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JERICO-S3 MILESTONE

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→ Please specify the type of milestone:

- ❏ Report after a workshop or a meeting (TEMPLATE A)
- ❏ Report after a specific action (TEMPLATE B) (test, diagnostic, implementation,…)
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- ❏ Other (TEMPLATE B) (to specify) …………………….

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1. Objectives and implementation process

The main objective is to identify the state-of-the-art and novel sensor candidates that can measure the targeted EOV packages for environmental monitoring. This work will be the basis for the selection of sensors for the design of packages. This report focusses on environmental pollutants – metals, hydrocarbons, microplastics, underwater bioacoustics, as well as biological contaminants such as phytoplankton abundance (EOVs listed by Global Ocean Observing System (GOOS)). The list is preliminarily analysed and consolidated based on the maturity of the sensors (reaching TRL 6), available for deployment and rapid responses, field use and with suitable detection limit.

2. Main report

2.1. Introduction

Ocean biogeochemistry, biology and ecosystems need to be monitored in the ocean as well as in the coastal boundaries at appropriate spatial and temporal scales. Monitoring methods used to rely on manual sampling followed by analysis in the laboratory using standard analytical techniques to online in-situ sensors. This laborious process includes sample collection and storage, transport and appropriate analysis following the SOP provided that increases the cost drastically and limits sampling in regions of limited access. *In situ* sensors are developing rapidly to monitor many different parameters in the environment in a cost-effective, sustainable way, providing extensive quality data. However, the maturity of these sensors is variable, and many are based on imaging technologies and generate enormous amount of data which lacks automated processing. However, algorithms are constantly being developed for instrument automation to identify biological pollutant, (for example Flow Cytobot from JERICO-NEXT). Several reviews are available for different pollutants monitored in the marine environment [1-3]. Here, we consolidate a list of existing and novel sensors available for environmental water monitoring for pollutants such as metals, microplastics, phytoplankton diversity/harmful algal toxins and microbial diversity.

2.2. Methodology

Scientific literature reviewing of the state-of-the-art sensors and biosensors in marine monitoring, as well as personal communication with recent research projects/researchers on existing sensors and novel sensors have been the basis for compiling a list of sensors which have reached a TRL of 6 and are ready for *in situ* implementation and integration into packages.

Series of dedicated meetings with sensor experts and WP5 Technical steering teams (ST5 BGC; ST6 (genomics); ST7-biology):

Date	What	Who	Outcomes
Oct. 6, 2020	Udate and requirements on sensors	WP1, WP7	First version of the review table
Feb 2, 2021	Biological sensors	WP7, WP5 (ST7)	update Biological sensors (plankton)
Feb 5, 2021	Autosamplers and filtering devices	WP7, WP5 (ST5, ST6, ST7)	backbone for the WASP

Feb 10, 2021	Biology (best practices	WP7, WP7 (ST7)	update biological sensor list for sensor package
Feb 15, 2021	Review from strategy	WP1, WP7	endorsement link to IRS/PSS
Feb 18, 2021	Biogeochemistry	WP7, WP5 (ST5)	Update BGC sensors
Feb 24, 2021	BGC	WP7, WP1 (CNRS)	Consolidation

An exhaustive list of existing sensors and novel sensors are provided as a table in Appendix 1, that gives information on the type of sensor developed, analytical methods involved, data collected, commercial availability and price. For further analysis and consolidation, H2020 FP7 –OCEAN–2013, Ocean of Tomorrow (OOT) projects were thoroughly reviewed. The OOT2013 was the last cross-thematic call of its kind under FP7, focussing on marine sensing technologies (including biosensors) to monitor the marine environment. Nine out of the 12 projects funded under this topic developed biosensors for marine environmental monitoring, analyte/pollutant detected, biosensor type, sensing material and the recognition type. Personal communication with the project leaders is ongoing to collect information on their maturity, TRL level, etc. Selected novel sensors which could provide complementary data are presented in Appendix 2.

Current strategies for marine monitoring of contaminants include sensors that produce a response due to change in the physical, chemical, or biological conditions, and collect sometimes large amounts of data. These sensors are designed in combination with analytical techniques to achieve sensitivity, selectivity, limit of detection, repeatability and reproducibility[4]. Biosensors that convert biological responses to electrical signals, immunosensors, aptasensors, genosensors and enzymatic biosensors are different types of recognition elements in a biosensor; based on the transduction principle they are classified into optical (optical fibre, surface plasmon resonance (SPR)), electrochemical (amperometer, impedence); and piezoelectric (quartz crystal microbalance biosensors). Such biosensors are the most used tools due to their rapid responses, portability, easy fabrication, and field deployability. For example, SPR is used for detecting toxins such as Saxitoxin, okadaic acid and domoic acid with a detection range from 0.36 ng/ml to 50 ng/ml [5].2nd generation ESP – in situ detection of marine organisms using sandwich hybridization, competitive ELISA, and qPCR assays. These were installed on ships, shore-based stations, moorings, and drifters. 3rd generation ESP – made to fit Tethys-class long range AUV that can collect and process 60 samples while roaming freely in the ocean from the surface to a depth of 300m in a single deployment for over many days. Appendix 1 gives an overview of existing and novel sensors of interest to JericoS3, deployed or having reached a technology readiness level of 6, including type of sensor developed, analytical methods involved, data collected, commercial availability and price. Further, H2020 FP7 –OCEAN–2013, Ocean of Tomorrow (OOT) projects were reviewed (Appendix B). The OOT2013 was the last cross-thematic call of its kind under FP7, focussing on marine sensing technologies (including biosensors) to monitor the marine environment.

3. *Conclusion and next steps*

The new technologies need to be designed in such way to easily integrate into the existing and available coastal and ocean observation programs in a sustainable and cost-effective

way. Use of autonomous vehicles and integration of biosensors to monitor contaminants and toxins such as phytoplankton biomass are developing.
Development of automation technology is progressing rapidly that enables us to widen the scale and scope of environmental monitoring by giving data-rich, cost-effective, sustainable outputs. Recent advances in *in situ* devices are designed to collect eDNA sample to identify species and population in water [6, 7], nanopore sequencing to identify microbes and planktons are built. Currently, focus of eDNA studies are on the analytical methods and bioinformatics pipeline. Automation will give us the advantage to collect samples in remote inaccessible regions by humans for monitoring. Environmental sample Processor (ESP), a robotic device is programmed for sample collection and preservation for further analysis in the lab.

The growing demand in marine environmental monitoring focus on the development of autonomous, in situ and advanced methods for sample preservation technologies. New technologies such as optofluidic, molecular sensors, ESP are most promising for miniaturization, automation and towards *in situ* monitoring.

Analysis of environmental DNA (eDNA) will be the future approach for reducing costs and impacts of traditional monitoring approaches.

- Autonomous sampler that collects water samples, filter the sample, and preserves the sample for further analysis – WASP to be integrated in the JIIM platform – OOT BRAVOO and COMMONSENSE
- Benthic and pelagic biosensors

4. *Appendix and references*

4.1. Appendix A: Sensor candidates for the integrated sensor packages

Benthic and pelagic sensors packages:

- Water chemistry (environmental parameters)
- Contaminants (microplastics, PCBs, toxins, PAH, hydrocarbons, general toxicity, DOC, DON
- Underwater noise
- Plankton diversity and toxic algae blooms
- Microbial diversity

High throughput data: Environmental parameters, Flow cytometry
Triggered when needed based on above data: imagery, underwater noise, biology, contaminants

Water sampler		
1. SYREAUCO (IFREMER, Romaric)	Sampler 15 x 500ml turbidity or fluorescence SPM, POC, OM content, Chla	In development. Sampler functional TRL7 in early 2020. Commercially available in late 2020.
OOT Mariabox and ENVIGUARD	Sampling, Filtering, washing of interest to Jerico	Check this is usuable/adaptable to jerico platforms for DNA/RNA extractions

Good for all samples requiring laboratory analysis, and also as a basis for plugging in a filtering unit containing preservation liquid. With some development, this technology could enable all DNA/RNA based analysis.		
In situ imaging /flow cytometry		
2. Video plankton recorder (HZG, Felipe)	Underwater images of zoo and phytoplankton, with size distribution. Plankton and particles 35µm-mm.	Commercially available Data sent via fibre optic cable or internally stored.
18. Underwater Vision Profiler 6 (coppola)	The Underwater Vision Profiler or UVP (CNRS patent) is designed to study large (>100 µm) particles and zooplankton simultaneously and to quantify them in a known volume of water. Marine particles > 100µm Particles and plankton possible identification > 500µm Depth 6000m	Commercially available TRL9 15Keuros
15. Video Plankton Recorder VPR (Felipe)	video-microscope system used for imaging plankton and other particulate matter in the size range from a few micrometers to several centimeters. The VPR is essentially an underwater microscope. High frequency images from plankton and other marine particles (100µm-1cm). Max depth 350m	Exists a new generation with realtime data transfer TRL9 Commercially available 80K euros
3. Underwater Vision profiler 5	Imaging of Large (>100µm) particles and zooplankton (>500µm) and quantification in known volume of water. Functional down to 6000m depth.	TRL9 commercially available 100K euros
4. Flowcam	Imaging particles sizes 2-1000µm, phytoplankton, microzooplankton abundance and diversity (harmful aglae). Can be triggered based on fluorescence, or programmed.	TRL9 commercially available. Not on platform yet. +
5. Stand alone Imaging device	Automated programmable image acquisition and processing. Macro and mega fauna diversity and spatial temporal dynamics.	TRL6 – prototype commercially available. Used in several fixed platforms and on board ARGO float.

	Floating, epi-benthic and benthic macro and mega fauna whose size is larger than 1cm	6,5K Euros including softward and hardware
6. Imaging flow cytoBot (NIVA, SYKE, felipe)	Images of phytoplankton and microzooplankton (5-150µm) triggered by autofluorescence. Cell abundance, diversity and biomass. Trained to recognise phytoplankton. QC needed	Used with ferrybox TRL? 150 Keuros for instrument only
7. ZooScan (CNRS, IFREMER, Felipe)	Digital images zooplankton (>50µm) classified by size or taxa.	TRL9, lab system 20Keuros
10. Cytosense (CNRS-MIO, CNRS-LOG, CNRS-BOREA, VLIZ, CEFAS, RWS) Felipe	Automated pulse shape and images recording flow cytometer. Phytoplankton at single cell level. Fluorescence emitted by pigments Resolves phytoplankton functional groups and average sizes. 1-800µm and up to 4mm for chains	EOL buoy autonomous test TRL9 commercially available 120-150keuros
8. 13. CytoPro (CNRS, Felipe, gregori)	Automated flow cytometer coupled to a staining module. Extends capabilities of Cytosense Heterotrophes (prokaryotes, nanoflagellates, ciliates, microzooplankton) cell abundance 0.4-500µm and viability/activity >15-20µm Autofluorescence recorded Sata and images analysed	Integrated on fixed platform 100-150K euros
Fluorometer		
9. FastOcean sensor (CNRS, VLIZ, RWS, NIOZ, Felipe)	Measures phytoplankton photosynthetic activity and primary production - Act2 Run semi-automatic system - APD profiler system for the water column 600m depth	Commercially available 34Keuros 94Keuros
11. FluoroProbe and AlgaeOnlineAnalyser (AOA)	Highly sensitive submersible spectrofluorometer.	Commercially available TRL9

(FRRF – AOA : CNRS-LOG, CNRS-BOREA, IFREMER, VLIZ – MultiExciter : SYKE) Felipe	Measures total phytoplankton chlorophyll a concentration and to discriminate four spectral algal groups. Fluorescence intensity after excitation at 470, 525, 570, 590, 610 and 370 nm (relative unit) - Total chlorophyll a concentration (µg chl a. L^{-1}) - Brown algae concentration (in eq. µg chl a. L^{-1}) - Cyanobacteria concentration (in eq. µg chl a. L^{-1}) - Green algae concentration (in eq. µg chl a. L^{-1}) - Cryptophytes concentration (in eq. µg chl a. L^{-1}) - CDOM concentration (arbitrary unit) - Water temperature (°C) - Transmission (%)	Data on cyanobacteria and 3 other microalgae in real time 28-40Keuros
13. NEXOS O1 (TriOS - PLOCAN Eric Delory)	Matrixflu Matrix fluorometer – NeXOS O1 – Visual or UV spectrum. CDOM PAH BTX TRP	Integrated onto several platforms 13Keuros
16. HyAbS - Hyperspectral Absorption Sensor (HZG)	a custom-made sensor and basically a modified and advanced version of the manual or semi-automated PSICAM. Absorption coefficient spectra, CDOM, Phytoplankton biomass (chlorophyll-a), SPM, algal groups	Used connected to ferrybox TRL6, custom made 50Keuros
Acoustic sensors		
12. NEXOS A1 (Eric Delory)	Passive acoustic digital sensor with smart interfacing and embedded processing. Measures underwater noise.	Integrated on several platforms (waveglider, deep glider, surface buoy…) Acoustic data 8Keuros
Benthic sensors (A Gremare)		

19. Sediment microelectrode profiler: Diffusive oxygen fluxes Benthic Chambers : Total oxygen fluxes at the sediment water interface Eddy correlation system: Total oxygen fluxes at the sediment-water interface Optical sensor + dedicated image analysis software of time series of images (AVIExpolore)	Vertical profiles of oxygen concentrations within the sediment column Decrease in oxygen concentrations within the sediment column Time series of oxygen concentration and turbulence in the water column Time series on the abundance and activity of benthic macrofauna	Sediment microelectrode profiler commercially available. Benthic chambers not commercially available
pH, nitrates, absorption, pollutants		
17. CONTROS HydroFIA pH (Kongsberg) (HZG)	Analyzer for pH value (pH7-9) in Seawater	Commercially available 50keuros Data sent in realtime
22. Valeport SUV51 nitrate sensor (J.Allen, E. Alou, SOCIB)	Nitrate sensor Absorption of UV radiation at 5 ultra UV wavelengths + 1 internal reference channel. Mapping of nitrate variability	TRL7 but no further development planned 20kNOK, not commercially available
23. Valeport SUV101 nitrate sensor (J.Allen, E. Alou, SOCIB)	Nitrate sensor Absorption of UV radiation at ultra UV wavelengths. 0,1µM Nitrate detection limit Mapping of nitrate variability	TRL2 but better than SUV51 20kNOK, not commercially available. Designed for multiplatforms
20. Microplastics: Autonomous microplastics sampler (Andrew King, NIVA)	Quantity (mass) and quality (microscopy/FTIR/NIR) of three microplastics size fractions. Mass, volume, size spectrum, plastic type	TRL? Under development
21. Autonomous, multiple sample particle filtration, preservation, storage system (Andrew King, NVA)	Filtration for lab analysis of POC/N/P, chl a, algal pigments, cDOM, DNA/RNA, particulate metals, etc.	TRL? Under development
OOT BRAAVOO – Microbial bioreporters (Jan Van der Meer, UNIL)	In situ autonomous Biosensors for contaminants based on bioluminescence Measures petroleum compounds and general toxicity	Limited autonomy. Functional in laboratory.
OOT SENSEOCEAN – Biogeochemical sensors (Matt Mowlem, NOC)	Optode sensors: O2, pH, CO2 Fluorescence sensor: BTEX, PAH, Tryptophan, Algae Nitrate and nitrite, pH, phosphate, iron, silicate, ammonium, DIC, Organic N and P Lab on chip, Silicate sensor.	Already an integrated sensor package Has been deloyed on many platforms

OOT SMS (Luca Sanfilippo, SYSTEA	Automated immune assay with magnetic beads to capture chemicals Detection of algal toxins (okadaic acid, domoic acid, saxitoxin) Detection of pharmaceuticals (sulphonamides) Detection of nutrients: ammonia, nitrate, orthophosphate	Tested on floating platform and buoy
OOT SCHeMA (A. Novellino, ETT)	Integrated in situ Chemical mapping. Parameters measured: chemicals Metals Pesticides Pharmaceuticals Pathogens Nutrients Pollutants CO2	Complete with data collection system
OOT ENVIGUARD (Dennis Growland)	Biosensor technology for monitoring harmful aglae, toxins and PCB Chemical contaminants Biotoxins PCBs	
OOT MariaBox (Matteo Bonasso)	Parameters (8 parameters): Pollutants (PAH, fluorinated surfactants, heavy metals, pesticides) Algal toxins (saxitoxin, microcystin, azaspiracid, domoic acid) Environmental parameters (temp, pH, salinity, DO) Aditional custumisable module	Filtration unit of interest to Jerico?
OOT Common Sense (Sergio Martinez, LEITAT)	Environmental parameters: Temperature, Conductivity, salinity, pressure, pH, DO Turbidity Chorophyll a, Cyanobacteria Nutrients (LOD, phosphate, nitrite, nitrate) heavy metals (Cd Hg, Pb) Microplastics Underwater noise	Interest for jerico: Heavy metals, microplastics
DNA/RNA based 'sensors': Methods requiring automated sampling and sample preservations		
14. Microbial species and genes	DNA/RNA qPCR based sensor.	Has been fully integrated/automated in ESP

	Quantifies species or genes in seawater: Algae bloom Pathogens Eutrophication Oil pollution	platform. Used with ferrybox in jericoNext. Expensive and not mature to integrate onto Jerico platforms as fully in-situ.
Metabarcoding	Biodiversity based on sequencing	
eDNA	Biodiversity based on sequencing of environmental DNA	
DNA probe/sandwich hybridisation	Several candidates including NORCE, OOT SMS Detection of toxic algae species	

4.2 Appendix B: List of selected sensors developed from OOT projects

Project	Biosensor type/Electrode, Sensing material	Recognition type	Analyte/pollutant detected
BRAAVOO	Fluorescent bioreporter bacteria, immunosensors, algal -microfluidic	Lab-on-a-chip Enzymes, antibodies	General toxocity, stress response, Algal toxins, heavy metals, organic compounds related to oil and antibiotics (ref – final summary of BRAAVOO project)
EnviGuard	Molecular probes, algal, chemical. Bacterial and viral -microfluidic -electrochemically, optical label-free responses	Nucleic acid Aptamers Antibodies	Microorganisms and toxins from biological sources – Betanodavirus, E.coli, okadaic acid and saxitoxin, PCB 128, PCB 118
MariaBox	Novel		Pollutants (PAH, heavy metals, pesticides) Algal toxins (saxitoxin, microcystin, azaspiracid, domoic acid)
SEA-ON-A-CHIP	Immunosensors Immuno-assays -microfluidic system, microelectronics	Antibodies?	8 selected contaminants Irgarol sulfonamides

SMS	DNA probes, sandwich hybridisation, colorimetric Immuno based assays	Algal toxins immunosensors	Okadaic acid, domoic acid, saxitoxin, toxic algal species – Dynophisis, P Nitschia, A. minutum
COMMON SENSE			eutrophication – Chlorophyll A, Cyanobacteria, heavy metals, microplastics, underwater noise
NEXOS	Fluorescence, absorption, carbon sensing	Optical, passive optical sensors	pH, inorganic carbon, carbonic acid, antifouling
SCHeMA	Metal probe, solid state electrodes electrochemical probes Optical sensor Optochemical probes		Metals, pesticides, pharmaceuticals, pathogens, nutrients, pollutants, CO_2
SenseOCEAN	Electrochemical microsensors Fluorescence sensor Silicate sensor, chemical sensor	Optical, chemical	Nutrients, phytoplankton, chlorophyll, detritus

4.3 Appendix C: Sensors for a phytoplankton/plankton package to be integrated into C-EGIM

C-EGIM ports	parameters	sensors	Manufacturer	sensor name
	biofouling	chorination of all optical sensors		
Standard+ physics				
1	conductivity/salinity, temp, depth	CTD	Seabird, Aanderaa	
3	current speed and direction	ADCP		
2	dissolved O2	optical sensor		
4	Chl a	fluorescence	SeaBird, Trios, Aanderaa, +++	
5	turbidity	optical sensor	SeaBird, Trios, Aanderaa, +++	
BGC				
6	N, C, O	optical sensor UV	TRIOS, DE	opus

	biogeochemistry (nitrate, nitrite, BOD, COD, DOC/TOC, TSS			
	Nitrate		Seabird, US	MBARI-isus, Seabird-SUNA
	Nitrate		Valeport, UK	SUV51, SUV101
7	pCO2		CONTROS	HydroC
	pCO2		Franatech	
8	pH	Optode	OOT SENSEOCEAN	pH, (O2, CO2)
		ISFET	Seabird	SEAFET V2 Ocean pH
			SensorLab	pH sensor 200-SM (max. 2 bar)
Biology				
9	pigments (Chloro, Fuco, PhycoCyanin, PycoErythrin) and CDOM	spectrofluorometer	bbe Moldaenke	FluoroProbe,
	pigments (Chloro, Fuco, PhycoCyanin, PycoErythrin) and CDOM	spectrofluorometer	Chelsea Technology	VLUX AlgaePro
10	PhytoPP - primary production (and Phytoplankton pigments)	FRRF Fast repetition rate fluorometry	Chelsea Technologies	FastOcean APD
11	Phyto cell size (micro, nano, picoplankton)	automated pulse shape and images flow cytometer, fluorescence emitted	CytoBuoy	CytoSub
12	zooplankton and large particles	underwater vision profiler		self triggered particle sensor - UVP
12 bis	particles, plankton, microplankton (-200µ)	imaging flow for particles, nano and microplankton	McLane	IFCB (Imaging Flow Cytobot) - Diatom, dinoflagellate

		phytoplankton sampler	McLane	PPS (phytoplankton, trace metals, particles (plastics)
		video microscope (if high concentration)		VPR Video particles/plankton recorder
Integrated sensors				
	CTD,ODO, pH, Turbidity, Chl-a		SeaBird	HydroCat
	CTD, DO, pH		SeaBird	SeapHOx
			Aanderaa	Seaguard (CTD, DO, Chl-a, pCO2, pH)

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