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JERICO-S3 MILESTONE Joint European Research Infrastructure network for Coastal Observatory **Science, Services, Sustainability**	
MS#, WP# and full title	JERICO-S3 MS.30 - WP5 - "Revised SOP provided to WP7 for adjustment of the WASP"
5 Key words	Environmental DNA, automated sample processor, molecular sensor, qPCR, ecogenomic sensor
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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,…)
- ❑ Document (TEMPLATE B) (guidelines,...)
- X Other (TEMPLATE B) (to specify) TABLE

Diffusion list			
Consortium beneficiaries	Third parties	Associated Partners	other

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A) TEMPLATE B - Other cases (not a workshop or meeting report)

1.B - Objectives

The main objective of this milestone was to provide a set of Standard Operating Procedures to WP7 for adjustment of the WASP so that eDNA sampling can be integrated. For that aim, a review of the different approaches for automated eDNA sampling has been performed so that a diagnostic of the potential and drawbacks of each type can be drawn.

2.B and implementation process

The review is based on a bibliographic search performed to identify these scientific publications reporting development and/of use of automatic DNA samplers and analysis devices, on the outputs of a workshop on Marine Omics Technology and Instrumentation Workshop which took place October 10th - 12th, 2023 at the Monterey Bay Aquarium Research Institute (MBARI) and on communication with scientists and technology developers. MBARi is one of the pioneers on the development of such devices and developed the Environmental Sample Processor (ESP; https://www.mbari.org/technology/environmental-sample-processor-esp/) which is commercially available through McLane Labs. The status of these technologies and future avenues was thoroughly discussed during the workshop (https://sites.google.com/mbari.org/moti-workshop/home), which joined together technology developers and users and several of the available and in development samplers were presented such as the RoCSI (by Julie Robidart), Smith Root (Austen Thomas), Pufferfish from Aquatic Labs (Allan Adams), SADIe (Kim Parsons), among others.

3.B - Main report

Although most studies performed so far require an active sample acquisition and processing, there are several ongoing initiatives which have made significant progress towards automated sampling and analysis of eDNA. The table below summarizes some of the achievements and available devices:

Paper / link	Year	Insitute	Type of study	Organisms targeted	Autonomous sampling equipment / device	Sampling capacity	In-device storage	Molecular profiling or biosensors on device	Device readiness and use in monitoring	In-lab analysis	Summary of results
Jones et al. (2008) A robotic molecular method for in situ detection of marine invertebrate larvae. https://doi.org/10.1111/j.1471-8286.2007.02021.x	2008	MBARI	Larvae monitoring proof of concept	Invertebrate spp.	Moored ESP	Continous	-	SHA for 5 invertebrate spp.	ESP commercially available	Valdiation	SHA profiles validated by lab-based assays
Scholin et al. (2009) Remote Detection of Marine Microbes, Small Invertebrates, Harmful Algae, and Biotoxins using the Environmental Sample Processor (ESP). Oceanography. https://doi.org/10.5670/oceanog.2009.46	2009	MBARI	Technical development	(depends on configuration)	1G? and Deep ESP	Varies with specs	RNALater	SHA and ELISA	ESP commercially available (see above)	-	
Preston et al. (2011) Underwater Application of Quantitative PCR on an Ocean Mooring. PloS ONE. https://doi.org/10.1371/journal.pone.0022522	2011	MBARI	Technical development / Proof of Concept	Prokaryotic (total RuBisCo and Synechococcus)	1G? ESP. Moored	Here RT monitoring for 28 days every 12 minutes with qPCR. 1L filtered amd 0.5 mL lysate used for	(samples for Ottesen et al collected in same deplyoment)	DNA extraction and RT-qPCR (Proof of concept). Also nutrient sensor and fluorometer	ESP commercially available (but likely not RT-qPCR module, developed at Lawrence Livermore Nat Lab.	-	First successful proof of concept of DNA extraction and RT-qPCR underwater, using algal bloom in Monterrey Bay

						extraction					
Ottesen et al. (2011) Metatranscriptomic analysis of autonomously collected and preserved marine bacterioplankton. ISMEJ. http://doi.org/10.1038/ismej.2011.70	2011	MIT (MA, USA)	Metatranscriptomic time series (weekly)	Prokaryotic diversity	1G?-ESP from MBARI. Moored	5 filtered samples	RNALater	Not used	ESP commercially available	Metatranscriptomics	Temporal variation of plankton metatranscriptome
Pargett et al. (2013) Deep water instrument for microbial identification, quantification, and archiving. Proceedings of 2013 OCEANS - San Diego. https://doi.org/10.23919/OCEANS.2013.6741066	2013	MBARI	Technical development / Proof of Concept	Prokaryotic spp. and total abundance	Deep-ESP (benthic lander)	Deep-sea version of ESP operating to 4000m. Filtering and on-device molecular assays	Not specified	RT-qPCR (?) and SHA	Developed in-house. Research use only (so far?)	-	Deployed at Axial Seamount Hydrothermal Emissions Study (ASHES) and used for sample collection an on-device assays (unclear which)
Seegers et al. (2015) Subsurface seeding of surface harmful algal blooms observed through the integration of autonomous gliders, moored environmental sample processors, and satellite remote sensing in southern California. Limnol Oceanogr. https://doi.org/10.1002/lno.10082	2015	UCLA and MBARI collaboration	Temporal monitoring of algal blooms / Proof of concept	HAB spp.	Moored ESPs at 10m depth, AUV monitoring of chlorophyll in 4D, and satellite	2 ESPs used for continuous monitoring	.	SHA and ELISA	Commercially available ESPs (MBARI) and AUVs used	-	Indicated importance of subsurface population st to source for "seeding" surface Pseudo-

					remote sensing						nitzschia HABs in S California.
Pargett et al. (2016) Development of a mobile ecogenomic sensor. Proceedings of OCEANS 2015 - MTS/IEEE Washington. https://doi.org/10.23919/OCEANS.2015.7404361	2016	MBARI	Technical development	(depends on configuration)	2G-ESP	Continuous RT for up to 3 month, max depth 50m	100+ pucks but only 3 for storing filters (?)	SHA and Immunosorbent assays (cELISA; for toxins) w ready to use assays for specific HABs	Commercially available from McLane Labs and used in routine monitoring in the US: https://mclanelabs.com/environmental-sample-processor/	-	-
Yamahara (2019) In situ Autonomous Acquisition and Preservation of Marine Environmental DNA Using an Autonomous Underwater Vehicle. Front Mar Sci. https://doi.org/10.3389/fmars.2019.00373	2019	MBARI	Benchmarking of eDNA filter preservation in ESP	Range of microbial to macro (fish) spp.	3G-ESP mounted on LR-AUV	15 x 1L ESP samples (1h each)	RNALater	Not used	3G-ESP still not commercial (?)	qPCR of ESP and traditionally collected samples	No significant differences in eDNA densities observed between ESP and traditionally collected
Ribeiro et al. (2019) Development of an autonomous biosampler to capture in situ aquatic microbiomes. PloS ONE, https://doi.org/10.1371/journal.pone.0216882	2019	Portugese consortium: CIIMAR, INESC TEC, and others	Technical development and proof of concept	Prokaryotic and phytoplankton	eDNA sampling by pumping and filtering with the developed IS-ABS field prototype	2L samples. Distinct size fractions. Unclear how many	Unclear	No	In development	Metabarcoding with both autonomously and manually	

						samples (0.2 uM or custom)				collected sample	
Zhang et al. (2020) Persistent Sampling of Vertically Migrating Biological Layers by an Autonomous Underwater Vehicle Within the Beam of a Seabed-Mounted Echosounder. IEE J Ocean Eng. https://doi.org/10.1109/JOE.2020.2982811	2020	MBARI	Proof of concept of acuoustics guided eDNA sampling	Invertebrats	3G ESP mounted on LR-AUV	24 samples taken (out of 60)	RNALater	acoustic modem	3G-ESP still not commercial (?)		
Hansen et al. (2020) Remote, autonomous real-time monitoring of environmental DNA from commercial fish. Sci Rep, https://doi.org/10.1038/s41598-020-70206-8	2020	DTU Aqua (Denmark)	Evaluation of ESP monitoring of fish	4x commercial fish spp.	2G-ESP on land, with pump, in large mesocosm	Continous RT, 3x1L samples @ .2 uM (may be too little vol.)	-	RT-qPCR	ESP commercially available	qPCR validation	Only the most abundant species (mackarel) was detected consistently with enough accuracy (not only due to ESP)
Sepuldiva et al. (2020) Robotic environmental DNA bio-surveillance of freshwater health. Sci Rep, https://www.nature.com/articles/s41598-020-71304-3	2020	US Geol. Survey	Proof of concept for freshwater	Pathogen and invasive fish spp.	2G (?) ESP on land with pump	1L samples	RNALater	-	ESP commercially available	qPCR (from stored samples instead of on ESP. Why?)	Detects equally well but not better in spite of more samples so less sensitive. Still adventagous due to

												reduced effort. A later article also takes into account env parameters and eDNA persistence / distribution modelling
Knapik et al. (2020) Metatranscriptomic Analysis of Oil-Exposed Seawater Bacterial Communities Archived by an ESP. Microorganisms. https://www.mdpi.com/2076-2607/8/5/744	2020	NORCE (Norway)	Mesocosm / indicator identification	env. MRNA	2x 2GESP w oil exposure mesocosm	7x 0.7L samples @ .2 uM	RNALater	-		ESP commercially available	Metatranscriptomics and 16S metabarcoding	
Tang et al. (2020) New insights into the distributions of nitrogen fixation and diazotrophs revealed by high-resolution sensing and sampling methods. The ISME journal. Doi: 10.1038/s41396-020-0703-6	2020	National Oceanography Centre (NOC)	Description of distribution of nitrogen fixation and diazotrophs	Prokaryotes	Robotic Cartridge Sampling Instrument —RoCSI	High-frequency filtering of between 1.5-4L of seawater.	RNALater			Don't know if it is sold. It is being used in the frame of iAtlantic and TechOceans projects.	RT-qPCR and metabarcoding	

Zhang et al. (2021) A system of coordinated autonomous robots for Lagrangian studies of microbes in the oceanic deep chlorophyll maximum. Science Robotics. http://doi.org/10.1126/scirobotics.abb9138	2021	MBARI	Multi-robot monitoring system proof of concept	Prokaryotic diversity	LR-AUV ("Aku") w 3G-ESP for DCM sample collection at 260m	0.2 + 0.45 +5 uL filters (1.6L during 1h)	RNALater	ChlA, nutrients	3G-ESP still not commercial (?)	Metabarcoding	"Deployed in the N Pacific Ocean within the core of a cyclonic eddy, this coordinated system autonomously captured fundamental characteristics of the in situ DCM microbial community in a manner not possible previously"
Moore et al. (2021) An Autonomous Platform for Near Real-Time Surveillance of Harmful Algae and Their Toxins in Dynamic Coastal Shelf Environments. J Mar Sci Eng. https://doi.org/10.3390/jmse9030336	2021	MBARI, NOAA, etc.	HAB monitoring proof of concept	HAB spp.	Moored ESP	Continous (up to 7 weeks)	.	SHA: 7 spp. Of HABs	ESP commercially available	-	Possible connection bloom formation – nutrients-wind-forced coastal-trapped waves.

Formel et al. (2021) Subsurface automated samplers for eDNA (SASe) for biological monitoring and research. HardwareX. https://doi.org/10.1016/j.ohx.2021.e00239	2021	NOAA, Uni. Of Miami, Missisipi State Uni.	Technical specs for automated open source low cost water sampler	(sampling only)	eDNA sampling by pumping and filtering	Down to 50 m (shallow only) and up to 4 samples but for a price of <300€	DNAgard	No	Freely available speces (but not built?)	qPCR, dPCR, metabarcoding ...	-
Flanigan et al. (2021) Proc from OCEANS 2021 San Diego, https://doi.org/10.23919/OCEANS44145.2021.9705708	2021	SeaSatellites (USA), Scripps institute	Technical development	(depends on configuration)	Programmable surface water AUV with solar power	6 individual pumps, for one sampel each. Does not filter but collects up to 1L /sample	No	Not currently	Commercially available (or soon) from SeaSats	Both filtering and molecular	-
Yoerger et al. (2021) A hybrid underwater robot for multidisciplinary investigation of the ocean twilight zone. Sci Robotics. https://doi.org/10.1126/scirobotics.abe1901	2021	Woods Hole, MBARI, Stanford	Technical development report for mesopelagic AUV. ROV for positoning	(depends on configuration)	eDNA sampling by pumping and filtering	Down to 1000 m depth able to track diel migrators. Up to 12 samples	RNALater?	-	In development	-	https://www.whoi.edu/what-we-do/explore/underwater-vehicles/auvs/mesobot/
Truelove et al. (2022) Expanding the temporal and spatial scales of environmental DNA research with autonomous sampling. Env DNA. https://doi.org/10.1002/edn3.299	2022	MBARI (CA, USA)	Proof of concept / benchmarking	Microbial to macro (fish) diversity	ESP (3G-ESP) mounted on long range AUV (Tethtys?)	1L filtering at 0.2 uM Millipore (slowly, "sipping")	Flushed with N2, then in RNALater. Max 60 samples	Not used in this study but see below for 2G ESP	3G-ESP and LR-AUV developed in-house at MBARI. Reasearch use so far (?)	Metabarcoding (12S, 16S, 18S, COI)	Results were comparable to samples taken from boat with Niskin bottle concurrentl

											y. Some differences may be explained by cells not lysing as with vacuum pump
Govindarajan et al. (2022). Improved biodiversity detection using a large-volume eDNA sampler with in situ filtration and implications for marine eDNA sampling strategies. Deep-Sea Research I. doi: 10.1016/j.dsr.2022.103871	2022	Woods Hole (and others)	Evaluation of biodiveristy detection by large-volume (40-60L) eDNA sampler compared to CTDs (2L)	Global diversity (v9-18S rRNA)	eDNA sampling by pumping and filtering	12 samples of 40-60L collected between 20-400 m depth (but can go deeper). Filters seawater at a rate of 2 l/min.	No	Machine-vision monochrome stereo cameras, color camera, and high-sensitivity radiometer (in the AUV)	No information in the paper, but Yoerger (developer of Mesobot AUV; Yoerger et al. 2021) is co-author here.	Metabarcoding of autonomously and CTD-collected samples	The higher the seawater liters filtered the higher the diversity detected. They detected 66% more diversity filtering 40-60L using the large-volume eDNA sampler in a the Mesobot AUV than filtering only 2L using the CTD.
https://dartmouthocean.com/products/edna-sampler	-	Dartmouth Ocean Technolo	Commercial product	(depends on configuration)	eDNA sampling by pumping and filtering	Up to 9 samples, max 20 m depth,	RNALater	Flurometer (optional)	Commercially available	qPCR, dPCR, metabarcoding ...	-

		gies Inc. (Canada)				0.2 uM polycarb (or different)					
https://www.eomoffshore.com/environmentalsamplingprocessor	-	EOM Offshore	Website describing routine monitoring of HABs in the Gulf of Maine	HABs	2G ESP (moored)	Routine near-realtime monitoring of HABs	-	SHA or qPCR	Deployed for monitoring	-	
eDNA Sampler: A fully integrated environmental DNA sampling system	2018	Smith-Root	Description of a fully integrated eDNA sampler	Any – but developed originally for fish	ANDe™	47 mm filter membrane	-	-	Fish monitoring	-	

4.B - Conclusion

4.1. Synthesis of main conclusion

The table showcases examples of developments and applications of devices for DNA sampling and/or analysis. This table will set the basics for future discussion and for the preparation of a review/synthesis document that will be shared with the relevant agents within and outside the consortium in form of a technical paper or a manuscript submitted for publication. This will be aimed at fostering the discussion on eDNA automated sampling, in particular regarding the challenges and obstacles associated with semi-autonomous to autonomous, deployable instrumentation developed for omics-based sample collection, processing and in-situ analysis. Yet, as it was concluded in the Marine Omics Instrumentation and Technology Workshop, whose goal is to survey the technology landscape for ocean deployable biomolecule sampling and sensing instrumentation by convening technology innovators, and summarized by the MBARI CEO Chris Scholin "There's no single sensor or sampler technology that can solve all of our problems. The future lies in finding the right combination of devices and the platforms on which they are deployed to address specific use cases.".

4.2. Next steps (work plan)

The table and document to be produced will be contrasted with the relevant scientist and end-users so that it is kept up to date. Additionally, the difficulty to select a single universal eDNA sampling device and the recognized need of adjusting for each case-study does not prevent the need for developing standardizing processing pipelines and harmonizing data acquisition, for which initiatives are currently ongoing such as the Ocean Best Practices Task Team on Omics/eDNA Protocol Management (https://www.oceanbestpractices.org/ocean-best-practices-systems/our-work/task-teams/omics/).