TO APPLY FOR
FCT 2025 CALL
FOR PHD STUDENTSHIPS

PHD RESEARCH PLAN PROPOSAL

CIIMAR

2025

In ovo immune stimulation—A tool to improve fish larvae welfare

SUMMARY
The production of high-quality juveniles is still a bottleneck in the farming of numerous fish species. Survival until the juvenile stage is typically as low as 10-15% for many species. Both scientific evidence and experience in hatcheries for a variety of fish, support the hypothesis that detrimental fish–microbe interactions are the cause of these problems. Bacterial outbreaks are most commonly treated with antibiotics, but their use has become strongly regulated in many countries due to the onset and transfer of resistance mechanisms among bacteria species as well as to the bioaccumulation/biomagnification and the uncertainty of long-term effects to the environment. Intensive cultivation conditions and climate change effects (rising temperature, hypoxia, salinity and acidification) may also induce stress, and thus depress larvae fish immune system. In many important cultured species such as the sea bream and sea bass, maturation of lymphomyeloid organs is delayed. Synthesis of specific antibodies is lagged until several weeks after hatching. Therefore, vaccination is not an option at larval stages. Among the recommended strategies to prevent these health problems in larval cultures is the induction of larvae innate immune capacity. In view of this, the major objective of this project is to evaluate the potential of stimulating the developing embryo immune system at the earliest possible point in time as a strategy to reduce fish larvae mortality. Therefore, we propose to explore a new and exciting approach; the delivery of immunostimulants while embryos are still inside the egg by a bath immersion. By using a bath immersion instead of microinjection, administration of the immunostimulant can be scaled up to simultaneously treat many eggs/embryos in a highly versatile and potentially cost-effective manner suitable for its application in the industry.

MAIN METHODOLOGIES
This project will start with in vitro cell culture assays in which the candidate, working closely with the PI, will identify the best potential candidate to serve as immunostimulant. To overcome the barriers that any compound faces before being taken up by the embryo, we will use a molecular transporter effective to penetrate the egg chorion. The chosen immunostimulant will be chemically modified to incorporate both the molecular transporter and a fluorescence group, making real-time monitoring of immunostimulant uptake by eggs/embryos achievable immediately after immersion treatment by fluorescence microscopy. Administration of the immunostimulants can be done to eggs (pre-fertilization) or embryos (post-fertilization), depending on the characteristics of the eggs and embryos in different fish species. Conditions during egg incubation will be optimized for zebrafish, rainbow trout and sea bass eggs. The PhD candidate will develop his/her work mostly in the wet lab and will receive one-to-one training in basic cell culture techniques, biochemical techniques and gene expression techniques essential for evaluating the effect of the immunostimulants both in vitro and at the embryo level. The candidate will also have hands-on training in fluorescence microscopy and gain technical skills in metabolism and biotransformation (MALDI-TOF), bacterial challenge tests for fish larvae and high resolution in situ spatial genomics. The candidate should be receptive to travel abroad, as this project may include research stays at partners institutions to develop specific tasks. The supervisor will engage the candidate in scientific activities and stimulate acquisition of important knowledge, skills and competencies raising the candidate scientific independence and confidence.

MAIN SUPERVISOR
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CO
Benjamín Costas/ A2S
Gregorio Molés/ A2S

PLACE OF WORK
CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, Terminal de Cruzeiros do Porto de Leixões

WILL THE PROPOSAL RESEARCH IDEA BE FUNDED BY A SPECIFIC PROJECT?
YES, DELIVER2EGG (OPERAÇÃO N.º 16725 NO BALCÃO DOS FUNDOS COMPETE2030-FEDER- 00790700)

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"Bridging the Future: Comprehensive Strategies for Scour Risk Mitigation Under Climate Change"

SUMMARY

Bridges face constant natural hazards, with scour around foundations, worsened by storms and floods, being a leading cause of collapse. In the U.S., scour accounted for 60% of bridge failures over 30 years, costing $50 million annually. Notable incidents, like the Schoharie Creek and Hintze-Ribeiro bridge collapses, highlight the critical need to address scour risks through improved design and monitoring to prevent such costly and severe failures. The proposed proposal aims to address the multifaceted challenges of bridge scour through a comprehensive five-step approach: (i) Climate change impacts: Assessing the potential impacts of climate change on bridges, including changes in precipitation, temperature and hydrological processes that lead to increased vulnerability to scour; (ii) Soft computing models: Moving from traditional deterministic approaches to exploring the probabilistic nature of scour variables through advanced soft computing models such as Artificial Neural Networks (ANNs), Adaptive Neuro-Fuzzy Inference Systems (ANFIS) and Support Vector Machines (SVMs); (iii) Advanced AI modelling and reliability analysis: Integrate advanced AI modelling and reliability analysis into the risk-based methodology. Using climate prediction approaches to improve the accuracy of assessing the impact of climate change on scour risk, with a focus on improving predictive capabilities and model reliability; (iv) Risk-based methodology and reliability analysis: Develop a robust risk-based methodology, which is critical for effective risk management and prevention of catastrophic consequences. Incorporate reliability analysis techniques to quantify uncertainties and prioritise bridges based on scour susceptibility and climate change resilience; and (v) Data requirements and numerical modelling: The importance of detailed scour hole morphology data and the use of Computational Fluid Dynamics (CFD) tools for numerical modelling is emphasised. These components contribute to a comprehensive approach to predicting scour at bridge foundations and provide a valuable database for model calibration and validation.

MAIN METHODOLOGIES

By prioritising climate change resilience and using state-of-the-art modelling techniques, this project aims to contribute to the long-term management of bridges and mitigate the potentially catastrophic consequences of scour-related events by combining innovative techniques and advanced technologies for a holistic risk management strategy.

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MAIN SUPERVISOR

Ana Margarida Bento/Marine Energy and Hydraulic Structures

PLACE OF WORK

Faculty of Engineering of the University of Porto (FEUP)

WILL THE PROPOSAL RESEARCH IDEA BE FUNDED BY A SPECIFIC PROJECT? NO

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Lipid Metabolism in Marine Mammals: Unique Adaptations and Conservation Challenges

SUMMARY
Marine mammals (MM), such as cetaceans and pinnipeds, have evolved a range of specialized adaptations to thrive in environments so distinct from those of their terrestrial ancestors. Among the most striking of these adaptations is the accumulation of large amounts of fat tissue, such as blubber, which serves essential functions including buoyancy, thermal insulation, and energy storage. Previous research has identified positive selection, gene loss, and specific genetic adaptations in MM genes, particularly those encoding enzymes involved in lipid metabolism (e.g.1). These findings highlight the distinctive metabolic traits of marine mammals. However, compared to terrestrial mammals, our understanding of their physiology and lipid metabolism remains limited. This project aims to address this knowledge gap by investigating and characterizing the distinctive features of lipid metabolism and its regulation in MM. Such insights are crucial for understanding MM metabolic needs, dietary habits, and foraging ecology. More importantly, this knowledge will contribute to identifying environmental threats and shaping effective conservation policies. Firstly, we will address the effect of environmental pollutants known to interfere with lipid metabolism namely obesogens (chemicals that trigger adverse effects in lipid homeostasis) to accurately assess the danger posed by these compounds (e.g. TBT, Dechlorane plus, Methoxychlor, PFAs). This task will be carried out at CIIMAR using in vitro transactivation's assays, a well, established procedure in which the supervising team has expertise (e.g 2, 3). Secondly, we will investigate the endogenous lipid metabolism by experimentally characterizing several enzymes involved in Long Chain Polyunsaturated fatty acids (LCPUFAs) biosynthesis (e.g. 4, 5). LCPUFAS play a vital role in maintaining membrane fluidity and functionality at lower or artic temperatures and signaling cascades among others.

MAIN METHODOLOGIES
Overall, the applicant is expected to have a general and solid background in Biological Sciences, with basic knowledge in molecular (PCR, cloning, sequencing) and cellular biology (cell culture). The candidate should be receptive to travel abroad, as this project may include one research stay at a partner institution to develop a specific task and for the participation and dissemination of results in international conferences. This project will start with an in silico approach in which the candidate will work closely with the PI to select relevant nuclear receptors (PPAR, RXR etc) and emerging chemical pollutants for in vitro transactivation assays. During this period the candidate will receive one-to-one training to identify target genes encoding for nuclear receptors and other significant enzymes involved in lipid metabolism (ELOVL, FADS etc). At the end of this task the candidate will be able to easily navigate public genomic databases (e.g. NCBI and Ensembl) and correctly identify target genes and extract corresponding sequences for gene synthesis, which will be used in the experimental assays. Next step will be wet lab and hands-on training in basic molecular biology techniques skills required to prepare and transfer synthesized genes to expression vectors, for both the transactivation assays and heterologous yeast expression. This will be followed by training in basic mammalian cell culture techniques, experimental design and transactivation assays.Finally, the PI will actively seek to involve the candidate in other scientific activities and stimulate the development of important skills boosting their scientific independence and confidence. These activities may include journal club, public speaking, networking in conferences, organization of conferences, mentoring of undergraduates, writing scientific manuscripts, revision of manuscripts, grant writing and science communication to the lay population.

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PLACE OF WORK
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WILL THE PROPOSAL RESEARCH IDEA BE FUNDED BY A SPECIFIC PROJECT? NO

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The role of Hybrid Nature-Based Solutions on Biodiversity Enhancement and Emerging Contaminant Mitigation

SUMMARY

Aquatic ecosystems are increasingly threatened by anthropogenic pressures, including persistent emerging contaminants (ECs) like pesticides, pharmaceuticals, and PFAS. These micropollutants pose significant risks to non-target species, disrupting aquatic ecosystem structure and function. Conventional wastewater treatment plants are ineffective in removing certain ECs, highlighting the urgency for innovative, cost-effective, and environmentally sustainable solutions. NATUREBIOPROMO project was approved under Biodiversa+ call to address these challenges. This project aligns with key international policies, including the EU Water Framework Directive and the Sustainable Development Goals (SDG 6, 7 and 15). The proposed PhD project will address this challenge through 1) the integration of hybrid Nature-Based Solutions (NBS)—such as constructed wetlands (CWs), artificial floating islands (AFIs), and microalgae ponds—under real conditions to enhance pollutant and nutrient removal efficiency. It also emphasizes 2) the dual role of NBS in wastewater treatment and biodiversity conservation, supporting biodiversity-oriented management aligned with the EU's Green Deal and Biodiversity Strategy for 2030. Moreover, it will 3) explore the valorisation of wetland biomass for bioenergy (biogas), fostering a circular economy model. The main objectives include: O1: Assessment of real-scale spatial and temporal performance of our case study NBS to evaluate its ecological significance; O2: Seasonal and temporal characterisations of biodiversity levels and their functional attributes; O3: Evaluation of present and future contributions of the NBS, regarding biodiversity enrichment, reduction of ECs and nutrients; O4: Valorisation of plant biomass for bioenergy production (biogas production) This project will greatly impact biodiversity conservation, pollutant load reduction, cost-effective wastewater management, clean energy production, and broader environmental sustainability, yielding economic and societal benefits. This PhD project will have the collaboration between CIIMAR and the Faculty of Engineering of Porto (FEUP), integrating a multidisciplinary approach that spans ecology, ecotoxicology, analytical chemistry, and environmental engineering, fostering innovative solutions at the intersection of biological and technological advancements.

MAIN METHODOLOGIES

Following the main proposed objectives, the respective methodologies comprise: O1 - Assessment of real-scale spatial and temporal performance of our case study NBS to evaluate its ecological significance 1.1 - Environmental surveys of the case study Field surveys to a CW integrated into a municipal wastewater treatment plant located in the North Portugal will be planned in a seasonal approach during the first year for measuring physicochemical parameters (e.g. temperature, pH, oxygen, water flow), nutrient concentrations (N, P), and ECs concentrations from the influent and the effluent of our case study. 1.2 - Biodiversity analyses Biodiversity analysis will be divided into the microbiological (bacteria and fungi) and macrofauna diversity associated with the NBS. Microbiological diversity: Microbial communities will be assessed through sequencing of 16S rRNA gene amplicons and ITS following the procedures as previously described1,2,3,4. Macrofauna diversity: Macrofauna samples will be collected using standardized protocols, sorted, identified as far as possible to species level and counted. These data will be analysed using different taxonomic and functional diversity metrics5,6 that reflect their richness, dispersion, and evenness7. For functional diversity, species will be classified according to functional traits5. 1.3 - Environmental characterization (water, substrate and plants) Water quality: Samples will be transported to the laboratory in a cool box, and preserved at 4 ºC until analysis. Target compounds will be extracted by solid phase extraction (SPE) and later, analysed. Substrate quality: Collected samples will be used for quantification of organic matter (450 ºC, 8h) and the remaining for later quantification of target ECs. Plants quality: For quantification of ECs in plant tissues, they will be first freeze-dried and then homogenized for later quantification. Plants Dynamics: Characterizing plant traits (e.g. density, plant growth parameters, root traits) & photosynthesis efficiency (via PAM fluorometry)8, pigments (chlorophyll a, b), and EC bioaccumulation will be done whenever possible. O2 - Evaluation of present and future contributions of the NBS, regarding biodiversity enrichment, reduction of ECs and nutrients 2.1 Selection and implementation of an optimized solutionDuring one-year, seasonal field surveys will be planned, to study the effect of the optimization strategies based on the biodiversity change and the improvement of ECs removal. A similar sampling strategy will be adopted as in 1.1. 2.2. - Toxicity effects of ECs exposure on non-target species Lymnaea stagnalis serves as an ecotoxicological model and bioindicator for aquatic contaminants due to its ease of maintenance in laboratory settings and wide geographical distribution9. Based on the results of ECs concentrations on the effluents of the case study, before and after the optimized solution, the model species will be exposed to different combinations of ECs concentrations for 21 days. DNA damages through citogenotoxicity analysis10 and effects on gonads maturation dynamics through histological analyses11 will be evaluated. O3 - Valorisation of CWs sub-products 3.1. Production of biogas using wetland biomass To evaluate the biogas production potential, the biochemical methane potential (BMP) of the plant biomass will be assessed. BMP tests will be conducted according to the VDI 4630 test standard12. Volatile fatty acids concentration and biogas composition will be analysed by GC-FID-TCD. The BMP tests will use inoculum from an urban WWTP anaerobic digestor, adapted with macrophyte biomass beforehand.

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PLACE OF WORK
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WILL THE PROPOSAL RESEARCH IDEA BE FUNDED BY A SPECIFIC PROJECT?
YES Biodiversa2023-935

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“Naturally diabetic”? Insights from Cetacea evolution

SUMMARY
Contemporary Evolutionary Biology deals with understanding how novelty evolved from an inherent gene set (phenotype-genotype link) and its relationship to adaptive landscapes. Besides gene duplication episodes and the rewiring of regulatory networks, adaptive gene loss has emerged as a powerful driver of adaptation: for instance, paralleling abrupt habitat transitions (e.g. Cetacea transition from land-to-water). As shown by our group and others, such scenarios of adaptive gene loss can be accompanied by extensive remodeling at the genomic and transcriptomic levels, encompassing novel rewirings’ at a physiological scale. This proposal delves into how the physiological adaptations occurring in the Cetaceans transition from land to water enabled them to significantly change their metabolism into becoming resistant to conditions that otherwise lead into diabetes in humans or their close land relatives. Dolphins and other delphinids naturally display diabetes-like metabolic markers—such as high blood glucose levels and insulin resistance—yet they do not develop the disease’s pathogenic symptoms. Could studying these “naturally diabetic” animals provide insight into diabetes in humans? We expect to map the cellular and molecular landscape of dolphin energy metabolism and uncover potential therapeutic targets for treating human diabetes. Type 2 Diabetes is among the most impactful chronic metabolic diseases worldwide, with serious multisystemic consequences that affect both individual health and the broader society. By comparing the diabetes resistant physiological landscape in Delphinidae with that of dolphin’s close land relatives and human we expect to provide advancements in basic research translatable into societal benefits (ranging from conservation awareness, education or tackling a human disease).

MAIN METHODOLOGIES
We will investigate these traits using advanced methods, including: (1) comparative genomics to identify unique genetic features, (2) integrative gene expression analysis at the single-cell and tissue level in dolphin pancreas, skeletal muscle, testis, liver, heart, brain and kidney—key organs in blood sugar regulation, (3) in vitro functional studies to characterize target proteins and develop fine-scale metabolic maps, (4) dolphin fibroblast culture assays to examine protein network interactions upon hormonal exposure, and (5) diabetic mouse models to address relevant metabolic differences and identify/test protective factors.

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PLACE OF WORK
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WILL THE PROPOSAL RESEARCH IDEA BE FUNDED BY A SPECIFIC PROJECT?
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Population genomics of the Mediterranean red coral, Corallium rubrum, a patrimonial species threatened by overharvesting and anthropogenic climate change

SUMMARY

Recent improvements in sequencing technologies and bioinformatics are greatly improving the potential inputs of population genetics in conservation biology (Formenti et al. 2022). Corallium rubrum, is a habitat-forming octocoral (Cnidaria, Anthozoa) distributed in the Mediterranean Sea and in the neighboring Atlantic with a key structural role in biodiversity-rich benthic communities. C. rubrum is under conservation concerns due to overharvesting and marine heatwaves. The intensity of these two anthropogenic stressors dramatically contrasts with the low resilience of C. rubrum and questions its evolutionary trajectory (Montero-Serra et al. 2019). Yet, the impact of these stressors on the species genetic make-up is still poorly understood and crucial questions regarding, admixture among lineages, demographic history, effective population sizes, selection, including local adaptation, are still open.Based on a chromosome level reference genome (Ledoux et al. 2025) and using whole genome resequencing, the objectives of the thesis are twofold. First, we will set up the evolutionary stage of the species covering a large part of the its distribution range. In particular, we will: i) characterize the full spectrum of genetic diversity and structure, including admixture pattern; ii) infer the species demographic history and, iii) explore the genomic landscape of the species (e.g. islands of differentiation, large structural variants). Then, we will conduct a case study focused on two Marine Protected Areas (MPAs). Here, we will characterize: iv) the hot / coldspots of genomic diversity; v) the patterns of local adaptation and connectivity in the two MPAs. Overall, this thesis will improve our basic knowledge on C. rubrum providing an in-depth characterization of the species evolutionary building-up. In the meantime, it will also support conservation policies including estimation of fishing quota, definition of evolutionary vs. management units or prioritization of management efforts in the two protected areas.

MAIN METHODOLOGIES

For the two objectives, the sampling is done and whole genome re-sequencing with high coverage (30X) is ongoing. For the Obj.1, we are sequencing approx. 120 individuals coming from 20 locations around the Mediterranean Sea and Southern Portugal while for Obj.2, we are sequencing approx. 90 individuals coming from 10 locations shared between the two protected areas, located in the Catalan Sea. The PhD student will be in charge of all the bioinformatics and statistical analyses from read mapping and SNP calling to population genetics analyses. For Obj. 1, analyses will include the characterization of genetic structure (e.g. PCA, Admixture, F-statistics) and diversity (e.g. run of homozygosity, He, Pi). Genome scan analyses will also be conducted to characterize the species genomic landscape (e.g. genomic islands of differentiation). The demographic history of the species will be reconstructed based on site frequency spectrum (e.g. FastSimCoal2) or using Approximate Bayesian Computation in a coalescent framework. To keep these inferences computationally realistic, we will consider models focused on particular divergence events and involving a subset of populations (e.g. Atlantic vs. Mediterranean populations; Mediterranean vs. Adriatic populations). We will put emphasize on the estimation of demographic parameters in particular effective population size. From a management perspective, these analyses will allow us to look for different evolutionary lineages and, potentially, to define evolutionary units. Moreover, we will provide estimates of current effective population size for the different locations, which could be used to adjust current fishing quotas. Obj. 2: We sampled approx. 90 individuals from 10 populations form the same depth (~20m) but contrasted environment (e.g. caves vs. overhang, thermal regimes) in two MPAs. Besides analyses on structure, diversity and connectivity at regional scale, we will test the imprint of local adaptation using different statistical frameworks (differentiation-based vs. genome environment association) as recommended in highly structured species, such as C. rubrum. From a management perspective, these analyses will allow: i) to characterize the conservation status of the different population; ii) estimate the scale of local adaptation to inform the definitions of conservation units in the two MPAs; and iii) to provide scientifically based recommendation for the prioritization of the management effort. This case study will be a relevant example of the inputs of population genomics for the management of protected areas.

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PLACE OF WORK
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WILL THE PROPOSAL RESEARCH IDEA BE FUNDED BY A SPECIFIC PROJECT?
YES REDCOR_CPrv_BIOPOLIS2023008_CPrv_ECConservation-AO1

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Population genomics for the conservation and restoration of the Mediterranean red gorgonian, Paramuricea clavata in the Parc Naturel des Calanques (France).

SUMMARY

Recent improvements in sequencing technologies and bioinformatics are greatly improving the potential inputs of population genetics for biodiversity management and conservation (Formenti et al. 2022). Yet, in practice population genetics is still poorly considered by biodiversity managers. The main aim of the PhD thesis is to demonstrate how population genetics can help management and restoration actions in a protected area. The PhD thesis is focused on the Mediterranean red gorgonian, Paramuricea clavata, in the Parc National des Calanques (PNC, France). P. clavata is a habitat-forming octocoral (Cnidaria, Anthozoa) with a key structural role in biodiversity-rich benthic communities. The shallow populations (until 40m depth) are under conservation concerns due to recurrent anthropogenic marine heatwaves. The intensity of this stressor dramatically contrasts with the low resilience of P. clavata and questions its evolutionary trajectory. The shallow populations of P. clavata from the PNC are severely impacted by marine heatwaves, with a dramatic mortality event in 2022 (Estaque et al. 2023). The impact of these events on the species genetic make-up is still poorly understood and crucial questions regarding for instance the ability of deep populations to recolonize shallow and impacted areas ("deep refugia hypothesis") or the genetic factors underlying the sensitivity to thermal stress are still open. The PhD student will: i) test the deep refugia hypothesis and ii) look for genetic factors and evolutionary processes driving the response to thermal stress. To reach these two objectives, the PhD student will implement state of the art bioinformatics and population genetics analyses (structure, diversity, demography, genome wide association study) to analyse a whole genome re-sequencing dataset. Overall, this thesis will improve our basic knowledge on P. clavata ecology and evolution in the context of climate change and provide support to help management prioritization in an emblematic protected area.

MAIN METHODOLOGIES

For the two objectives, the sampling is done and whole genome re-sequencing with high coverage (30X) is ongoing. For the Obj.1, we are sequencing approx. 150 individuals coming from 15 populations located in the Parc National de Calanques and neighboring area until the mesophotic depth (100 m). For Obj.2, we are sequencing approx. 100 individuals coming from one population located in the PNC and sampled after the 2022 marine heatwave. Based on the occurrence/absence of tissue necrosis during the sampling, these 100 individuals were identified as "sensitive" (50) and "resistant" (50). The PhD student will be in charge of all the bioinformatics and statistical analyses from read mapping and SNP calling to population genetics analyses. For Obj. 1, analyses will include the characterization of genetic structure (e.g. PCA, Admixture, F-statistics) and diversity (e.g. run of homozygosity, He, Pi) among populations. Genome scan analyses will also be conducted to characterize the species genomic landscape (e.g. genomic islands of differentiation). We will put particular emphasize on the characterization of the pattern of connectivity within the PNC and between the PNC and neighboring area (spillover effect), the pattern of local adaptation (e.g. to depth) and the estimation of the effective population sizes. Based on this genetic baseline, we will characterize the conservation status of the different populations (e.g. genetically rich vs. depleted, source vs. sink) providing support for the prioritization of the management effort within the PNC. For Obj. 2, analyses will include the characterization of genetic structure (e.g. relatedness) and diversity among individuals. Then a genome wide association survey (GWAS) will be conducted to contrast the genomic background of resistant vs. sensitive individuals and to look for gene potentially involved in the response to thermal stress. The obtained results will be discussed in the light of Obj. 1 to define protocols for climate-resilient restoration protocols.

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Comparative Population Genomics Illuminates Species Boundaries in Eunicella and Alcyonium Octocorals

SUMMARY

The process of speciation is a central topic in evolutionary biology due to its role in shaping biodiversity patterns. Yet, the study of speciation is challenging particularly in recently diverged species, in which reproductive isolation is not complete and gene flow and hybridization can still occur. The main objective of the PhD thesis will be to investigate species boundaries in two octocoral genus representing six accepted octocoral species: Eunicella verrucosa (Pallas, 1766), E. cavolini (Koch, 1887), and E. singularis (Esper, 1791), Alcyonium digitatum , A. acaule and A. coralloides. The six species are considered as habitat-forming species supporting biodiversity rich communities and show contrasted distribution range in the Atlantic and/or in the Mediterranean. Noteworthy, some of these species are under strong conservation concerns owing to recurrent mass mortality events linked to anthropogenic climate change. Preliminary works conducted by our team using Kmer analyses and whole genome re-sequencing show potential for on-going hybridization between E. singularis and E. cavolini while the high divergence among Atlantic vs. Mediterranean populations of A. coralloides suggest the occurrence of two different species. In this context, the PhD student will: i) follow the characterization of the genetic relationships within and among the six different species; ii) infer their demographic history; iii) look for the genomic landscapes of differentiation and genes/genomic regions potentially involved in speciation process. To reach these objectives, the PhD student will implement state of the art bioinformatics and population genetics analyses to analyze a whole genome re-sequencing and a RNAseq dataset.Overall, this thesis will improve our basic knowledge regarding speciation in the marine species providing support to refine biodiversity estimation in octocorals.

MAIN METHODOLOGIES

Using whole genome re-sequencing data from approx. 60 individuals coming from 13 localities in the six species, the PhD student will i) characterize the full spectrum of genetic diversity and structure, including admixture within and among the six species; ii) infer the species demographic history using approximate Bayesian computation in a coalescent framework, iii) explore the genomic architecture and landscape of differentiation among species (e.g. islands of differentiation, large structural variants). These results will be complemented by an in-depth analysis of RNAseq dataset on the same species to further refine the identification of genetic regions potentially involved in speciation process.

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Evolutionary dead-ends or the novelty of adaptation? The evolution of excretion in fish

SUMMARY

As the Bilateria animal first took shape in the murkiness of the ancestral seas, so did a unique scene of novel behaviors. These were molded by conflict. It was a “new and competitive world” with an immense and diverse assembly of stem bilaterian lineages. How did this animal diversity come about? What physiological and genetic processes upgraded the capacity for predation and escape? A simple hypothesis puts forward that complex systems require compartmentalization and cell/organ specialization. Thus, the appearance of highly specialized excretory organs arises as an essential physiological process, capable of generating efficiency for larger and more active body plans. Strikingly, the comparative molecular architecture of excretion systems shows a surprising developmental and structural conservation signature While this typical arrangement is mostly conserved in vertebrate lineages, some notorious exceptions exist. In some marine teleost fish species, urine production via glomeruli has been eliminated. In effect, urine production is severely compromised in teleost fish that lack glomeruli, so-called aglomerular. Currently, seven teleost lineages presumably display kidneys without glomeruli, providing a striking case of evolutionary parallelism. The loss of such a successful evolutionary innovation – the Principle of Ultrafiltration, is puzzling. What caused this radical organ reorganization? What are the immediate (genetic) drivers for this striking difference in teleosts? What developmental patterns were modified, if any? Are these differences somewhat ontogenetic, with absence of glomerular being a life stage trait? In this project, we aim to decipher this evolutionary riddle. We aim to clarify the molecular signature paralleling the elimination of filtration in urine formation and provide hints on the causes leading to such drastic adaptation in seven teleost lineages. We will use a multidisciplinary approach to comprehend the evolutionary foundations of this phenotype from embryo to adult life stages.

MAIN METHODOLOGIES

Comparative Genomics, Genome and Single-cell RNA seq of glomerular and aglomerular species, Developmental analysis, Bioinformatics

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Mechanisms of reproductive and developmental impairments in invertebrate endocrine systems

SUMMARY

The endocrine system, pivotal in maintaining organismal homeostasis, is tightly regulated by nuclear receptors (NRs), intracellular transcription factors that respond to endogenous and exogenous molecules to orchestrate vital processes such as reproduction, development, and metabolism (1). In invertebrates, NRs unique to this group, such as two DNA-binding domain NRs (2DBD-NRs) (2), are crucial for development but remain understudied, particularly in the context of endocrine-disrupting chemicals (EDCs) (3-5). These pollutants, abundant in marine ecosystems, interfere with NR signalling, leading to adverse physiological effects and posing significant risks to biodiversity and ecosystem health (6). Unlike vertebrates, where endocrine disruption mechanisms are better understood, data on invertebrate-specific NRs and their responses to EDCs are sparse, limiting accurate risk assessment and conservation strategies (7-10). The project addresses this gap by investigating the Mediterranean mussel (Mytilus galloprovincialis), a model organism for ecotoxicology. The project aims to decipher the role of 2DBD-NRs in regulating reproduction and development in invertebrates while elucidating how EDCs disrupt these pathways. To achieve this goal, this project is structured into three objectives: (1) functionally characterize 2DBD-NRs by defining i) their spatiotemporal expression in mussel larvae using in situ hybridization chain reaction and ii) their binding profile to ligands and gene promotor region using in vitro molecular assays; (2) assess developmental impacts of 2DBD-NR disruption using CRISPR/Cas9 genome editing and transcriptome profiling; (3) establish a mussel-immortalized cell line for in vitro studies. This work will contribute to inform risk assessment frameworks and conservation policies for aquatic ecosystems by linking EDC exposure to physiological and ecological consequences.

MAIN METHODOLOGIES

"in situ" hybridazation; CRISPR/Cas9 technology; Transcriptome analyses by mRNA-sequencing; DEG analysis; "in vitro" transactivation assays; Thermal Shift Assays; Electrophoretic mobility shift assays; cell lines manipulation; new cell line establishment

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Chemical signals mediating microbial colonization and biofouling

SUMMARY
Submerged surfaces in the Ocean, either biological or artificial, are prone to colonization by microbial biofilms and eventually lead to successive biofouling communities [1]. However, several biological surfaces naturally present effective protection against epibiosis [2]. The outer surfaces of certain species of ascidians (Tunicata) present massive overgrowth by micro- and macrofoulers, while certain other are effective in maintaining their tunics free of fouling [3,4]. This observation sets the stage for this project – what could be the basis for such differential colonization? We hypothesize that small molecules produced at the tunic of non-fouled ascidians can prevent the attachment of fouling organisms, including biofilm-forming microbes. Still, very little is known about the microbiota of these tunicates and their ability to produce small molecules that protect the hosts' surface [5]. This presents as an opportunity to understand the chemical ecology of these marine organisms, but also to discover small molecules that can have anti-biofilm properties, which, in turn, could find application as antibiotics or antifoulants. The work is divided into three objectives: 1) characterize the microbiota of colonized and non-colonized ascidian surfaces; 2) study the ability of microbes from seawater to colonize the pure tunic material from the two types of organisms; 3) identify and characterize small molecules present in the tunics of non-fouled tunicates that prevent colonization.

MAIN METHODOLOGIES
The student will engage in an ambitious and highly multidisciplinary project that will involve microbiology, metagenomics, marine biology, ecological assays and natural products chemistry. The microbiota of the tunics of multiple specimens of two ascidian species (highly colonized vs hardly colonized) will be compared, and their potential for the biosynthesis of secondary metabolites evaluated. In addition, colonization assays by seawater microbes using the tunic surfaces will be performed in vitro. Bioassay-guided isolation of natural products with antibiofilm properties from the invertebrates' tunic material will be performed. Thus, the student will have the opportunity to learn multiple techniques, namely gDNA extraction and metagenome analysis, bioinformatics, biological assay development and natural products chemistry techniques, including several chromatographic techniques, mass spectrometry and nuclear magnetic resonance. The work will be mainly carried out at the BiO and CNP labs at CIIMAR.

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Marine Fungal Metabolites and their Semisynthetic Analogs to Circumvent Multidrug-Resistant Bacteria

SUMMARY
NOVEL STRATEGIES to solve the increased incidence of infections with multidrug-resistant (MDR) pathogens are URGENTLY NEEDED. Marine fungi are privileged sources of antimicrobial compounds. Bacterial efflux pumps have been associated with MDR. Based on previous collaboration with the University of Szeged, the aims of this proposal are to isolate and characterize new marine natural products and to obtain analogs by synthesis that circumvent MDR and reach broad spectra sustainable antimicrobials.

MAIN METHODOLOGIES
1) isolate and characterize bioactive compounds from the marine-derived Aspergillus species 2) a structure-based design and semi-synthesis of analogues of marine products against efflux pumps responsible for multidrug-resistance in bacteria 3) investigate their potential as antimicrobial agents in pathogens relevant for human diseases and screening for their capacity to modulate microbial transporters 4) perform detailed toxicological and other preformulation studies for the most promising derivatives.

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CephaMine: Mining for Next-Generation Antibiotics Encrypted in Cephalopods' Salivary Glands

SUMMARY
Antibiotic resistance (ABR) is a growing global crisis projected to cause 10 million deaths annually by 2050. Exacerbated by the misuse of antibiotics during the COVID-19 pandemic, ABR is further complicated by bacterial biofilms, such as those formed by the ESKAPEE group, which shield bacteria from antibiotics and immune defences. ABR also poses significant economic challenges, increasing healthcare costs and disrupting food industry processes due to bacterial contamination. Antimicrobial peptides (AMPs) hold promise for combating ABR, offering broad-spectrum activities such as antibacterial, antibiofilm, anti-inflammatory, and immunomodulatory effects. Despite their potential, the discovery of AMPs from omics data remains underexplored, particularly for 'encrypted AMPs' hidden within longer transcripts or proteins. The CephaMine project addresses this gap by systematically screening 5,466 unique non-toxic AMPs mined from omics data of cephalopod salivary glands, a rich source of bioactive compounds. The project involves: 1. Machine learning and deep learning predictions to evaluate antibacterial activity and targets within the ESKAPEE group. 2. Antibiofilm activity evaluation to identify candidates with dual antibacterial and antibiofilm properties. 3. Network-based analyses to uncover additional properties such as immunomodulatory and anti-inflammatory effects. 4. In vitro testing of shortlisted candidates for activity against multidrug-resistant (MDR) bacteria. By leveraging cephalopod omics data and integrating cutting-edge computational and experimental approaches, CephaMine aims to identify unique peptide structures with potential as next-generation (NG) antibiotics. This project seeks to streamline antibiotic discovery and contribute innovative solutions to the fight against ABR.

MAIN METHODOLOGIES
Webservers and standalone prediction tools based on machine and deep learning techniques for the Antibacterial and Antibiofilm activities of the cephalopod antimicrobial peptides (CAMPs); Complex similarity networks for identifying those CAMPs with immunomodulatory and anti-inflammatory effects, 3D-structure modelling and in vitro assays for the antibacterial and anti biofilm activities.

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A Gateway to Metal Resistance: Siderophores to Fight Heavy Metal Toxicity in the Biological Environment

SUMMARY
Human activities such as mineral mining or oil exploitation threaten the vital roles played by microbes in deep-sea ecosystems. Microbes on the seafloor provide crucial ecosystem services, and metal discharges into the environment can have disastrous effects on the balance of the ecosystem. Understanding microorganisms' resistance mechanisms can help develop effective bioremediation strategies to minimize metal toxicity. Chelating agents, such as siderophores, can help microorganisms survive under iron-deficient conditions and protect them from metal toxicity. It is crucial to study the influence of chelating agents in the sequestration potentials of iron, copper, and cadmium by bacteria and their impacts on metal toxicity in deep-sea bacterial functioning. This research presents an opportunity to test an environmental mitigation strategy before anthropogenic activities begin to shape an ecosystem substantially. As a part of a project aimed at studying the impact of deep-sea human disturbance on microbial ecosystem functions, the student will be involved in investigating siderophores and siderophore-mimetics as potential molecular tools to counteract the harmful effects of metals. The task will focus on conducting metal complexation, stability, and biodegradability studies on the synthesized siderophore and siderophore-mimetics.

MAIN METHODOLOGIES
The chelating behavior of the natural siderophore with metals (Fe3+, Cu2+, and Cd2+) will be evaluated by complexation tests using an analytical reversed-phase HPLC protocol. The amount of siderophore-metal complex formed and the different affinities for the different metals will be assessed.Natural water varies in the number of dissolved minerals, organic matter content, and pH, depending on its source, location, and season. As such, factors such as temperature, solubility, concentration, humidity, and pH can affect the stability of some compounds. Stability assays will be conducted considering these variables and according to the methods described in ASTM (2008) and Huanga et al. (2014) using natural seawater. Additionally, the ready biodegradability of the siderophores and siderophore mimetics will be tested according to the Organization for Economic Co-operation and Development (OECD) guideline (301A: DOC-Die Away method) for the testing of chemicals. Although nowadays it is desirable, whenever possible, to use biodegradable compounds, the use of non-readily biodegradable chelating agents is preferred in some situations where the chelating agent should remain available for a certain period of time. In these circumstances, the use of readily biodegradable compounds should be avoided as they can be easily degraded by permanent contact with microorganisms.

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Development of MALDI-TOF Methodologies for Identification and Environmental Monitoring of Cyanobacteria

SUMMARY
In the past 20 years MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight) mass spectrometry (MS) has been a game-changer in clinical microbiology, in food safety and quality control (also using Low Mass Biomarkers), in monitoring environment, to support quality control in biofertilizers for a sustainable agriculture and to monitor bloom- forming toxigenic cyanobacteria (a high health risk in the drinking and recreational water). MALDI-TOF MS enables rapid, accurate, and cost-effective identification of microorganisms and rely on the existence of comprehensive spectra database, integrated with advanced algorithmic tools for processing, identification, characterization of microbes and surveillance. Furthermore, cyanobacteria are a very diverse group of photoautotrophic bacteria, found primarily in freshwaters and also in marine environments. Several species of cyanobacteria are toxic and produce potent toxins, being identified as the leading cause of animal poisoning and contamination of aquatic environments. Humans can also be exposed to cyanobacterial toxins through direct consumption of improperly treated water and contaminated food products or through dermal contact and ingestion during recreational activities. Numerous illness symptoms in humans related to acute exposure to cyanotoxins have been reported. The development of methods allowing rapid and reliable identification of cyanobacteria in the environment is therefore critical to prevent poisoning incidents and intoxications cause by cyanobacteria. The PhD project will focus in the development of MALDI-TOF MS protocols and standard operating procedures for analysis of cyanobacteria and identification of cyanobacteria taxa, as well as MALDI-TOF MS protocols for analysis (identification and quantification) of cyanotoxins. The methodologies developed will be tested for monitoring toxic cyanobacteria and water quality assessment.

MAIN METHODOLOGIES
cyanobacteria growth in lab conditions; cyanotoxins extraction and purification; phycobilins extraction; sample preparation and MALDI mass spectrometry analysis.

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MAIN SUPERVISOR
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PLACE OF WORK
CIIMAR

WILL THE PROPOSAL RESEARCH IDEA BE FUNDED BY A SPECIFIC PROJECT?
YES MALDIBANK (PROJECT: 101188201)

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From Sea to Remedy: Exploring Marine Actinobacteria for Novel Antifungal Molecules

SUMMARY
Actinobacteria are a vast group of Gram-positive bacteria, highly prolific in the production of bioactive secondary metabolites with a wide range of biological and pharmaceutical properties. While most known actinobacterial species originate from terrestrial environments, recent evidence has shown that they are also true inhabitants of marine ecosystems and a proven source of novel bioactive molecules, including antibiotics, antitumor agents, anti-inflammatory, antiviral, and antioxidant compounds [1,2]. The microbial metabolism of untapped marine sources may thus provide valuable drug leads, making marine actinobacteria a promising—yet still underexplored—resource. The increasing threat of antifungal resistance poses a serious challenge to global public health, emphasizing the urgent need for new molecules with potent antifungal activity. According to the World Health Organization (WHO) priority list of fungal pathogens, several fungi represent significant health risks, including Candida auris, Aspergillus fumigatus, Cryptococcus neoformans, and species from the Mucorales order, among others. These microorganisms are responsible for life-threatening invasive infections, particularly in immunocompromised individuals, and current antifungal treatments are often limited by toxicity, narrow-spectrum activity, or emerging resistance. Given the biotechnological potential of actinobacteria in producing antimicrobial secondary metabolites, exploring marine-derived strains could lead to the discovery of novel antifungal compounds effective against these priority pathogens. As such, this proposal aims to find novel molecules with antifungal activity produced by marine actinobacteria, by screening a large collection of these microorganisms available at CIIMAR. The identification and characterization of such molecules would not only contribute to the development of new therapeutic options but also highlight the role of marine biotechnology in addressing emerging fungal infections and drug resistance challenges.

MAIN METHODOLOGIES
CIIMAR houses a large collection of marine actinobacteria, comprising over 1,000 strains isolated from diverse marine sources, including macroalgae, sponges, corals, marine sediments, and deep-sea environments. This collection will be screened for antifungal activity against clinically relevant fungal pathogens. Organic extracts from selected actinobacterial strains will be initially tested for antifungal potential. The most promising samples will undergo further evaluation to determine the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC). Strains with the highest bioactivity will be analyzed to identify the compounds responsible. Genomic sequencing of these strains will explore their biosynthetic potential for producing novel antifungal molecules, while the effects on fungal growth and virulence factors will be assessed in complementary studies.

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PLACE OF WORK
CIIMAR and UTAD

WILL THE PROPOSAL RESEARCH IDEA BE FUNDED BY A SPECIFIC PROJECT? NO

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Assessing Multi- and Transgenerational Impacts of Per- and Polyfluoroalkyl Substances (PFAS) in Aquatic Ecosystems: Gaps in Environmental Hazard and Risk Assessment Frameworks?

SUMMARY

The progress of industrialization has had significant consequences for the environment. One key implication is the accumulation of persistent organic pollutants, such as per- and polyfluoroalkyl substances(PFAS), which pose a high risk to environmental health. PFAS are a diverse group of synthetic chemicals that have been used in industrial applications and in the production of consumer goods including electronic, military, agricultural, packaging, and textile industries(1,2). PFAS are commonly found in aquatic ecosystems due to their resistance to removal by wastewater treatment processes. This persistence, combined with their potential for bioaccumulation, endocrine/thyroid-disrupting effects and potential transgenerational epigenetic effects(3), poses a significant threat to aquatic life. In response, restrictions on their use and reduction of their environmental levels have been implemented, with recent addition of PFAS to several regulations, such as (EU) 2023/915 and (EU) 2024/3019. These regulations aim to limit PFAS presence in seafood and monitor their concentrations in urban wastewater treatment plants, respectively. However, despite their widespread presence, regulatory restrictions, and potential for long-term adverse effects, PFAS are often overlooked in studies evaluating multi- and transgenerational impacts of environmental pollutants. This oversight limits our ability to fully assess PFAS risks in aquatic ecosystems. To address this gap, this study will investigate the multi- and transgenerational effects of key PFAS using two aquatic animal models: Danio rerio(teleost fish) and Gammarus locusta(crustacean). Both, models with short life cycles, are well-established at CIIMAR-EDEC(4,5,6), providing an ideal platform to explore the multi- and transgenerational effects of PFAS across different biological systems. The primary objective of this research is to link potential multi- and transgenerational phenotypic adverse effects of PFAS to epigenetic modifications and to uncover the underlying mechanisms. A secondary goal is to determine whether the inheritance of altered phenotypes is transmitted through paternal or maternal lines. This study will contribute to the advancement of the PFAS hazard and risk assessment frameworks.

MAIN METHODOLOGIES

We will expose the two model organisms (Gammarus locusta and Danio rerio) to environmentally relevant concentrations of key PFASs during 4 consecutive generations (F0-F3) – Multigenerational group. In parallel, a sub-set of offspring produced in F0 generation will be raised under PFAS-free water for the subsequent three generations (F1-F3) forming the Transgenerational group. Additionally, we will conduct crossbreeding between exposed and unexposed males and females to investigate whether the transmission of effects occurs through the paternal or maternal lineage.We will benefit from the short-life cycle these two model organisms, ideal to perform multi and transgenerational studies. In each generation key phenotypic alterations (morphologic, reproductive histopathology and behaviour) will be evaluated and the results obtained will determine the adverse phenotypic outcomes and define if the effects are multi and/ or transgenerational. These findings will be integrated with a molecular approach to characterize the mechanisms underlying the effects observed and to determine if/how the transgenerational effects have an epigenetic basis. Transcriptomic and/or proteomic analyses will be used to identify the gene/protein expression alterations and epigenetic methods (DNA methylation, and/or chromatin accessibility) will be used to establish a fingerprint of epigenetic alterations. By establishing the relationships between observed epigenetic changes and altered gene/protein expression, we will identify the pathways responsible for the phenotypic outcomes associated with PFAS exposure. Finally, to evaluate whether the inheritance of effects is transmitted by paternal or maternal lines, cross reproduction (two sexes exposed; exposed males x unexposed females; exposed females x unexposed males) in F0 will be performed and the animals from each condition will be followed until F3 generation in clean water. The student will join the EDEC team at CIIMAR, with his work will be supported by the ongoing projects at EDEC.

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MAIN SUPERVISOR

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PLACE OF WORK

CIIMAR

WILL THE PROPOSAL RESEARCH IDEA BE FUNDED BY A SPECIFIC PROJECT? YES, SEVERAL ONGOING PROJECTS AT EDEC

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Microplastics distribution on the North Portuguese coastal region: Hydrodynamic and socioeconomic impacts

SUMMARY

Microplastics (MP) are contaminants of emergent concern with strong impacts on organisms and ecosystems in general. Present in a wide range of products and originating from larger plastic debris, MP can be found in various environments, including oceans, rivers, estuaries, land, air, and even in remote areas. Beyond their direct effects on organisms, MP can contain chemical additives and adsorb pollutants from the environment, amplifying their harmful effects. Considering that the distribution and abundance of MP in the marine environment is increasing, so does their impact on the ecosystems. Understanding MP transport pathways and sources is crucial for mitigating their environmental impact. Only with this knowledge effective strategies can be developed to reduce their presence in the environment. Achieving this requires a multidisciplinary approach, integrating hydrodynamic analysis with socioeconomic assessments to identify key transport routes and sources. This PhD topic proposal aims to use the MP database previously generated from several research projects (e.g. Atlantida, MAELSTROM, INSPIRE, BlueWWater, CIIMAR Watch), and upcoming opportunities, to understand the link between the land and marine MP sources and their distribution. During this PhD proposal it is intended that the student participate in in situ campaigns, perform laboratory analysis, apply statistical tools and implement numerical models to understand, in a multidisciplinary way, the main sources and distribution of MP along the north Portuguese coast.

MAIN METHODOLOGIES

In situ campaigns to sampling MP and physical parameters. Laboratory analysis to quantify, characterize and classify MP. Statistical analysis to represent concentration, trends and effects of site-specific conditions (hydrodynamic and socio-economic parameters). Numerical modelling tools to understand the main transportation patterns and their distribution along the coastal region.

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PLACE OF WORK
CIIMAR

WILL THE PROPOSAL RESEARCH IDEA BE FUNDED BY A SPECIFIC PROJECT?
YES, SEVERAL ONGOING PROJECTS AT LBC AND LOAI

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Development of advanced tools to improve risk assessment of Pharmaceuticals of Emerging Concern

SUMMARY
Today, over 100,000 chemicals are continuously produced. Many of these chemicals will ultimately reach the aquatic ecosystems thus threatening the integrity of a vast array of organisms. Recently, concerns have increased on the toxicological risk of Contaminants of Emerging Concern (CECs), i.e., pharmaceuticals, personal care products, nanoparticles, microplastics. Many of these chemicals are not new, but the knowledge on their behavior and toxicological/ecological hazard is too scarce, and therefore there is an urgent need to assess their risk. Given the concern both to human and environmental health, a new European directive was introduced on November 2024 to assure that Waste Water Treatment Plants (WWTP) monitor and implement solution to reduce the discharge of CECs, particularly pharmaceuticals. Although most studies focusing in the risk of pharmaceuticals were initially driven to evaluate the outcomes of chemical exposure on the parental generation, recent evidences suggest that several stressors, including pharmaceuticals, may induce effects that are not circumscribed to the parental generation. One of the earliest studies demonstrated that Exposure of female rats to the anti-androgenic compound vinclozolin resulted in male gonads abnormalities in F1 that passed at least up to the F4 generation, disrupting spermatogenesis and increasing of infertility incidence. This reproductive outcome was correlated with alteration on the epigenome in the male germ line. If chemical exposure of one generation can be passed to multiple subsequent non-exposed generations, the risk assessment of these chemicals should incorporate this time interval: from exposure to responses in subsequent, non-exposed, generations. Here, we expect to significantly contribute to develop tools to upscale knowledge on the transgenerational effects of pharmaceuticals, thus contributing to improve human and environmental risk assessment, and the implementation of EU directives. This will be addressed by a combination of molecular and biochemical analysis, the use of advanced molecular and cellular approaches to implement gain-and-loss of function experiments, and bioassays with the model animal, zebrafish (Danio rerio).

MAIN METHODOLOGIES
Ecotoxicological assays with zebrafish; Next generation sequencing (RNA-Seq), genome and epigenome editing techniques based on CRISPR-Cas 9, Epigenome biomarkers

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MAIN SUPERVISOR
Miguel Santos/Endocrine Disrupters and Emerging Contaninants - EDEC

PLACE OF WORK
CIIMAR/FCUP/possibility to work in collaboration with labs in Europe/US

WILL THE PROPOSAL RESEARCH IDEA BE FUNDED BY A SPECIFIC PROJECT?
YES, BLUEWWATER: HTTPS://BLUEWWATER.EU/EN/BLUEWWATER/

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Population dynamics of the European sardine Sardina pilchardus Walbaum, 1792 using palaeogenomics

SUMMARY
Overfishing has significantly reduced the population sizes of many marine species, yet our understanding of its evolutionary impact remains limited due to the lack of accurate fishery catch data. One of the most affected species is the European sardine (Sardina pilchardus), a key pelagic fish with high economic importance, particularly in Southern Europe and Morocco. Sardines are the primary target of purse-seine fleets, supporting local economies. Their distribution extends from the southern Celtic and North Seas to Mauritania and Senegal, including the Azores, Madeira, the Canary Islands, and the Mediterranean. A recent population genomic study analyzing 12 sardine populations across their entire range revealed at least three genetic clusters. One includes individuals from the Azores and Madeira, another comprises Iberian populations (which exhibit some genetic admixture), and the third groups Mediterranean and Canary Islands samples. To better understand how overfishing has influenced sardine populations, we will use advanced ancient DNA techniques to analyze pre-exploitation samples and compare them to modern populations. We have obtained specimens from several Roman-era archaeological sites: Adro Vello, Galicia (Spain), Cacilhas and Portimão (Portugal) Tahaddart (Morocco) Nantes (France). By sequencing full genomes from these ancient specimens, we can assess historical population sizes and genetic diversity before overfishing. This research provides crucial data for fisheries management, helping define sustainable fishing efforts within FAO-designated areas. Our approach offers valuable genomic insights across time, informing conservation and stock management strategies frame usually not available to decision makers. Most sequencing data is already available for analyses.

MAIN METHODOLOGIESAncient DNA techniques will be used to retrieve DNA from these samples. Subsequently double strand DNA libraries will be constructed and multiplexed and samples will be sequenced in an Illumina platform. The data collected from these specimens will be mapped to the available reference genomes (26), following demultiplexing, trimming of adapters and quality control steps. Data quality filtering will ensure that errors introduced by next generation sequencing (NGS) technologies and by typical degradation of DNA in museum and fossil material will be discarded. A number of population genetic analyses will be undertaken using SNP variants, or, when implemented on ngsTools, using genotype likelihoods, as the latter has been shown to improve population genetic inferences, especially for low depth data. Population structure will be studied by means of Principal Component Analysis (PCA) using PCAngsd, NGSadmix, highlighting any change in the genetic structure of this population over time.

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PLACE OF WORK
CIIMAR

WILL THE PROPOSAL RESEARCH IDEA BE FUNDED BY A SPECIFIC PROJECT?
YES, 2022.03142.PTDC

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Harnessing Microbes to Tackle Fluorinated Pharmaceuticals Pollution - Investigating Microbial Pathways for Biodegradation

SUMMARY
During the last decades, environmental contamination by fluorinated organic compounds has received increasing attention because of their several uses. Among these, fluorinated pharmaceuticals pose a growing environmental concern due to their persistence and potential ecotoxicity (Murphy, 2016). Many of these compounds, used in various therapeutic applications, are not fully metabolized by the human body and are excreted unchanged or as metabolites, ultimately reaching wastewater treatment plants. However, conventional treatment systems are often ineffective at completely removing fluorinated drugs, leading to their accumulation in aquatic environments and soil. In addition, recent changes in the OECD definition of per- and polyfluoroalkyl substances (PFAS) now classify certain fluorinated drugs as PFAS, emphasizing their environmental persistence (Wang et al., 2021). Despite their widespread use, the biodegradation pathways of many fluorinated pharmaceuticals remain unknown. Given the rising detection of fluorinated pharmaceuticals in ecosystems, further research is needed to elucidate their fate and degradation mechanisms. In this context, this proposal aims to investigate the potential of natural microbial communities from diverse environmental matrices, as well as pre-enriched microbial consortia known for degrading other fluorinated compounds, to biodegrade two fluorinated pharmaceuticals, one of which classified as a PFAS. The outcomes of this investigation will allow a better understanding of the fate of these compounds in the environment and the development of effective remediation strategies.

MAIN METHODOLOGIES
A variety of environmental samples will be collected from diverse sources to serve as inocula for enrichment experiments. Additionally, microbial cultures already available at CIIMAR, known for their ability to degrade other fluorinated compounds, will also be tested. The biodegradation of the target fluorinated pharmaceuticals will be examined in batch mode under aerobic conditions, with the potential for co-metabolism in the presence of an additional carbon source, such as acetate. Biodegradation progress will be monitored by HPLC, while fluoride release, an important indicator of degradation, will be measured using potentiometry with a fluoride-selective electrode. If degradation is confirmed, microbial consortia will be characterized using molecular biology techniques, and the metabolic pathways responsible for the degradation of the target compounds will be elucidated.

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PLACE OF WORK
CIIMAR

WILL THE PROPOSAL RESEARCH IDEA BE FUNDED BY A SPECIFIC PROJECT?
YES, MAR2PROTECT GA NO 101082048- PREVENTING GROUNDWATER CONTAMINATION RELATED TO GLOBAL AND CLIMATE CHANGE THROUGH A HOLISTIC APPROACH ON MANAGED AQUIFER RECHARGE - EUROPEAN UNION. 2022-2026.

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Novel appetite modulating compounds from cyanobacteria and microalgae for the combat of obesity

SUMMARY
In most parts of the developed world, being overweight is now the norm. In Europe, over 50% of the adult population is overweight and more than 20% is obese (WHO, 2016). Triggered by a sedentary lifestyle and high caloric food options, this steady increase in mean body weight is correlated with a dramatic increase in the metabolic syndrome, resulting in morbidities such as type 2 diabetes, non-alcoholic fatty liver disease (NAFLD), cardiovascular disease and cancer. Cyanobacteria/microalgae are known as producer of proteins, but also of valuable compounds for human health benefits (e.g. polyunsaturated fatty acids PUFA´s; minerals, vitamins or even pharmaceutical compounds). In this context, we have successfully identified cyanobacteria/microalgae from freshwater and marine environments in previous projects that suppressed the appetite in zebrafish larvae. The regulation of appetite is an interesting avenue for obesity treatment, characterized by a complex neuroendocrine network. In this work, we aim to identify the responsible metabolites in promising strains of cyanobacteria/microalgae by bioactivity-guided and mass-guided isolations. The mechanism of action of isolated compounds will be analyzed by molecular techniques, ranging from mRNA expression, metabolomics, protein-ligand interactions and others, to understand future biotechnological applications. The overall objective is to identify the responsible compounds from the previously observed appetite reducing activities from cyanobacteria/microalgae, and to evaluate the molecular mechanism in the zebrafish model. Such compounds may be developed in the future as novel nutraceuticals for obesity and related diseases.

MAIN METHODOLOGIES
- growth of cyanobacteria/microalgae - organic extractions of biomass and chemical fractionation - structural elucidations (MS, NMR); - bioassays for appetite in zebrafish. - protein-ligand interactions; - metabolomics using LC-MS/MS - mRNA expression

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