based diet (Control, CTRL), CTRL supplemented with 100 mg/kg of Vitamin E (VITE), and two diets where 2% of flour from either pineapple peels or stems were added to VITE (PP and PS diets respectively). Flours and diets were analyzed for proximate composition, free/bound polyphenols, carotenoids, TBARS, DPPH, ABTS and ORAC. Also, diets were stored in vacuum for 3 months, at 4 and 24°C, and analyses were replicated for evaluation of antioxidant content/potential over time.

Diets were assigned in quadruplicate to homogeneous groups of 17 fish (IBW = 13.5 ± 0.8g) in 50L tanks. Fish were fed to satiety with automatic feeders until tripling their initial weight. Post-trial, all fish were weighed and measured. 20 fish p/treatment were sampled for proximate composition. Additionally, 16 fish p/treatment were either immediately sampled or exposed to confinement stress (5 min; 100 kg/m<sup>3</sup>) followed by air exposure (1 min), replicating capture in an aquaculture scenario. Non-stressed and stressed fish will be analyzed for immunological status, blood stress biomarkers, liver antioxidant enzymes and TBARS, and muscle TBARS, DPPH, ABTS and ORAC. Results will allow us fully assess the potential of these fruit flours as additional sources of natural antioxidants in aquafeeds.

**Acknowledgments:** work funded by project ATLANTIDA (NORTE-01-0145-FEDER-000040), supported by NORTE2020, under PORTUGAL2020 and through ERDF, and the FCT project UID/Multi/50016/2020. RP was financed by FCT (SFRH/BD/144631/2019)

## Investigating microplastic contamination in farmed European seabass and its implications for human consumers

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Microplastics (MPs) are a growing concern in global food safety. The MP presence in commercial fish tissues has been previously reported, potentially leading to higher MP exposure risk for consumers. Currently, aquaculture produces over 50% of fish for human consumption, and relies on diverse production systems. Recirculation aquaculture systems (RAS) produce fish under constantly controlled conditions in almost closed circuits where plastic gear (e.g., tanks, pipes, filters) are used. These components can contaminate RAS, as well as aquafeed, water and the surrounding atmosphere, becoming available for fish to uptake and bioaccumulate. Plastic-suspect particles were extracted from dorsal muscle samples of 55 specimens of European seabass (fish weight: 122.0±0.7g). Items were catalogued by shape, colour and size. Then, 50% of the items were chemically characterized by micro-Fourier Transform Infrared Spectroscopy (μFTIR). The remaining items were compared with MPs previously identified by μFTIR analyses. A total of 124 particles were recovered from dorsal muscle samples. Most of them were blue and black, ranging from 34-2181μm. Natural/synthetic cellulose (43%) and polyethylene terephthalate (16%) fibres, and polyvinyl chloride (6%) fragments were the most common particle type. The total mean of MPs in muscle samples was 0.4±0.3 MP items/g, thus similar to the concentration found in dorsal muscle samples of wild seabass from the NE Atlantic Ocean [1]. Based on EFSA recommendations on fish consumption by adults, namely 300 g/week [2], a human exposure of 5616 MP/year from RAS-farmed seabass fillet consumption was estimated. Reported MP levels correspond well to those found in wild specimens, predominant polymer types seem to differ. The practical implication of these findings warrants further studies.

1. Barboza L.G.A., et al. (2020) Microplastics in wild fish from North East Atlantic Ocean and its potential for causing neurotoxic effects, lipid oxidative damage, and human health risks associated with ingestion exposure. *Sci. Tot. Environ.* 717: 134625.

2.EFSA (2014) Scientific Opinion on health benefits of seafood (fish and shellfish) consumption in relation to health risks associated with exposure to methylmercury. *EFSA J.* 12 (7), p. 3761

**Acknowledgements:** Work funded by ATLANTIDA (NORTE-01-0145-FEDER-000040), supported by the Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement and through the European Regional Development Fund (ERDF).

## FINAL REMARKS

We, the PhD students' committee of CIIMAR, would like to thank all the participants of the Blue Think Conference 2022. A special thank you goes to Professor Vitor Vasconcelos, and our keynote speakers, Professor Josefin Stiller, Doctor Flora Julie Vincent, Professor Elke Dittmann and Doctor Manuel Manchado who all kindly accepted our invitation to share their experience and expertise at this conference. We greatly appreciate the work of the Scientific Committee during the organization of the Blue Think Conference 2022 and would also like to thank the communication office of CIIMAR who helped us reach so many people.

Since the creation of the PhD students' Committee, we had the objective to organize an in-person conference to promote a more approachable scientific interaction between the CIIMAR community. At last, this year we accomplished this dream, and gathered everyone in CIIMAR's headquarters. Once again, the conference exceeded all our expectations with 67 abstract submissions, 152 registrations and more sponsorship collaborations from CIIMAR, APDL, Chaperone, Câmara Municipal de Matosinhos, Stabvida, Santander, Sotocal and Conservas Pinhais!

We hope next year the Blue Think Conference can grow even further and at the same time will keep bringing us closer together, allowing us to share knowledge and human connections.

Thank you for attending,  
CIIMAR's PhD Students' Committee



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