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JERICO-S3 DELIVERABLE

Joint European Research Infrastructure for Coastal Observatories
Science, Services, Sustainability

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EXECUTIVE SUMMARY

This deliverable documents the main design elements and testing activity of the three technological demonstrators, developed in the course of the JERICO-S3 project and implemented in Pilot Super Sites and Integrated Regional Sites. It concerns two integrated systems and sensor packages addressing respectively the pelagic environment (cEGIM-Powered Plankton Dynamics Sensor Package - PSP) and the benthic environment (Autonomous Coastal Observing Benthic Station - ACOBS), and the Water-sampler, filtration and preservation device (WASP), designed to enhance our understanding of specific coastal ecosystem processes through fit-for-purpose observations. The PSP is dedicated to the smart observation of Plankton dynamics through the concomitant measurement of essential physical, biogeochemical and biological variables. ACOBS is designed to assess benthic biological and biogeochemical processes, including oxygen fluxes, by integrating multiple sensors and technologies. The WASP system combines a water sampling module, and filtration/preservation module to collect and preserve environmental DNA (eDNA) samples, and is further integrated in NIVA´s Ferrybox system. Bench testing results demonstrate the effectiveness and reliability of these systems in both controlled and field environments, providing valuable insights for future deployments and advancements in marine observation technologies, under the framework of JERICO.

1. INTRODUCTION

The JERICO initiative represents a significant advancement in marine science and environmental monitoring. As a collaborative effort funded by the European Union, JERICO aims to enhance our understanding of coastal and shelf sea environments through the development and deployment of advanced observation systems. By integrating a network of coastal observatories, JERICO addresses key scientific challenges related to coastal dynamics, marine ecosystems, and environmental changes.

One of the critical areas of focus within JERICO is the advancement of technologies for integrated observation of ecosystem processes, including marine biology.

This deliverable is documenting the development of demonstrators. Detailed information of the technical innovation and their demonstration are to be found respectively in deliverables D7.7 and D7.9. The Plankton Dynamics Sensor Package (cEGIM-Powered PSP) and the Autonomous Coastal Observing Benthic Station (ACOBS) represent cutting-edge tools for optimal observing and analysing key biological and environmental variables describing pelagic and benthic processes respectively. The WASP (Water and Sediment Profiler) system further enhances this effort by providing advanced capabilities for the collection and preservation of phytoplankton and environmental DNA samples.

The deliverable highlights the significance of these testing phases, demonstrating the potential of these technologies to contribute valuable insights into coastal and marine ecosystems.

2. PLANKTON DYNAMICS SENSOR PACKAGES - PSP

2.1. The integration platform - cEGIM[1]

In the context of *JERICO-S3*, the EGIM, developed within the frame of the European Union funded H2020 project EMSOdev, has been transformed and adapted to perform in coastal environments and to address JERICO´s Key Scientific Challenges (KSCs). This Coastal EGIM (c-EGIM) is able to operate on mooring line, sea bed station, and surface buoy. A central function of the c-EGIM is to provide a novel capacity for observing complex and coupled coastal processes, through the integration of dedicated sensors, providing process-targeted datasets, which are measured in a manner that optimises the understanding of the dynamics of specific coastal processes. The c-EGIM approach builds upon the use of homogenised hardware, sensor references, qualification methods, calibration methods, data format and access, and maintenance procedures.

The cEGIM (with its COSTOF-2 core) has been used as the IoT-enabling platform on which the Plankton dynamics Sensor package has been developed. The cEGIM is providing an IoT framework for the integration of multiple sensors, and enables the development of smart AI-based data applications. The PSP aims to optimally observe physical, biogeochemical and biological variables of importance for the description and understanding of the dynamics of phyto- and zooplankton.

Twelve data ports are available. Four have been used for standard EOVs, namely CTD, dissolved O2, turbidity, and ocean currents. Seven ports have been dedicated to variables (sensors) describing plankton dynamics, namely biological variables (algal pigments, cell size, Phyto-primary production) and biogeochemistry (nitrate, DOC, pCO2, pH).

2.2. List of sensors

The selection of variables and sensors was based on the comprehensive lists of Essential Oceanographic Variables (EOV) and Essential Biodiversity Variables (EBV) defined by the Global Ocean Observing System (GOOS) and necessary for studying phytoplankton. The final list of sensors for the test and demonstration phases was chosen by balancing the importance of the variables, the feasibility of developing suitable drivers for COSTOF2 (presented in the next section), and the availability of hardware among the project teams.

The various sensors that were mounted on the cEGIM for a demonstration deployment in the Bay of Seine (English Channel PSS) are listed in the following table:

[1] The JIIM has been renamed cEGIM (Coastal-EGIM)

Table 1 - List of sensors used for cEGIM-powered PSP, during the demonstration in the Bay of Seine.

Sensor name	Brand	Parameters measured	Resolution
Fluoroprobe	BBE Moldaenke	Total chlorophyll [eq. µg chl-a/l] Concentration [eq. µg chl-a/l] of: • Green algae • Blue-green algae • Diatoms/brown algae • Cryptophytes • Various fingerprints of classes/species, also user-defined • Yellow substances Depth (m)	0.01 eq. µg chl-a/l
MP6	NKE	Pressure (m) Temperature (°C) Conductivity (mS/cm) Dissolved Oxygen (0.01%) Fluorescence (µg/l) Turbidity (NTU)	0.006 m 0.05 °C 0.0012 mS/cm 0.01 % 0.03 µg/l 0.012 NTU
ISUS	Satlantic	Nitrate (µM) Bromide (µM) Sulfite (µM)	0.2 µM

2.3. Main functionalities

The cEGIM's array of sensors is managed by an electronic control unit called COSTOF2 (COmmunication and STOrage Front-end - 2nd generation), a multi-sensor acquisition and power supply system developed by Ifremer. COSTOF2 represents the culmination of extensive research and development from various deep-sea observatory projects. In its coastal applications, COSTOF2 was modified to fit a "shallow" Polyoxymethylene (POM) enclosure, suitable for depths up to 200 metres. This "shallow" version is used in the cEGIM platform.

COSTOF2 powers and configures the connected sensors, synchronises their measurements, and stores the resulting data. It can be connected to the surface via a cable for power supply and/or data transfer. Alternatively, COSTOF2 can operate completely autonomously when powered by an energy enclosure. In this autonomous mode, the COSTOF2 is fully configured on the surface before being deployed at sea, with data stored internally until retrieval.

The other added value of the PSP is to include an AI driven sampling controller which allows phytoplankton bloom detection based on the measurement of two parameters:

- Measurement of fluorescence (Fluo) via the BBE_Fluoroprobe sensor,

- Measurement of nitrate content (Ntr) via the ISUS_Satlantic sensor.

An algorithm developed conjointly by the Université du Littoral de la Côte d'Opale and Ifremer ensures bloom detection using the two Fluorescence and Nitrate measurements received periodically. The challenge is to integrate the algorithm into COSTOF2 to enable in situ bloom detection, and to increase the sampling frequency of all sensors while bloom is being detected.

For example, we can go from an acquisition period of 2h in normal conditions to 20 min during the bloom period, then back to 2h when the bloom is over. This behavior optimizes the quality and relevance of measurements, while preserving the batteries of energy-independent systems.

Data is pushed to a cloud in quasi-real time.

2.4. Integration and testing

During the Sainte-Anne du Portzic demonstration starting December 16, 2022, the cEGIM-powered PSP system was put to the test. This package combines specialised hardware and software for studying plankton at an adaptable sampling rate, to cope with the multi-scale dynamics of plankton ecology. The cEGIM was deployed at a depth of 8 meters, connected to a cable for power and data transfer. This setup allowed real-time testing of the PSP's sensors, including the Fluoroprobe for measuring fluorescence and the ISUS for nitrate levels. By operating in this setup, the team could verify that the cEGIM was effectively handling high-resolution data collection and analysis.

This demonstration, carried out under JERICO-S3, validated the PSP integration within the cEGIM and showcased the system's reliability for studying plankton dynamics. It was an essential step to ensure that the technology would perform as expected during the subsequent, more extensive deployment in the English Channel in the spring of 2023.

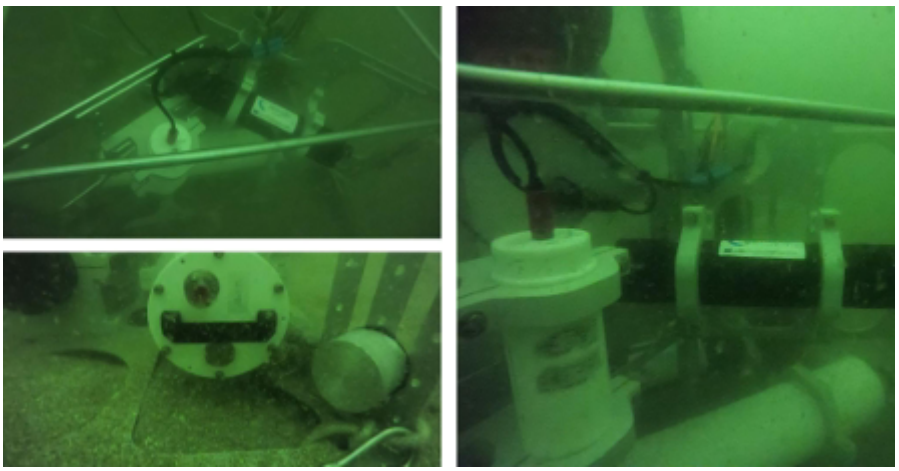


Figure 1 Several pictures taken during the PSP recovery in Sainte-Anne du Portzic (Pre-demonstration phase).

3. AUTONOMOUS COASTAL OBSERVING BENTHIC STATION - ACOBS

3.1. List of sensors

ACOBS is including several devices in order to integrate simultaneously acquired data on: (1) bottom seawater characteristics, (2) Diffusive oxygen fluxes at the sediment-water interface, (3) Total oxygen fluxes at the sediment-water interface, and (3) Benthic activity at the sediment-water interface and in the upper sediment column.

Bottom seawater characteristics
- SDOT NKE (oxygen and temperature)
- STPS NKE (pressure, temperature and salinity)
- STBD NKE (pressure, temperature and turbidity)

and/or
- Multiparametric sonde NKE: (pressure, temperature, salinity, chl-*a*, oxygen, pH, turbidity)

Diffusive Oxygen fluxes at the sediment-water interface
- Unisense microprofiler fitted with oxygen, pH, resistivity, sulphur microelectrodes and equipped with a AADI oxygen optode

Total Oxygen fluxes at the sediment water-interface

Benthic Chamber
- Newly designed benthic chamber allowing for repeated oxygen flux measurements and fitted with a pyroscience oxygen (and temperature) micro-optode, an AquaPHOx-L-O2 logger and an APHOX-FTC recirculation chamber

Eddy Covariance system
- 1 ADV Vector Nortek

- 1 AquapHOx-T-O2 Transmitter Pyroscience fitted with an high speed oxygen micro-optode

BEATRISS
- 5 oxygen sensors(HOBO U26, ONSET)
- 1 acoustic doppler currentmeter ADV (Nortek Vector velocimeter, Nortek)
- 1 Aquadopp profiler HR (Nortek)
- A set of autonomous sensors: salinity (HOBO U24, ONSET), pressure (HOBO U20-Ti, ONSET) and light (HOBO UA-002, ONSET)

Benthic activity and sediment reworking at the sediment-water interface and in the upper sediment column
- Ocean Imaging Systems Digital Sediment Profiling Camera Model 3731-D REMOTS

- Ocean Imaging Systems Plan View acquisition system:

  - Digital Still Camera DSC 24,000
  - Strobe Model 3831
  - Deep Sea Laser Model 400-37
  - Mounting Hardware D-373-60
  - Bottom Trigger Switch DSC

3.2. Main functionalities

ACOBS is primarily aiming at assessing the relationships between mineralization processes taking place at the sediment-water interface and potential controlling factors including bottom seawater characteristics (e.g. temperature, oxygen concentration/saturation…) and benthic macrofauna activity including sediment reworking.

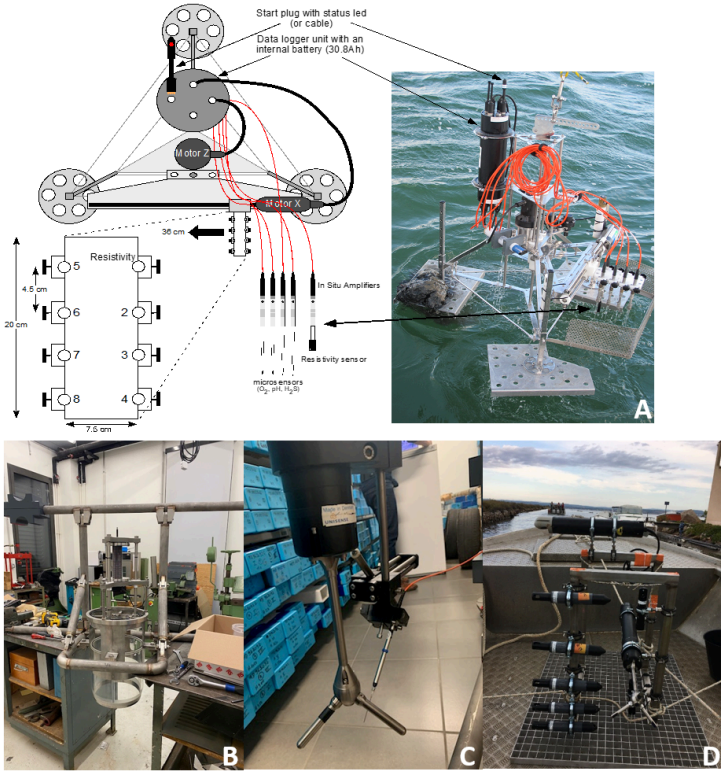
Together with the acquisition of a set of essential variables in the seawater bottom layer by a set of dedicated sensors (see above), ACOBS is primarily designed to provide time series measurements of both diffusive and total oxygen fluxes at the sediment-water interface. Diffusive fluxes are assessed through the deployment of an oxygen microprofiler (see above), which is allowing tor replicated measurements at different locations of the sediment-water interface. Total fluxes are assessed through: (1) a newly designed benthic chamber (see above), allowing for repeated water renewals and thus repeated flux measurements over a single area of the sediment-water interface, and (2) an eddy covariance system (see above) and/or the specifically designed BEATRISS system (see above) both allowing for continuous measurements of total oxygen fluxes over a large area of the sediment-water interface. ACOBS is also allowing for the continuous assessment of benthic activity and sediment reworking through the parallel deployment of a sediment Profile Imager also allowing for the imaging of the sediment-water interface (see above). In order to facilitate deployments in littoral environments, this last piece of equipment is not incorporated in the ACOBS main frame.

Due to the inherent fragility of some of its components (e.g. oxygen microelectrodes), ACOBS has been designed to achieve short-term (i.e., typically a few days) repeated deployments in both transitional and coastal (i.e., over the whole continental shelf) environments. Along the same line, data storage is achieved by internal loggers for all ACOBS components.

ACOBS is largely based on the integration of developments (e.g. eddy covariance, oxygen microprofiling) achieved within previous JERICO projects. Specific developments within JERICO-S3 include: (1) the integration of the BEATRISS system, (2) the design and building of a new benthic chamber allowing for repeated measurements of total oxygen fluxes, and (3) the implementation of a plane view system and associated software developments for the processing of time series of images of the sediment-water interface.

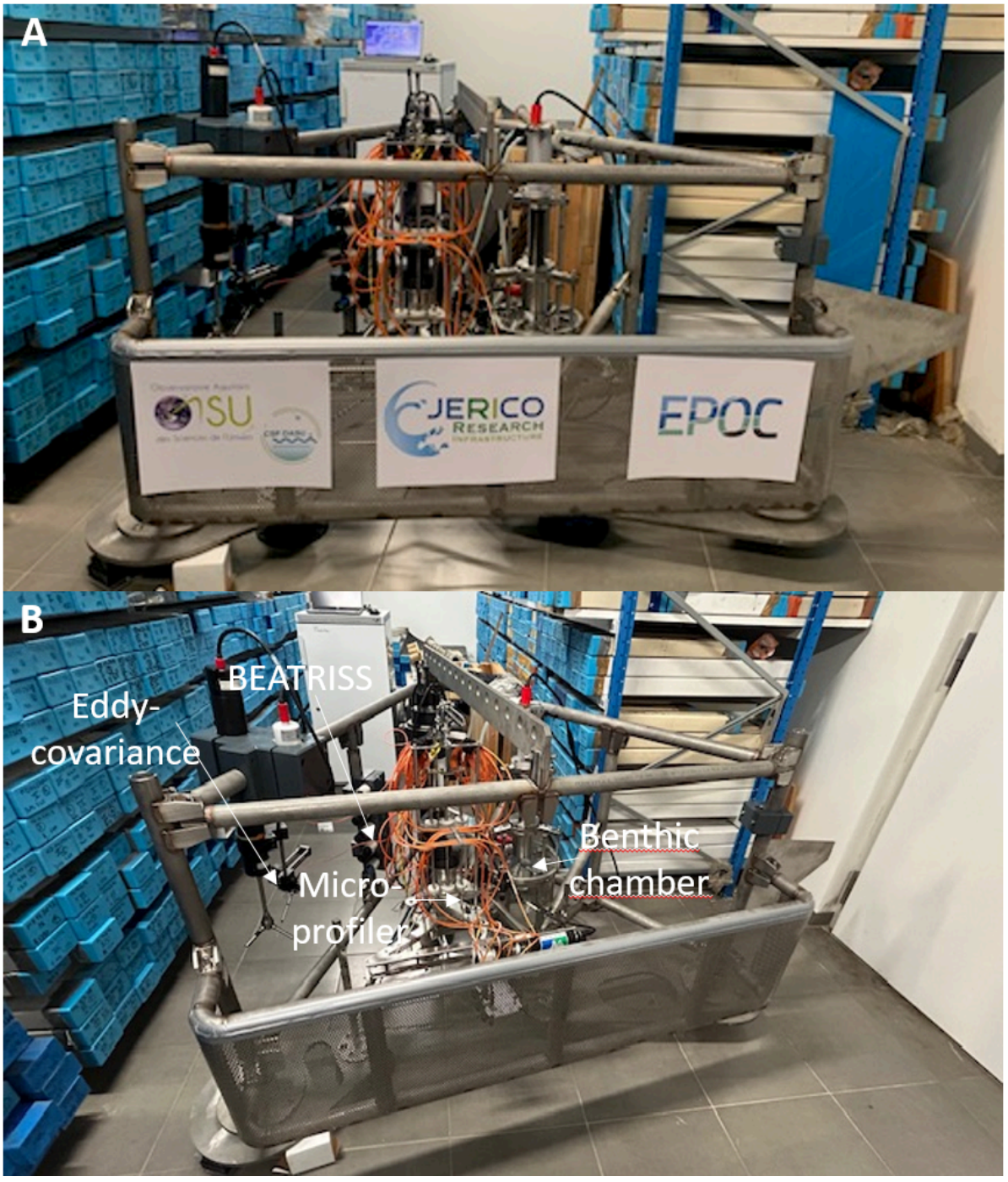
3.3. Prototype as before testing phase

The figure below shows individual views of the four main devices integrated in ACOBS, which are allowing for the quantification of oxygen fluxes at the sediment water interface. These are: (A) the Unisense oxygen microprofiler, which is used for the assessment of diffusive fluxes; (B) the new benthic chamber; (C) the eddy covariance system using now an oxygen microoptode instead of the glass oxygen microelectrodes previously used in a previous JERICO project ; and (D) the BEATRISS device, which are all three used for the quantification of total oxygen fluxes.

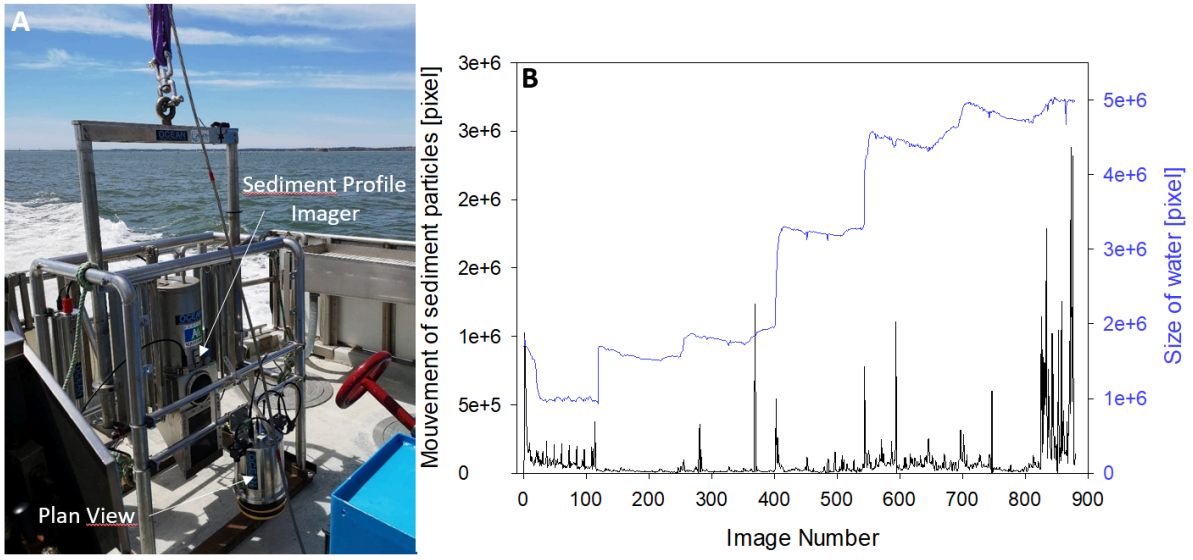


Nota: Panel A is taken from JERICO-NEXT D3.10

The figure below shows two general views of the main frame of ACOBS fitted with these four devices.



The figure below shows the Ocean Imaging systems Sediment Profile Imager and the Plan View image acquisition device (A), which constitute the last component of ACOBS. These devices are not integrated in ACOBS main frame for maniability reasons. These two devices allow for the quantification of benthic macrofauna activity and sediment reworking as shown in pane B, which is presenting a sediment reworking time series obtained during test deployments recently achieved in the Arcachon Lagoon.



The field deployment of ACOBS in its full configuration is yet to be tested. This will be achieved during late winter/early spring 2024 within the Arcachon Lagoon.

4. WATER-SAMPLE FILTERING & PRESERVATION DEVICE - WASP

4.1. List of sensors

The WASP from subtask 7.3.3 consists primarily of two sampling devices: a modified Mclane Phytoplankton and Particle Sampler (PPS) and an ISCO refrigerated autosampler (Fig. X). The samplers operate while integrated with a FerryBox platform that is equipped with SeaBird SBE38 and SBE45 sensors for measuring salinity and temperature, and a Turner Designs C3 sensor for measuring chlorophyll a fluorescence, coloured dissolved organic matter fluorescence (cDOM), and turbidity. The FerryBox system that will be used for T7.3.3 demonstration cases is the MS Color Fantasy ship of opportunity that operates between Oslo, Norway and Kiel, Germany in the eastern North Sea (Skagerrak and Kattegat seas).

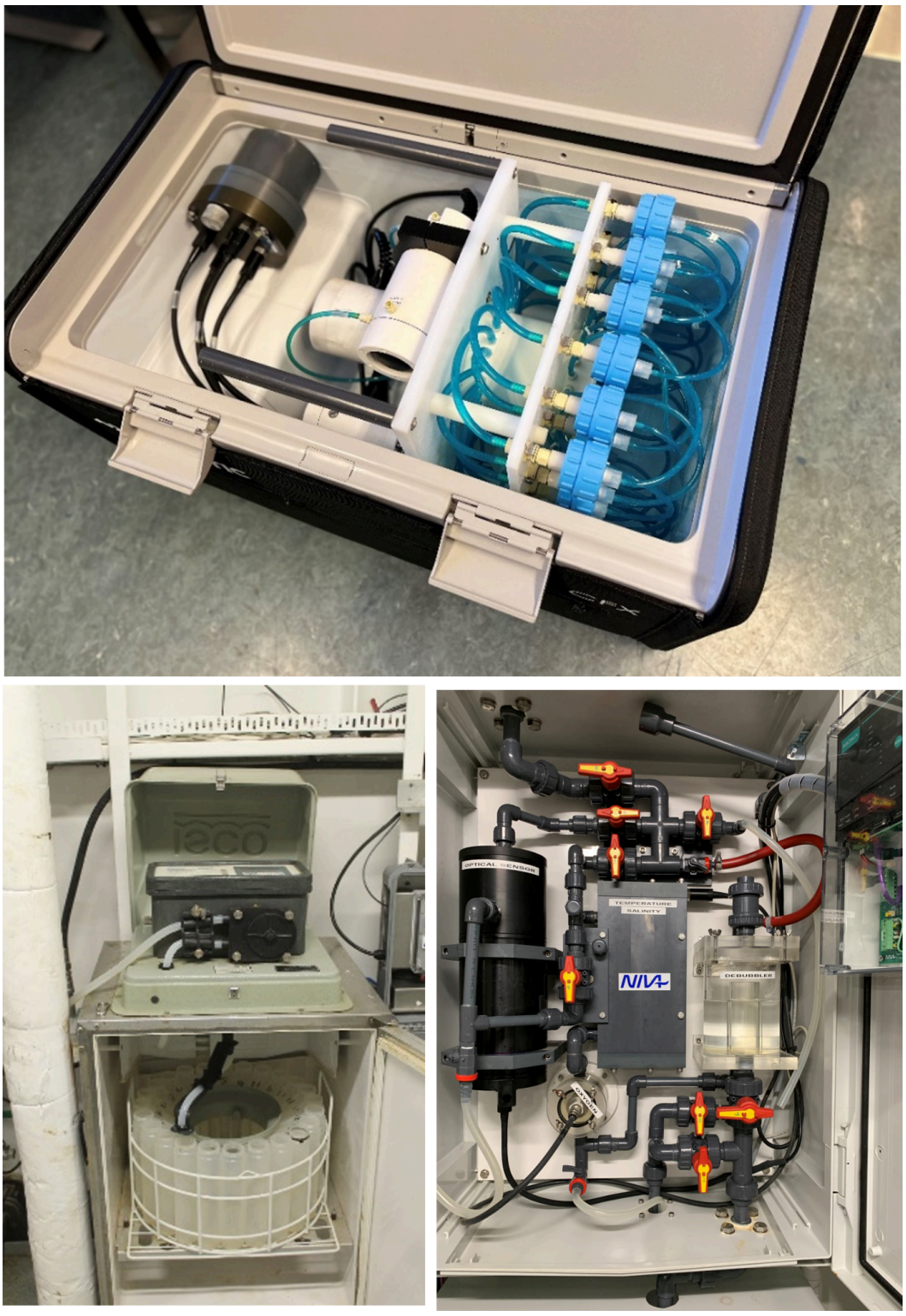


Fig. X. WASP sampling system composed of several instruments and samplers. Top: modified PPS for particulate matter sampling; Bottom left: ISCO refrigerated autosampler for water samples; Bottom right: FerryBox sensors including SeaBird SBE45 salinity-temperature and Turner Designs C3 for chlorophyll a, cDOM, and turbidity.

4.2. Main functionalities

The Mclane PPS portion of the WASP has been disassembled from its original in situ deployment configuration and modified for use on a FerryBox system. The main function of

the PPS portion of the WASP is to filter seawater for the collection of phytoplankton for environmental DNA (eDNA). The PPS portion of the WASP has 24 filter holders (for filters 47 mm in diameter) and a port selection valve to allow filtration through any one of the 24 filter holders. The pump flow rate is variable but is generally <~100 ml/min, and the total volume filtered is recorded for each sample. Additionally, the pump flow path is equipped with a solenoid valve which was originally designed to provide a fixative or other reagent flow over the sample after collection. This solenoid valve in the WASP configuration has been used to pump 96% ethanol through the samples to preserve eDNA as well as modified so that air can be passed through the sample tubing and filters so that the samples are filtered to dryness. The latter technique is used for freezing samples when the modified PPS is placed in a portable cooler/freezer box (Dometic CFX3-100). Each sampling event can be manually triggered by a remote operator or automatically triggered based on time and/or geofence, as well as data from other FerryBox sensors on the vessel or instruments located elsewhere. The location, date, and time of each sample is then recorded by the FerryBox software to enable a link to the auxiliary data.

The ISCO refrigerated autosampler portion of the WASP is utilised in its stock configuration. The autosampler contains 24 polypropylene sampling bottles (1 L volume) that are placed in a 5 °C refrigerator. A peristaltic pump first purges and rinses the sampling line and then pumps water to a rotating sampling discharge tube that sequentially fills the sampling bottles. Similar to the PPS, each sampling event can be manually triggered by a remote operator or automatically triggered by time or a geofence, and location/time metadata are logged. The refrigerated autosampler can collect bulk seawater for any lab-based analysis after collection. Preservatives or other reagents can be preloaded into the sampling bottles - e.g., a formalin stock solution can be added to the bottles and upon adding sample water to the bottle, the sample will be preserved with formalin. For subtask 7.3.3, nutrient samples will be collected using the ISCO refrigerated autosampler concurrently with collection of phytoplankton samples by the modified PPS.

4.3. Results of bench-testing

Several bench tests were carried out for the WASP at NIVA's Solbergstrand Field Station (Oslofjord) and at NIVA's laboratories in Oslo and Grimstad, Norway. The bench-testing was done in cooperation with the Horizon 2020 NAUTILOS project (New Approach to Underwater Technologies for Innovative, Low-cost Ocean obServation; Grant No. 101000825) in which an automated sampling device adapted for use on FerryBox observing platforms was also developed for collecting other suspended particulate material. A series of bench-testing activities were carried out to ensure the WASP performed according to metrics required for field sampling:

Volume filtered testing

The WASP is equipped with a magnetic drive pump that measures water flow and represents the volume of seawater filtered on each filter. For this test, 1 L of seawater was programmed for filtration and the water filtered was collected and measured volumetrically. A total of 17 replicates were carried out in which 1 L was the programmed volume while the actual volume filtered was 1018.6 ± 16.1 ml (mean ± standard deviation) with a relative standard deviation of 1.9%.

Sample recovery compared to conventional benchtop filtration

The WASP filtration system was compared with conventional benchtop vacuum filtration for chlorophyll a (chl-a). The test was carried out with *Skeletonema* diatom cultures which were filtered using both techniques, wrapped in foil and stored at -20 °C until analysis. Chl-a was measured using a protocol with methanol extraction and spectrophotometric absorption at 680 nm. The results from five tests indicated that the chl-a recovery using the WASP filtration system was 86 ± 8.4% (mean ± standard deviation). Recovery was observed to be as high as 98% when filtering larger sample volumes, which indicates that some sample material might be lost to the sampling tubes or filter holders in the WASP filtration system.

An additional test was also carried out to compare the qualitative recovery of phytoplankton genomic material using the WASP filtration system and benchtop vacuum filtration. This test assesses whether the WASP filtration technique affects cellular and nuclear integrity and therefore if genomic material is effectively sampled. Here, seawater samples collected from coastal Norway were filtered in duplicate using both techniques, preserved with ATL buffer (Qiagen) and then frozen at -20 °C until analysis. DNA was extracted from each sample and amplified with *Skeletonema* spp. primers and generic eukaryotic phytoplankton primers (primer pair 1391F and EukBr). Gel electrophoresis runs of PCR products indicated that *Skeletonema* DNA was not present in seawater samples, but eukaryotic phytoplankton DNA was present (Fig. Y). The results also show that the quality of DNA recovered from the WASP filtration system and benchtop filtration was comparable.

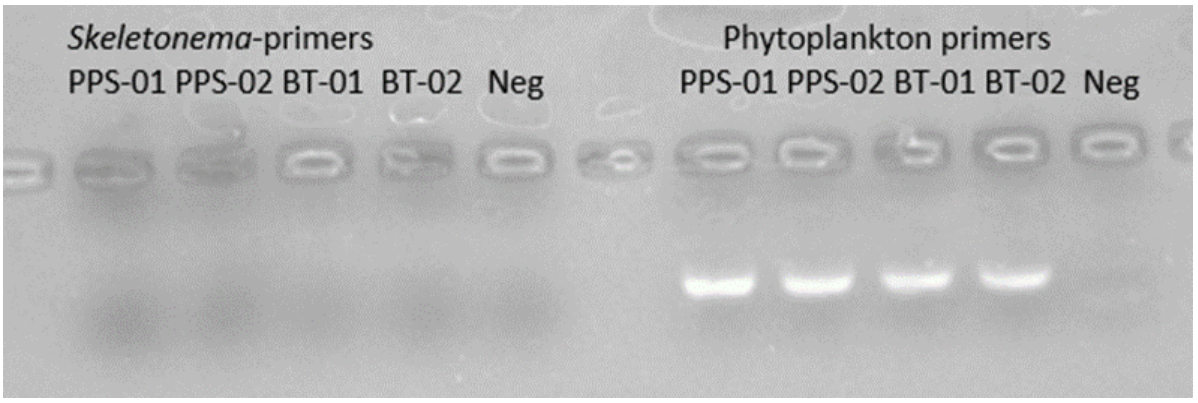


Fig. Y. Gel electrophoresis runs for WASP-PPS (PPS-01 and PPS-02) and benchtop vacuum filtration (BT-01 and BT-02). The left side of the image is for Skeletonema spp. primers and the right side of the image is for general eukaryotic phytoplankton primers. Negative control (Neg) is shown to the right of the benchtop runs.

Testing solutions for eDNA preservation

After sample collection, the WASP filtration system has been integrated into a portable freezer/cooler box (Dometic CFX3-100). Most protocols for preserving filtered biological material (i.e., phytoplankton chl-a, organic carbon, etc.) involve freezing followed by analysis, and some protocols for molecular targets involve reagent-based preservation. However, because the PPS is equipped with a reagent preservation system, several types of sample preservation techniques were tested that included the sole use of reagents and cooling/freezing as well as combinations of the two, and a preservation test was designed

to test possible sample preservation and handling scenarios (i.e., WASP collection, reagent addition, user collection, storage in the lab, followed by analysis). The sample types included phytoplankton representing different phytoplankton functional groups: raphidophyte *Heterosigma akashiwo*, dinoflagellate *Karlodinium veneficum*, and the diatom *Skeletonema marinoi*. The treatments included: ATL buffer (Qiagen) followed by refrigeration, direct refrigeration, direct freezing, ethanol followed by refrigeration or freezing, and RNAlater (Thermo-Fisher) followed by refrigeration or freezing. The refrigeration and freezing that followed ATL buffer and RNAlater addition, respectively, were protocols recommended by the manufacturers. The samples were collected in triplicate for each sample preservation technique at t=0 hours (directly after sampling) as well as triplicate samples for storage times ranging from 6 hours to 3 months depending on the sample preservation technique.

At each timepoint, eDNA was isolated for selected species and quantified with quantitative polymerase chain reaction (qPCR). The results for *Heterosigma akashiwo* are shown in Fig. Z below, and the effect of preservatives on recovery of DNA was similar for the other two phytoplankton types. The effective preservation techniques included storage in ATL buffer + refrigeration for up to 3 months, direct freezing (also for months, but additional time points were not tested here), and ethanol + freezing for up to 3 months. The less effective preservation techniques were cooling (even as short as 6 hours from the time of collection to the time of freezing) and RNAlater + freezing or refrigeration. The lower yield for ethanol + freezing at the t=1 month sampling timepoint in Fig. Z was likely due to poor sample handling during the experiment (samples were not dried properly prior to DNA extraction). While the ATL buffer was effective as a preservation solution, it is quite expensive and the WASP requires a relatively large amount of preservation solution when used with a pump and automation. The ATL buffer can also be viscous at low temperatures and it was determined that this might pose issues when used in an automated system with pumps/valves and it was therefore decided to not use this in further work. Therefore, based on the results, further developments with the WASP filtration system will involve direct freezing possibly in combination with ethanol.

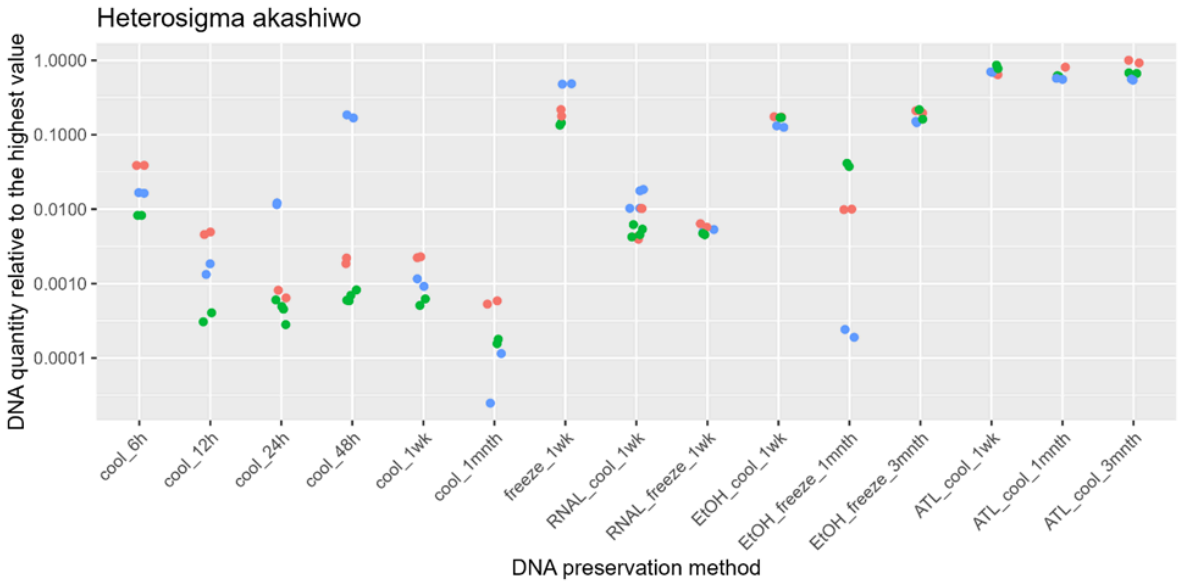


Fig. Z. Results from sample preservation test based on amplification of Heterosigma akashiwo 28S ribosomal DNA. Sample preservation techniques on the x-axis: refrigeration only ("cool_time") at t= 6h, 12h, 24h, 48h, 1 week, and 1 month; freezing at -20 °C for 1 week ("freeze_1wk"); RNAlater + refrigeration for 1 week ("RNAL_cool_1wk"); RNAlater + freezing at -20 °C for 1 week ("RNAL_freeze_1wk"); ethanol + refrigeration for 1 week and freezing at -20 °C for 1 month and 3 months ("EtOH_temp_time"); ATL buffer + refrigeration ("ATL_cool_time") for 1 week, 1 month, and 3 months. The y-axis represents qPCR yield (relative scale). Symbol colours represent sample replicate number (orange, blue, green) and each sample replicate was analysed in duplicate (two symbols per colour for each treatment).

Testing the use of a solenoid valve to enable filtering to dryness

Our initial tests with automated filtration using the WASP filtration system revealed that residual water sample could remain near/on sampling filters due to incomplete vacuum filtration. This could pose issues when recovering filters in that some suspended material in the residual water could be lost (sample loss) and/or if filters are collected at a later time, the leftover moisture could result in unwanted metabolic activity. This was especially evident when using polycarbonate membrane filters as opposed to glass fibre filters. The primary reason was that the automated nature of the sample filtration meant that seawater would always be in the sample tubing. Therefore, a modification was introduced into the filtration system to utilise the solenoid valve that was originally designed for reagent addition. This enabled a switch to nitrogen gas, air, or filtered air which would then be drawn through the sample tubes and through the filter to allow for complete filtration of the water sample as well as all seawater being filtered through the filter in a similar fashion to a conventional benchtop vacuum filtration system.

5.CONCLUSIONS

The development and testing of the JERICO-S3 technological demonstrators, including the Plankton Dynamics Sensor Package (PSP), Autonomous Coastal Observing Benthic Station (ACOBS), and the Water-sampler, filtration, and preservation device (WASP), have demonstrated significant advancements in marine observation technologies. These systems have shown effectiveness and reliability in both controlled and field environments.

The PSP and ACOBS provide comprehensive tools for monitoring and analysing plankton dynamics and benthic processes, respectively. Meanwhile, the WASP system, integrated with a Ferrybox, offers enhanced capabilities for collecting and preserving environmental DNA samples. Successful testing and demonstration validate these technologies' potential to deliver valuable insights into coastal and marine ecosystems, thereby supporting the goals of the JERICO project.

These advancements mark a critical step forward in optimising our understanding of marine biological and biogeochemical processes, facilitating better-informed management and conservation efforts.