Pathogens and biological invasions: biological threats to coastal marine environments – Summary and main outcomes from Task 4.3.5

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Abstract

Within the framework of the PNRR-RETURN project – VS4 “Environmental degradation” – Task 3.5 was designed to address biological threats to coastal marine ecosystems. Activities within the Task leveraged advanced techniques, such as Next Generation Sequencing (NGS), lagrangian transport models, and Machine Learning, with the aim of investigating i) the spread of microbial pollution and ii) the expansion of invasive alien species, which represent critical threats to the economy, society, and environmental health. A multi-approach strategy combining sampling surveys, the collection of existing data, and numerical modeling was the core methodology.

Natural aquatic environments play a pivotal role in the dissemination of Antimicrobial Resistance (AMR) and pathogenic bacteria. Coastal marine environments, in particular, are final receiving bodies of microbial pollutants from multiple sources (e.g., rivers, wastewater discharges, agricultural runoff and stormwater). Due to the increasing anthropogenic pressure and to their direct connection to the land, coastal marine environments have the potential of disseminating and promoting the establishment of antibiotic resistance genes (ARGs) and opportunistic pathogens, with the cascade effect of promoting the spread of AMR and exposing millions of people to health risks (e.g. through food consumption and recreational activities).

Figure 1 – Sampling sites in the Gulf of Trieste.

The Gulf of Trieste was under study to assess the presence, prevalence and distribution of microbial pollutants (e.g. genes conferring resistance to antimicrobials, potentially pathogenic bacteria) through NGS techniques, in line with the modern principles of “Genomic Surveillance”. The sampling strategy was designed to describe the main sources of mesoscale variability (Figure 1); a one-year timeframe was chosen to capture the seasonal variability at each site.

Four amplicon-based sequencing approaches were tested for the detection of potentially pathogenic bacteria in seawater and sample sediment. Long-read metabarcoding allowed sequencing the full-length 16S gene (~1500 bp), providing improved taxonomic resolution and pathogen detection compared to short-read metabarcoding. Shotgun metagenomics was applied to elucidate the presence, prevalence, and distribution of ARGs and genes conferring resistance to disinfectants and other antimicrobials. To cover the maximum range of the ARG diversity we choose a “read-based” approach, using RGI (Resistance Gene Identifier) against CARD (Comprehensive Antibiotic Resistance Database).

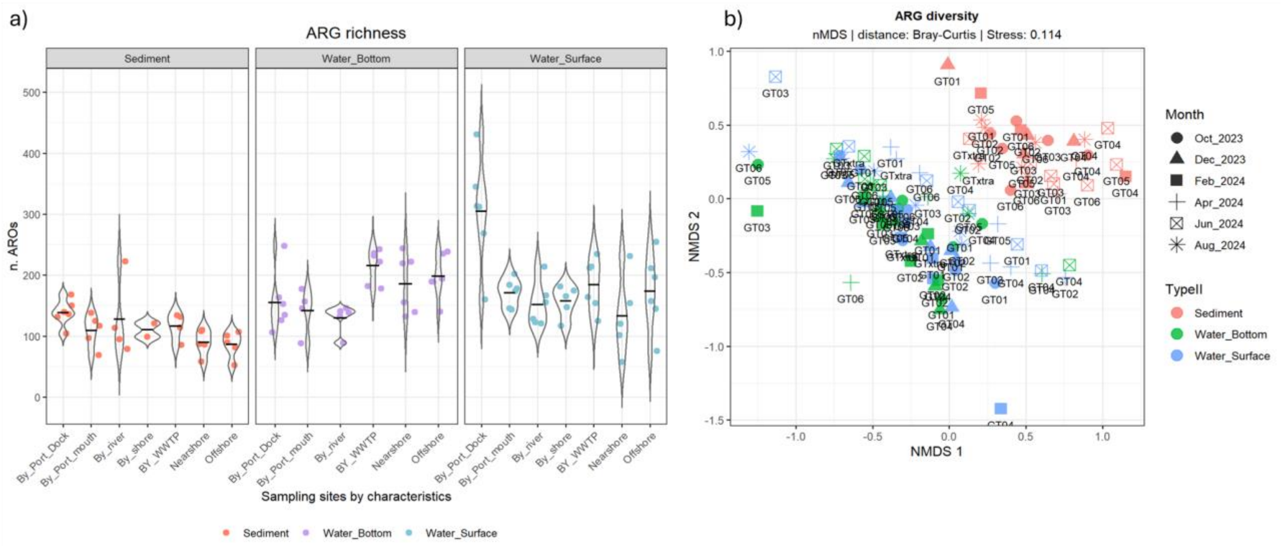

Figure 2 – ARG richness (a) and diversity (b) in the Gulf of Trieste.

Marine sediment revealed a more spatially and temporally stable resistome than the water column (Figure 2a), which on the contrary was characterized by a clear seasonal pattern (Figure 2b). The most abundant and recurrent genes found in the Gulf of Trieste cause resistance to the following antibiotic classes: fluoroquinolones, macrolides, cephalosporins, beta-lactams, tetracyclines, aminoglycosides, carbapenems, peptide antibiotics, rifamycin, lacosamide and streptogramins. However, gene conferring multidrug resistance (often based on efflux pump mechanisms) were found with high abundances and frequency, both in the sediment and the water column.

Data obtained by whole-genome and amplicon sequencing were coupled with the Gulf circulation data, organic load characterization, and other environmental variables, with the final scope of shedding light on the transport and fate of microbial pollutants in an archetypical coastal marine environment. At the same time, the untargeted metagenomic investigation is an extremely powerful tool to identify ARGs and to reveal their partitioning across environmental matrices, providing crucial information for planning high-resolution and site-specific AMR monitoring programs via targeted approaches (i.e. based for example on sensitivity tests, qPCR, or the deployment of biosensors).

The transport of pathogens and antibiotic resistance genes (ARGs) in the Gulf of Trieste was also addressed with a Lagrangian modelling approach. Simulations of ARG release from wastewater treatment plants, including during the November 3rd 2023 storm surge, reveal how the general circulation and meteo-marine events affect dispersal. Results identify critical areas, support the interpretation of field data, helping optimize sampling strategies and strengthening integrated monitoring.

Within the Task, we developed a modelling framework to assess current and future invasion risk of benthic invasive alien species in the Mediterranean. The approach builds on *MaxEnt* ecological niche models, but improves their reliability through extensive hyperparameter tuning and an objective, multi-criteria model selection procedure. Site-weighted performance metrics and novel delta metrics are used to reduce sampling bias, diagnose overfitting, and favour models that generalise well across space and time. Explicit uncertainty analyses, including extrapolation-risk mapping and sensitivity of suitability classes to threshold choice, provide spatially and temporally resolved confidence levels for predictions. Annual projections of habitat suitability, tested on the invasive alga *Caulerpa cylindracea*, enable detection of emerging hotspots and long-term trends, supporting prioritisation of monitoring and management actions. The framework is highly modular and transferable, and provides decision-ready maps and indicators for risk-aware planning.

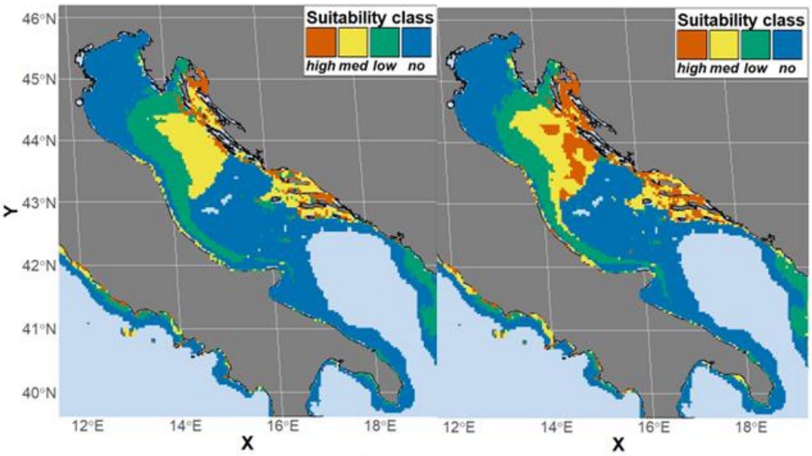

Figure 3 – Extrapolation risk map. Colours towards red indicate where future (2030-2050) conditions deviate significantly from the environmental combinations used to train the models ('strict extrapolation').

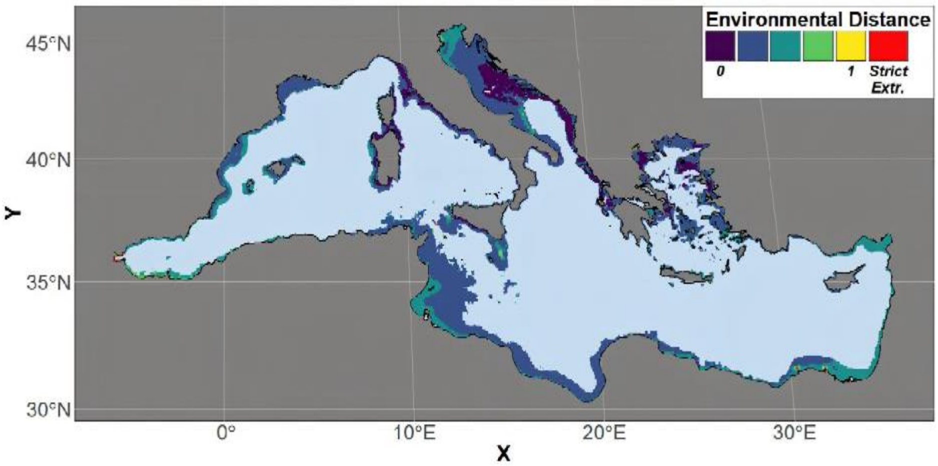

Figure 4 – A detail of the Caulerpa cylindracea's projected suitability for the future (2030-2050). The choice of a conservative (left) or stricter (right) threshold to classify the probability of occurrence to suitability strongly affects the results.