TA PROJECT REPORT

Project Information

Proposal reference number[1]	22/1002932
Experiment Acronym (ID)[2]	PoGo
Title of the project[3]	Po delta to Gulf of Trieste: Microbiological connectivity study and field testing of a Video-CTD probe prototype
Host Research Infrastructure[4]	S1-GB
Starting date - End date[5]	3 May 2023 – 31 October 2023
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Project objectives[7] (250 words max.)

The first objective of the PoGo project was to compare ADCP (acoustic doppler current profiler) and microbial ecology data at two different North Adriatic areas, Po Delta – site S1-GB and Gulf of Trieste – site Vida, characterized with different degree of anthropogenic pressure and riverine water discharges. Besides understanding how the differences in physio-chemical and biological parameters affect microbial community dynamics, our aim is to provide the first insights on ecological connectivity between sites by coupling microbial analysis with oceanographic observations and numerical modelling.

The second objective of the PoGo proposal was to test a Mini Video-CTD probe (V-CTD) prototype developed by the National Institute of Biology in waters with high salinity variations. The probe prototype has already been tested in Slovenian national waters and additional testing would be required in a different location. The waters at S1-GB feature high salinity differences and present a perfect testing site for such a device.

Main achievements and difficulties encountered (250 words max.)[8]

The S1-GB is an elastic beacon equipped with meteo-oceanographic sensors, located 4 nautical miles south of the mouth of Po di Goro (Po River Delta-Northern Adriatic). The station consists of an aerial platform at 6.5 m above sea level, a steel pipe structure, a submerged float and an elastic joint for mooring to the sinker. The beacon is located at a key monitoring point for the study of interactions between the Upper Adriatic and the Po River, experiencing a wide range of oceanographic conditions.

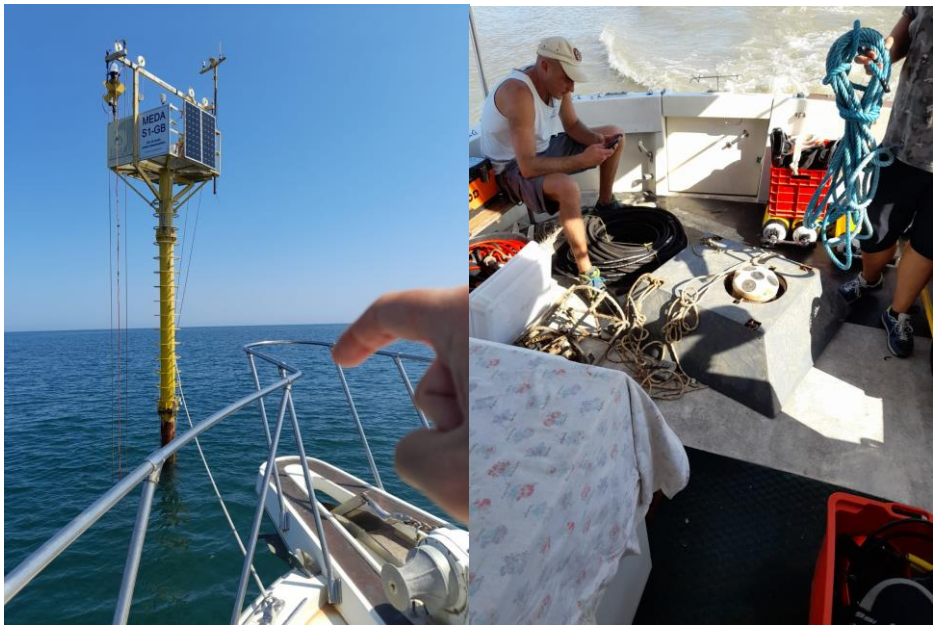


Figure 1: S1-GB beacon and the recovered ADCP still in the housing (Foto: Francesco Riminucci).

The project faced extreme difficulties in securing a suitable time window for site access. This was mainly due to many extreme weather events and frequent wind during the duration of the project. Despite these, we were able to perform four field campaigns, two of these as a joint team from both institutions and two additional campaigns by the host institution.

We successfully deployed an acoustic doppler current profiler (ADCP; Nortek AWAC - 1 MHz) which continuously measured currents at the location from 19 July 2023 to 29 September 2023. We performed two samplings for microbiological analysis. Several casts with the V-CTD prototype were performed on one of the campaigns.

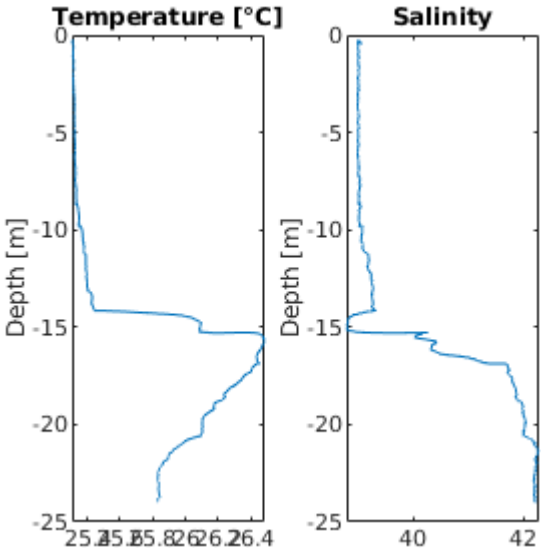
The ADCP has been positioned at the soft bottom near the station and secured by a rope which was fixed at the S1-GB pylon. Unknown perpetrators have cut the rope and the ADCP was considered lost for some time, only to be later recovered by two additional campaigns by the host institution with specialized team of professional divers .

Dissemination of the results[9]

All work carried out in PoGo, will be disseminated through scientific conferences and scientific articles in peer-reviewed journals. The ADCP and V-CTD raw data are already made publicly available in Zenodo repository dedicated to this project (DOI: 10.5281/zenodo.10123570; https://zenodo.org/records/10123570). Other obtained data will be added to the repository later, after analysis and inspection of results. All the emerging omics data will be deposited at publicly available databases (i.e.European Nucleotide Archive).

The work done in the scope of PoGo project matches well with Biodiversa PETRIMED project. Further field campaigns are planned in the scope of PETRIMED, and the data obtained during PoGo will be used in PETRIMED as well.

Technical and Scientific preliminary Outcomes (2 pages max.)[10]



The V-CTD was deployed three times on 17 October 2023. The interface between fresher and colder water (Po outflow) and saltier and warmer water can be observed at around 15 m depth. Salinity and temperature profiles were obtained with a calibrated SeaBird CTD (operated by the host institution) as well. These will be compared with prototype measurements. The accompanying video, recorder with the camera, which is part of the V-CTD prototype, shows a school of small fish at 15 m depth and a ctenophore at about 5 m depth. A recalibration of the prototype V-CTD will be performed in the following months and recalibrated values will be again compared with the SeaBird CTD measurements.

Figure 2: Temperature and salinity profiles as measured by the mini-CTD prototype.

The ADCP was mounted to a custom-made housing. The bottom of the housing was blocked with a wooden plate to reduce sinking into the mud that covers the bottom at the site. The method proved successful as the ADCP recorded the currents throughout the deployment period. The tidal signal can be observed in the pressure sensor data and in currents as well. As expected, there are several instances with pronounced currents in the northward or eastward directions which could contribute to water transport towards the Gulf of Trieste. These measurements will be related with the microbial analysis and circulation models to indicate whether such transport did indeed

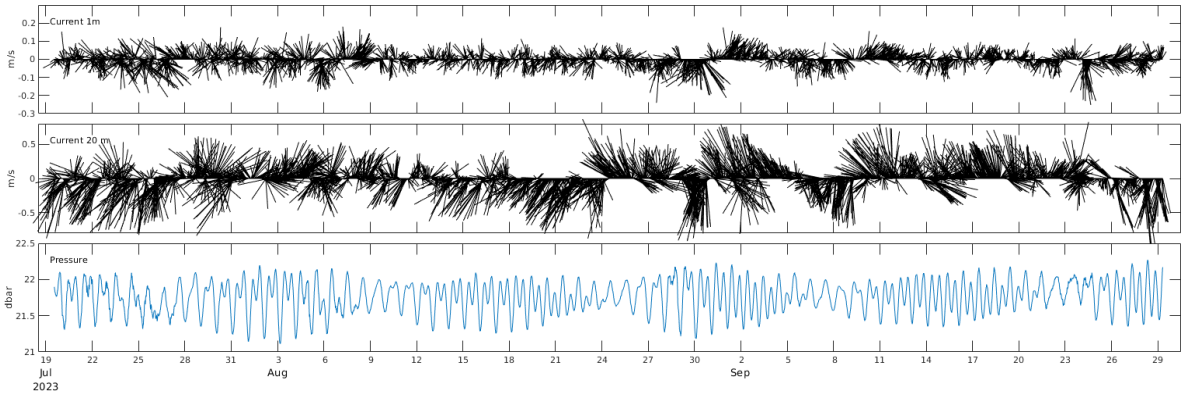


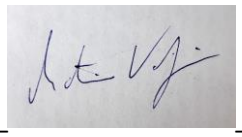
Figure 3: Currents measured 1 m (top) and 20 m (middle) above the ADCP, and pressure (bottom) as recorded by the ADCP current sensor.

The abundance, diversity and functional potential of microbial communities occupying surface layer of water column will be analyzed. For microbial abundance we sampled in triplicates bulk seawater and fixed samples with glutaraldehyde immediately after sampling. Samples are flash-frozen in liquid nitrogen and will be kept at -80C until processed. For HPLC-based analysis of phytoplankton

community composition 1L of bulk seawater samples was collected onto GF/F filters (glass fiber, Whatman), in triplicate, and flash-frozen in liquid nitrogen and will be kept at -80C until further processed. For analysis of microbial community composition and function bulk seawater samples were collected onto 0.2 um polyether sulfonic filters. We collected several replicates of 500mL samples, flash-frozen collected filters in liquid nitrogen, which will be kept at -80C until further processed.

Microbial abundance will be determined using flow cytometry (as described elsewhere, Orel et al., 2022), diversity of phytoplankton community will be determined via HPLC-based analysis of phaeopigments (as described elsewhere, Flander-Putrle et al., 2022) and via metabarcoding (i.e., targeting 18S rRNA genes, as described in Turk Dermastia et al., 2023). The composition of bacterial community will be determined via 16S rRNA bacterial genes using next-generation sequencing platforms (as described in Orel et al., 2022). The insights into the functional potential of microbial community will be provided by applying metagenomic approach (as described in Tinta et al., 2023). All constructed datasets will be analyzed using state-of-the-art bioinformatics and statistics tools.

[Location], [Date (dd/mm/yyyy]
__Ljubljana, 13/11/2023_____



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Location and date

Signature of principal investigator