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iNEST Interconnected Nord-Est Innovation Ecosysyem
Via VIII Febbraio 1848, 2 - 35122, Padova
CF 92315730280 | Cap.Soc. Euro 100.000,00 i.v.
Email: info@consorzioinest.it
PEC: consorzio_inest@pec.it

Progetto Ecosistema dell'innovazione ECS00000043
"Interconnected Nord-Est Innovation Ecosystem (iNEST)"
CUP F43C22000190006
BANDO PER IL FINANZIAMENTO DI PROGETTI DI RICERCA
destinato a giovani ricercatori

RELAZIONE FINALE

Nome e Cognome PI	Federica Relitti
Titolo del progetto	RivEr DischargEs in the GulF of TrIeste: a coNtribution to the improvEment of the digital twin of the northern Adriatic Sea (REDEFINE)

Descrizione delle attività di progetto

Background e obiettivi

Climate and physical model projections indicate that the Northern Adriatic Sea (NAd) will undergo progressive oligotrophication, acidification, and deoxygenation in the coming decades, with an intensification of extreme hydrological events such as floods and droughts [1, 2]. These changes are expected to substantially modify riverine freshwater and nutrient inputs, key drivers of coastal biogeochemistry and ecosystem functioning [3, 4]. Despite the recognised importance of river discharge, most modelling approaches rely on climatological averages and do not adequately consider exceptional events, potentially leading to biased assessments of coastal biogeochemical dynamics and climate change impacts [2]. The NAd is a highly dynamic and biogeochemically complex system whose functioning is strongly controlled by riverine inputs [1, 2, 5–8]. While the Po River has been extensively studied as the dominant freshwater source to the basin [6, 9–14], much less attention has been devoted to minor rivers, despite their relevance at sub-basin scales [7, 11]. This knowledge gap limits the reliability of high-resolution modelling and hinders the development of a robust digital twin of the NAd. The REDEFINE project aims to fill these knowledge gaps studying the contribution of the main rivers of Friuli Venezia Giulia (Isonzo, Timavo, and Tagliamento) to the Gulf of Trieste (GoT), the northernmost basin of the NAd (Fig. 1). The GoT is a shallow basin highly sensitive to riverine inputs of freshwater, alkalinity, nutrients, and organic matter, which largely regulate its hydrology, productivity, and carbonate system dynamics [15, 16]. Among the rivers discharging into the GoT, Isonzo represents the main freshwater source and exhibits strong seasonal variability, with flood events driven by snowmelt and precipitation [17]. The second freshwater source is Timavo, which flows underground for about 38 km before re-emerging in proximity of its mouth. The Timavo has complex hydrological characteristics related to

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its karstic nature, as the flow at the mouth is also influenced by underground circulation within the karst aquifer, and several minor springs are scattered along the coastline [18]. Although the Tagliamento River is known to have the highest discharges compared to Isonzo and Timavo [11], it is rarely considered a relevant tributary to the GoT due to the prevailing cyclonic circulation characterising the NAd. Nevertheless, intense and frequent wind conditions can generate highly variable and even opposite surface layer circulations [19, 20], making the contribution of Tagliamento relevant for the GoT.

Rivers also act as vectors of contaminants and microbial contamination to coastal waters. Catchments affected by agriculture, urbanisation, and industrial activities can export antibiotic-resistant bacteria (ARB), antibiotic resistance genes (ARGs), and other cofactors promoting antimicrobial resistance (AMR) dissemination [21–23].

Recent investigations by the chemical and biological oceanography OGS group (BIOCHEM) have highlighted a lack of recent, high-frequency data on riverine nutrient concentrations delivered to the GoT, underscoring the urgent need for a systematic, multi-parameter characterisation of freshwater inputs. This gap limits our ability to quantify riverine contributions to coastal biogeochemistry, to assess their role in blue carbon cycling, and to properly constrain predictive models under ongoing climate change. The REDEFINE project aims to contribute to blue carbon research by filling the knowledge gaps on FVG freshwater inputs to the GoT. Specifically, REDEFINE main objectives are: (i) provide a monthly biogeochemical characterisation of each FVG river flowing into the GoT (Isonzo, Timavo and Tagliamento) in terms of nutrients, carbonate system parameters and organic matter loading; (ii) quantify the role of these rivers as sources of microbial contamination, with particular focus on faecal contamination indicators, potentially pathogenic bacteria, and genetic determinants involved in AMR dissemination; (iii) evaluate the variability of biogeochemical parameters and microbial contamination during flood events in different seasons; (iv) compile a comprehensive, FAIR (Findable, Accessible, Interoperable and Reusable)-compliant dataset to support the scientific community, stakeholders, and policymakers.

By integrating high-precision biogeochemical measurements with molecular approaches for microbial and AMR surveillance, the REDEFINE project aims to establish a baseline for evaluating present and future impacts of freshwater inputs on the GoT. The project will support the development of forecasting tools and climate change impact assessments for the NAd, while providing new insights into the environmental ecology of AMR dissemination in coastal systems.

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Metodologie

Sampling stations were located in proximity of each river mouth, upstream of the saltwedge to avoid the potential influence of seawater (Fig. 1, Tab. 1). Since Timavo has several minor springs scattered along the coastline, the permanent spring located in Aurisina (AUR) was selected for this study [18].

Figure 1 – Study area.

Table 1 – Sampling stations.

Station	Station code	Latitude	Longitude
Isonzo	ISO	45°48'20.4"N	13°25'45.6"E
Tagliamento	TAG	45°46'13.0"N	12°59'50.0"E
Timavo	TIM	45°47'16.2"N	13°35'28.2"E
Aurisina	AUR	45°44'34.2"N	13°39'57.0"E

The physico-chemical properties (temperature, pH, conductivity) of each river were measured using a portable multi-parameter probe (YSI Professional Plus). To characterise the biogeochemical properties of rivers, discrete surface water samples were collected using a horizontal Niskin bottle for the determination of total alkalinity (TA); dissolved inorganic nutrients (nitrite – $N\text{-}NO_2$, nitrate – $N\text{-}NO_3$, ammonium – $N\text{-}NH_4$, phosphate – $P\text{-}PO_4$, and silicate – $Si\text{-}Si(OH)_4$); dissolved inorganic carbon (DIC); dissolved organic carbon (DOC); dissolved cations (Ca, Mg, Sr); total suspended matter (TSM); chlorophyll *a* (Chl *a*) and particulate organic carbon (POC). Samples were also collected for the analyses of the isotope composition of dissolved inorganic carbon ($\delta^{13}C\text{-}DIC$); dissolved oxygen ($\delta^{18}O\text{-}H_2O$)

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and particulate organic carbon (δ^{13}C-POC). Samples for inorganic nutrients were analysed as described in Ingrosso et al. [16] using a four-channel continuous flow analyser QuAAtro (Seal Analytical Inc., USA) AutoAnalyzer. Total alkalinity was analysed by an open cell potentiometric titration, using a Mettler Toledo G20, according to the SOP 3b [24], modified for the analysis of freshwater. Dissolved organic (DOC) and inorganic carbon (DIC) were determined using a Shimadzu TOC-V CSH analyser as described in De Vittor et al. [25]. Total suspended matter (TSM) was determined gravimetrically [26, 27]. The concentration of cholorophyll *a* (Chl *a*) was determined spectrofluorometrically as detailed in Kralj et al. [28]. Particulate organic carbon (POC) was analysed as described in Quero et al. [29], using an elemental analyser (Vario PYRO Cube, Elementar, Germany), coupled with an isotope ratio mass spectrometer (IRMS - IsoPrime 100, Elementar, UK) for the simultaneous determination of the carbon stable isotope composition (δ^{13}C-POC). Carbon stable isotopes are stated on VPDB-LSVEC Carbon Isotope Delta Scale.
Two external scientific partners were involved in REDEFINE activities for the determination of the stable isotope composition of dissolved inorganic carbon (δ^{13}C-DIC) and dissolved oxygen (δ^{18}O-H_2O) and for the analysis of the concentration of dissolved cations (Ca, Mg and Sr), as detailed in the following section.
Basic and multivariate statistical analyses were performed with the software PRIMER-e (Version 7.0.24; [30]).
Additional samples were collected to assess the potential of each river as a source of microbial contamination. Genomic DNA was obtained by filtration through sterile 0.22 µm PES membrane filters and subsequent extraction using the DNeasy PowerWater Kit (Qiagen) with a few modifications to the manufacturer's protocol to maximise extraction yields. The concentrations of antibiotic resistance genes (ARGs) against some commonly used antibiotic families were assessed by qPCR using a SYBR Green detection chemistry (SsoAdvanced universal SYBR Green supermix, Bio-Rad). Primer sets, standard calibration curves and the analytic conditions used here were described in Fonti et al. [31]. The riverine microbiome composition was resolved by full 16S rRNA gene amplicon sequencing by Oxford Nanopore technology. The metagenomic library was prepared according to the manufacturer protocol using a PCR-free barcoding strategy, loaded onto a R10.4.1 flow-cell and sequenced on the MinION 1Kb platform system, with a run time of 24 h. The basecalling (i.e. the conversion of the electric signal into strings of nucleotides-like information) was performed in the CINECA LEONARDO_B cluster under the IsCd2 project GOThAMr (project ID HP10C36WCV). Culture-based analysis of faecal indicators bacteria was performed according with ISO 9308-1:2014 [32], which provides enumeration of β-D-galactosidase-positive *E. coli* and coliform bacteria.

Attività di collaborazione

The REDEFINE project benefitted from a consolidated collaboration with the University of Trieste and the Slovenian international research centre Jožef Stefan Institute (Ljubljana).
The interdisciplinary research of the department of Mathematics, Informatics and Geosciences (MIG) of the University of Trieste (Trieste, Italy) focuses on investigating the biogeochemical cycle of trace and major elements and studying their abundance and distribution in surface environments. Thanks to cutting-edge instrumentation, MIG provided the complementary expertise on cations dissolved in water (Ca, Mg and Sr) via Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) using an Optima 8000 spectrometer equipped with an S10 autosampler (Perkin Elmer).
The department of Environmental Sciences (DES) of the Jožef Stefan Institute (Ljubljana, Slovenia) focuses on interweaving the physical, chemical, and biological processes that shape the environment. DES interdisciplinary and multidisciplinary research covers many areas, such as environmental analytical chemistry, biogeochemical cycles, microbial ecology, environment and health, environmental technologies, risk and environmental assessment, and

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environmental monitoring. DES laboratories specialise in inorganic and organic analytical chemistry, radiochemistry, and isotope ratio analysis. Researchers from DES were involved for their complementary expertise on the isotopic ratio of stable carbon in the dissolved inorganic carbon (δ^{13}C-DIC) and oxygen (δ^{18}O-H_2O). δ^{13}C-DIC was analysed from the headspace using Continuous-Flow IRMS Europa 20-20 with ANCA TG trace gas separation module, whereas δ^{18}O-H_2O through a IRMS delta Q (Thermo) in continuous flow mode with Gasbench. Delta values are stated on VSMOW-SLAP Oxygen Isotope Delta Scale, and VPDB Carbon Isotope Delta Scale, for δ^{18}O-H_2O and δ^{13}C-DIC, respectively.
The data provided by the external scientific partners are extremely valuable for characterising rivers' carbonate system.

Risultati finali e impatto del progetto

Risultati finali
The aim of the REDEFINE project is the biogeochemical characterisation of Isonzo, Timavo and Tagliamento Rivers to define nutrients, alkalinity and organic matter inputs into the GoT in different hydrologic conditions and seasons. Monthly sampling was carried out from October 2024 to June 2025. Thanks to the strategic location of the BIOCHEM laboratories and the availability of real-time data discharge data of Isonzo River provided by the Protezione Civile – Friuli Venezia Giulia river current meter (station CURRISO [32]), it was also possible to collect samples during three flood events (Fig. 2, Tab. 2) in Autumn (October 2024) and Spring (March and April 2025). The data produced during the project activities are available in the Zenodo repository [34] under an open-access policy.

Table 2 – Sampling dates and discharge regime conditions.

Sampling date	Discharge regime
04/10/2024	Flood
15/10/2024	Low
11/11/2024	Low
12/12/2024	Low
17/01/2025	Low
17/02/2025	Low
13/03/2025	Flood
16/04/2025	Flood
13/05/2025	Low
18/06/2025	Low

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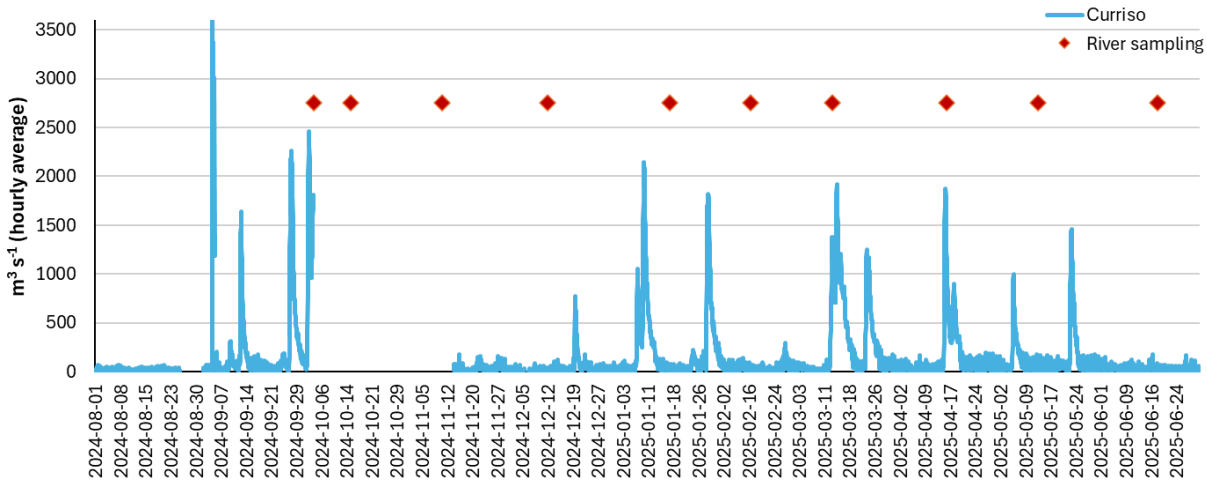

Figure 2 – Sampling events (red diamonds) and Isonzo River flowrate (blue line, $m^3\ s^{-1}$) recorded by the Isonzo current meter at CURRISO station [32] in the timeframe of REDEFINE project activity (from 01/10/2024 to 30/06/2025).

The main results obtained from the REDEFINE activities are summarised by the multivariate analysis (non-metric Multidimensional Scaling- nMDS; Fig. 3). Overall, the rivers investigated exhibited distinct biogeochemical characteristics, although with some degree of overlap (nMDS stress: 0.1; analysis of similarities ANOSIM one-way R=0.699). Timavo (TIM) was generally characterised by higher TA and DIC concentrations (AT: 4428 ± 263 µmol kg^{-1}; DIC: 4380 ± 239 µmol L^{-1}) and lower δ^{13}C-DIC values (-12.3 ± 0.9 ‰$_{VPDB}$) compared with Isonzo (ISO; AT: 2957 ± 313 µmol kg^{-1}; DIC: 2758 ± 226 µmol L^{-1}; δ^{13}C-DIC: -9.5 ± 1.3 ‰$_{VPDB}$) and Tagliamento (TAG; AT: 3334 ± 426 µmol kg^{-1}; DIC: 3201 ± 312 µmol L^{-1}; δ^{13}C-DIC: -7.9 ± 0.3 ‰$_{VPDB}$). These differences are mainly attributable to the distinct characteristics of the catchment basins and to the nature of the river courses (hypogeous *vs*. surface flow). The relatively high Mg/Ca ratios observed in Isonzo (0.27 ± 0.03) and Tagliamento (0.43 ± 0.03) indicate the dissolution of dolomite in addition to calcium carbonates. In contrast, Timavo catchment basin is primarily characterised by carbonates (Mg/Ca TIM: 0.12 ± 0.03; Mg/Ca AUR: 0.06 ± 0.01). Along the hypogeous course of Timavo, enhanced mineral dissolution leads to higher concentrations of DIC, TA, nitrates and phosphate, whereas reduced CO_2 degassing results in lower δ^{13}C-DIC values. These features are especially evident at the Aurisina station (AUR), where sampling is conducted directly at the spring. Here, CO_2 degassing is limited and biological nutrient uptake is constrained due to low light availability, as also confirmed by the low Chl *a* concentrations (0.06 ± 0.09 µg L^{-1}). Despite the differences among catchment basins, no differences were observed in the quality of POC delivered by rivers. Indeed, δ^{13}C-POC values for all rivers (-27.82 ± 0.75 ‰$_{VPDB\text{-}LSVEC}$) fall within the typical freshwater POC range [35], suggesting low contribution of terrestrial inputs.

No seasonal pattern was identified for the parameters investigated. However, during high-discharge events, all rivers - especially Isonzo and Tagliamento - showed increased concentrations of TSM, DOC and POC, as indicated by the observations plotting outside the groups identified by the similarity profile analysis (SIMPROF, Fig. 3). This suggests a direct relationship between discharge and detritus and organic matter concentration. Conversely, the concentrations of inorganic nutrients, total alkalinity and DIC do not appear to be affected by flood events. This likely suggests the combined effect of increased water volume and reduced residence time, which limits the extent of mineral dissolution.

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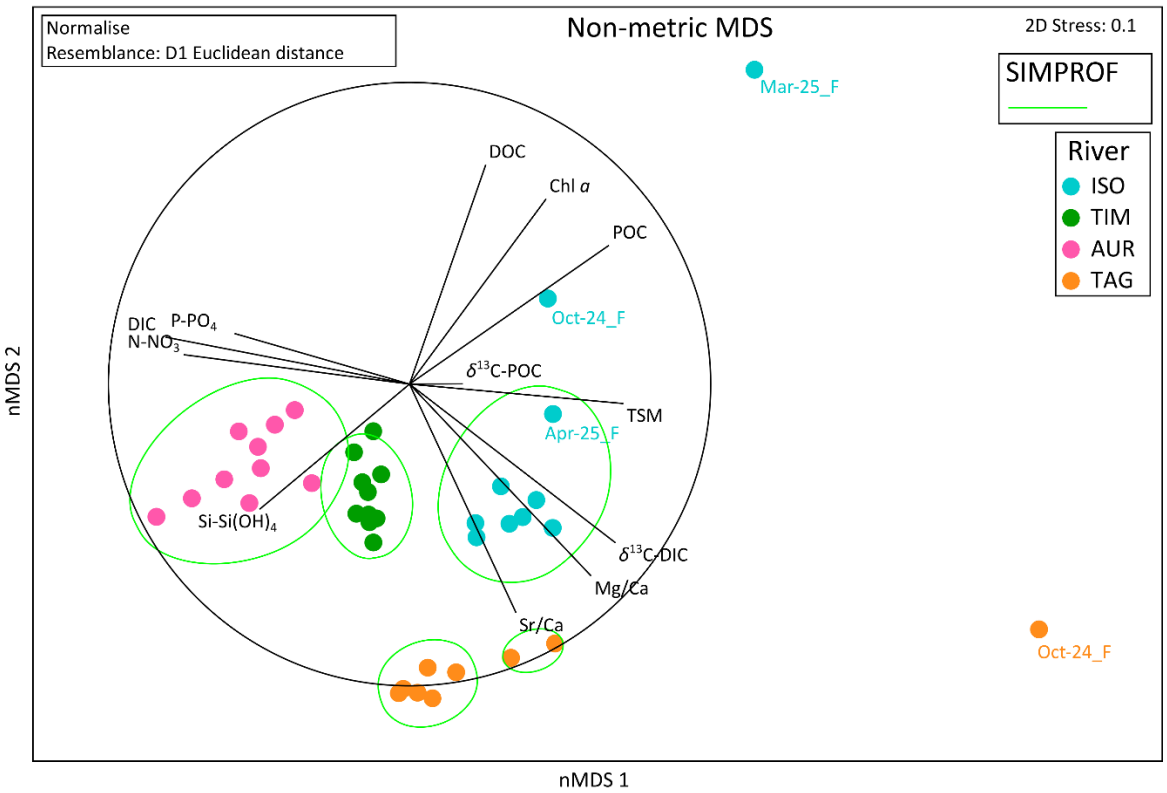

Figure 3 – Non-metric Multidimensional Scaling (nMDS) ordination plot of REDEFINE results. Dissimilarity was calculated using the Euclidean distance metric. The multivariate analysis was conducted only including the variables that did not result autocorrelated. The green lines represent the groups identified by the similarity profile (SIMPROF) analysis.

Overall, the results on riverine nutrient and carbon input to the GoT contribute to improving the knowledge on the biological and biogeochemical processes of the coastal ecosystem (e.g. phytoplankton bloom, mucilage, acidification). The differences observed among Isonzo, Timavo and Tagliamento, especially for carbonate system parameters, provide relevant information for assessing their respective influence on the GoT. The REDEFINE results also highlight the potential relevance of the coastal springs. Specific discharge data for these freshwater inputs are currently lacking; however, several springs are scattered along the coastline, suggesting that this area may represent an important, yet largely overlooked, source of freshwater to the GoT, which should be considered especially when studying coastal processes.

The investigation on the biogeochemical characterisation of FVG river inputs to the GoT will be further explored by coupling REDEFINE results with the discharge data provided by the local authority ([36] for Isonzo and Tagliamento) and a water management company ([37] for Timavo), which will be made available within a few months.

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From microbiological point of view, the Tagliamento and Isonzo Rivers showed significantly higher load of FIB (Faecal Indicator Bacteria, i.e. *Escherichia coli* and total coliforms) compared to the Timavo and Aurisina sources (Kruskal-Wallis test, H= 26.8 and H= 31.2 for *E. coli* and coliforms, respectively, p <0.001, followed by Dunn's post-hoc test) and despite the highest count fluctuation observed for the Isonzo River (Fig. 4A). Timavo and Aurisina display lower baseline contamination, characterised by intermittent *E. coli* detection.
As regards the degree of potential AMR dissemination, a persistent albeit fluctuating presence of genes conferring resistance to sulfonamides (*sul*2), macrolides (*erm*B), fluoroquinolones (*qnr*S) and beta-lactam antibiotics ($bla_{CTX\text{-}M}$) was observed across multiple samples collected in the Tagliamento and the Isonzo Rivers (Fig. 4B). Conversely, those genes were found mostly occasionally in the samples collected for the Timavo River. In particular, *sul*2 and *erm*B emerged as the most prevalent genes for the Timavo River, at both the sampling sites, despite with concentrations often lower than those recorded in the Tagliamento and Isonzo Rivers. Samples collected at the Aurisina spring exhibited only a sporadic presence of the *bla*CTXM gene.
The *mcr-1* gene, which encodes for colistin resistance by a mechanism of target-modification, was also investigated but it was found absent (i.e. below the virtual limit of detection of 0.75 copies/ml) in all samples.

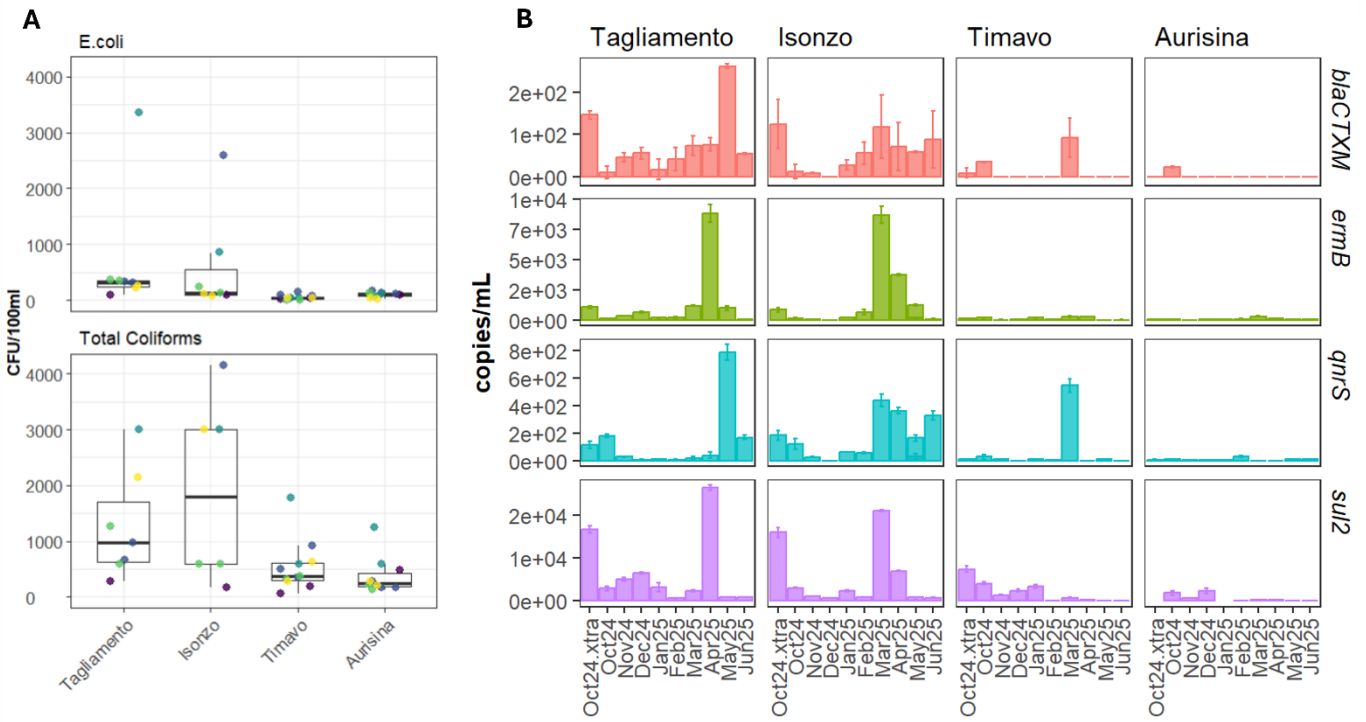

Figure 4 – Distribution of fecal indicator bacteria (A) and antibiotic resistance genes (B) across the four sampling sites. Individual data points in (A) are coloured by month: lighter colours indicate warmer months, darker colours the colder ones. Error bars in (B) represents the standard deviation.

In this study the microbiome analyses via Oxford Nanopore was designed to resolve the microbial community by addressing the 16S rRNA gene in its full length, which is not possible with current gold-standard NGS. The sequencing generated 6.27 million reads (i.e. 10.62 GB dataset). Basecalling and reads preprocessing have been

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performed successfully. Taxonomic reconstruction is currently underway with the aim of extracting the full list of potentially pathogenic bacteria present in the dataset.

Impatto del progetto

The dataset containing the results of the REDEFINE project activities has been published in the Zenodo repository [34] under an open-access policy to ensure data availability for the scientific community and stakeholders. The data collected within REDEFINE project contribute to improving knowledge on nutrients, carbonate system parameters and organic matter discharged into the GoT by FVG rivers under different hydrological regimes. They also support studies on the role of FVG rivers in the dissemination of AMR and microbial contamination in the GoT. These results, combined with freshwater discharge data measured by a local authority [36] and a water management company [37], will be essential for estimating the total river input of nutrients, alkalinity and organic matter to the GoT.
REDEFINE results integrate the expected outcomes defined in Spoke 8 of iNEST and support the datalake of the iNEST winning project Adr.IA (eXact lab Srl), which aims to implement the digital twin of the NAd. In addition, REDEFINE results support the validation of existing models, such as the forecasting system for the physical and biogeochemical conditions of the NAd (MedEAF [38]) developed by OGS within the framework of the EU Copernicus programme [39], to predict future climate change scenarios. REDEFINE is also closely interconnected with the iNEST Subtask RT 1.3.1 project and the Project NEREIDES-Bando Giovani Ricercatori (Dr. Davide Lombardo). These initiatives focus on the influence of Isonzo river plume on the benthic environment and evaluate surficial currents in the GoT, respectively. The synergy between these projects and REDEFINE will enhance the overall impact, providing a comprehensive understanding of the GoT functioning and an extensive dataset for the development of NAd digital twin, which aims to support informed management, operational, and policy decisions on the real twin.
The REDEFINE project provides entirely novel baseline data about the ARGs in the study area, which have a high potential to benefit local authorities in developing advanced AMR monitoring programs and AMR management strategies. Moreover, the outcomes of REDEFINE will support stakeholders involved in blue carbon research and management, contributing to climate policy integration and habitat conservation aimed at enhancing carbon sequestration in coastal ecosystems.

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iNEST Interconnected Nord-Est Innovation Ecosysyem
Via VIII Febbraio 1848, 2 - 35122, Padova
CF 92315730280 | **Cap.Soc**. Euro 100.000,00 i.v.
Email: info@consorzioinest.it
PEC: consorzio_inest@pec.it

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