1 Project Information

Proposal reference number[1]	JS3_CALL_1_REF_4023
Project Acronym (ID)[2]	ATLAS
Title of the project[3]	Advanced ecosysTem monitoring in ecoLogicAl obServatory
Host Research Infrastructure[4]	OBSEA
Images: **eDNA sampling:**	06/09/2021 – 19/09/2021 14/09/2021 - 17/09/2021
Name of Principal Investigator[5] **Home Laboratory** **Address** **E-mail address** **Telephone**	Sergio Stefanni Stazione Zoologica A. Dohrn, Villa Comunale, 80121 Naples (ITALY) sergio.stefanni@szn.it +39 081 5833228

2 Project objectives[6] (250 words max.)

This pilot project aims at setting a baseline of eDNA integration with other techniques/end users communities, that can be applied on fixed monitoring points such as cabled observatories. It comprises several aspects:

a-monitoring capability aspects on the integration of multiple independent datasets (images, sounds, environmental parameters with molecular genetics approaches), methodological for eDNA (time point sampling using Niskin bottles + filtration vs. autonomous sampler over 24 hours' period) and use of time-series as benchmark for comparison.

b-methodological aspects to expand the use of eDNA to evaluate the biodiversity monitoring efficiency in a coastal technological hub. The use of different primers combinations would provide feedbacks on hidden biodiversity components never measured with present sensor assets.

c-Validation aspects on the pathway or the creation of in-situ operating eco-genomic sensors: Automated eDNA sampler coupled with time point sampling could be redefined in terms of number (more sampling throughout the day) or filtering larger amount of water. Comparisons in monitoring capabilities by manual sampling and remotely scheduled filtering to be used as benchmark to set optimum volumes and frequency of filtering.

d-Data processing and statistic elaboration aspects: extract (prior) information from the long time series of OBSEA images , abiotic measurements and how eDNA can complement the monitoring process, to test hypotheses and cause/effect relationships associated to in situ manipulations.

1 Reference number assigned to the proposal by the TA-Office.
2 User-project identifier used in the proposal.
3 Title of the approved proposal. The length cannot exceed 255 characters
4 Name of the installation/infrastructure accessed with this project. If more than one installations/infrastructures are used by the same project, please list them in the box.
5 Fill in with the full contact of the Principal Investigator (user group leader).
6 Write the short-term, medium and long-term objectives of the project. Use no more than 250 words.

3 Main achievements and difficulties encountered (250 words max.)[7]

The OBSEA (www.obsea.es) (Fig. 1) is equipped with Ocean Optics camera (360 dg. rotation capability) in the main node, movable tripod AXIS P1346 camera with 800 m cable length, and Imaging from a crawler (with 80 m tether). All cameras are equipped with lights. Underwater sensors include Temperature and Salinity (CTD), Chlorophyll and Turbidity (AWAC) (Fluorescence), Passive Acoustic Monitoring (PAM; Hydrophone). OBSEA has also a large database with information stored since 01 January 2012.

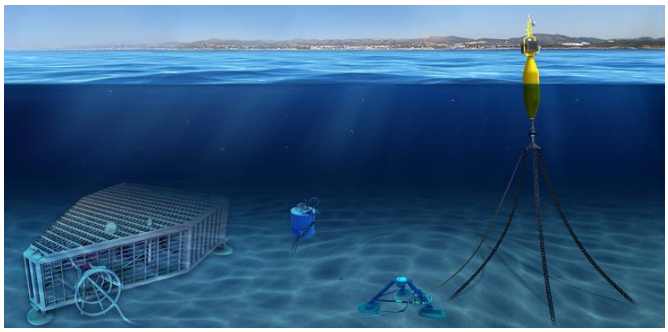

Figure 1. Schematic representation of OBSEA Observatory

The testing period for ATLAS had to be compacted in a single slot of time between 07-19/09/2021 for the imaging while the sampling for eDNA was concentrated in 3 days (14-17/09/2021). The main camera collected images from the water column (Fig. 2A), while the tripod camera was positioned and set to have in the field of view part of the artificial reef and part of the sandy bottom. Within the field of view of the cameras, the 2 autonomous samplers (Fig. 2B) trapped eDNA in time laps filtering up to 50L seawater, within a 24 hours' period, and deployed/retrieved at 20:00 of every day of the experiment. For the same days, and the same site, at 8:00 and at 20:00 respectively, an operator collected water using a Niskin bottle (3 independent replicates) and filtered 1L at the time on sterile Sterivex using a syringe (Fig. 2C).

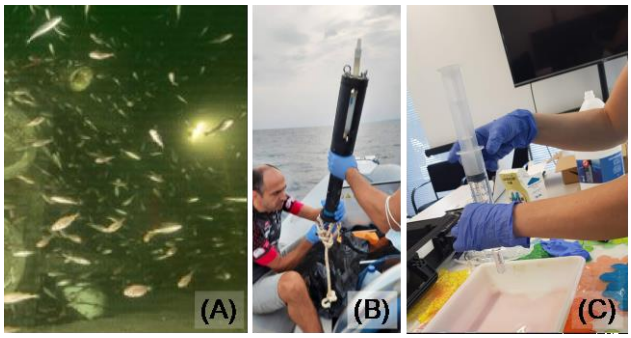

Figure 2. Image from the main camera (A), autonomous sampler (B) and syringe with sterivex filter ©

Capitalising from the 3 days experiment allowed us to explore more in details the methodological aspects of the efficiency in retrieving biodiversity using eDNA data comparing large volumes of seawater filtered every 24 hours (in duplicate) versus small volumes of water filtered in 2 time points a day (in triplicates)(Fig. 3). All filters were sent to Applied Genomics (UK) for eDNA extractions, amplifications (using 2 sets of primers targeting fishes), HT sequencing and bioinformatic analysis. Taxonomic association was compared to the list of species

7 Describe briefly the main achievements obtained and possible impacts, as well as possible difficulties encountered during the execution of the project. Use no more than 250 words.

identified by image analysis.

4 Dissemination of the results[8]

All work carried out in ATLAS, will be disseminated through scientific conferences and scientific articles in peer-reviewed journals. The methodological output will work as reference base-line for further monitoring programmes in OBSEA as well as other ecological observatories.
Most of the original data will be closed for usage by our team until the complete dataset is analysed and published in peer-reviewed journal and become publicly available.
However, we have no problems in sharing the original data prior to publication to other user of OBSEA.

5 Technical and Scientific preliminary Outcomes (2 pages max.)[9]

Sequence analysis of eDNA was carried out at Applied Genomics (UK). All samples were processed using specific primers (12S and cytb) targeting fish assemblages. The number of reads assigned to fish varies across replicates and samples, either using small (sterivex) or large volumes (autonomous samplers) (Fig. 3). All samples collected with strerivex filtration provided larger amount of sequence reads attributed to fish.

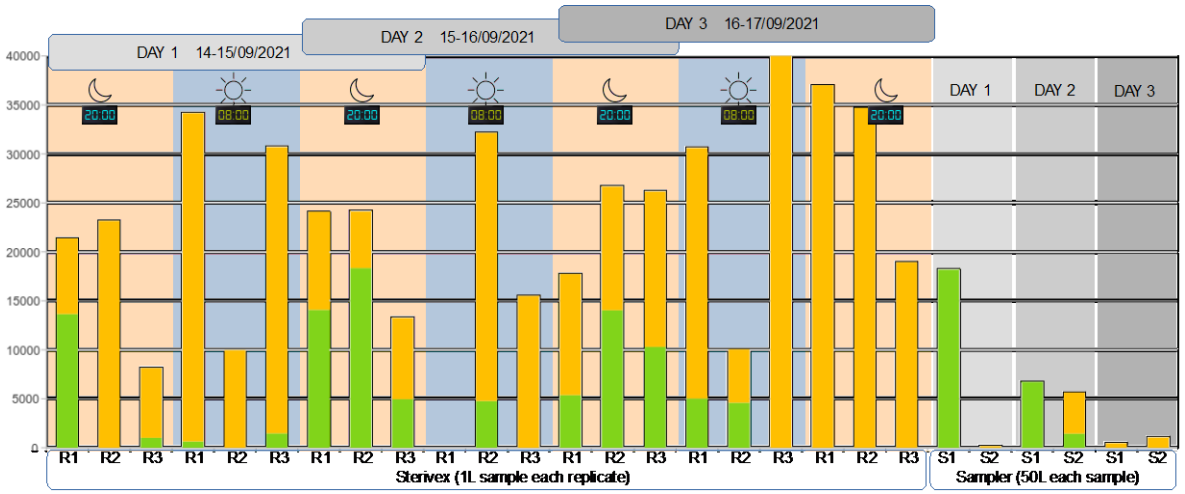

Figure 3. Distribution of reads among sterivex and autonomous samplers. The contribute of 12S (in orange) and cyb (in green) are grouped in replicates (R1-3 and S1-2)) for sterivex and autonomous samplers, respectively. On top, it is shown how the data derived from large volume sampling and sterivex in the 3 days experiment were compared.

In total, combining all the sampling methods, the eDNA approach detected the presence of 38 species of fish (34 only by 12S and 4 only by cytb, 2 of which by both markers, Tab. 1) and belonging to 18 families (Fig. 4).

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8 Describe any plan you have to disseminate and publish the results resulting from work carried out under the Transnational Access activity in JERICO -S3: scientific articles, books - or part of them -, patents, as well as reports and communication to scientific conferences, meetings and workshops. Highlight peer-reviewed publications. **Note that any publications resulting from work carried out under the JERICO -S3 TA activity must acknowledge the support of the European Commission – H2020 Framework Programme, JERICO -S3 under grant agreement No. 871153.**

9 Describe in detail results and main findings of your experiment at the present stage.

Family	Genus	Species
AMMODYTIDAE	Gymnammodytes	*Gymnammodytes cicerelus (12S)*
ATHERINIDAE	Atherina	*Atherina boyeri/A.hepsetus (12S)*
CARANGIDAE	Seriola	*Seriola dumerili (cytb)*
CARANGIDAE	Trachurus	*Trachurus mediterraneus/T. Trachurus (cyb + 12S)*
CARANGIDAE	Trachurus	*Trachurus picturatus (12S)*
CLUPEIDAE	Sprattus	*Sprattus sprattus (12S)*
CLUPEIDAE	Sardinella	*Sardinellla aurita (12S)*
CLUPEIDAE	Sardinella	*Sardinella longiceps (12S)*
CONGRIDAE	Conger	*Conger conger (cytb)*
COTTIDAE	Cottus	*Cottus gobio (12S)*
COTTIDAE	Taurulus	*Taurulus bubalis (12S)*
GADIDAE	Micromesistius	*Micromesistius poutassou (12S)*
GOBIIDAE	Gobiusculus	*Gobiusculus flavescens (12S)*
GOBIIDAE	Pomatoschistus	*Pomatoschistus minutus (12S)*
GOBIIDAE	Pomatoschistus	*Pomatoschistus microps (12S)*
HAEMULIDAE	Pomadasys	*Pomadasys incisus (cytb)*
LABRIDAE	Coris	*Coris julis (12S)*
LABRIDAE	Symphodus	*Symphodus tinca (12S)*
MORONIDAE	Dicentrarchus	*Dicentrarchus labrax (12S)*
MULLIDAE	Mullus	*Mullus surmuletus (cytb + 12S)*
MULLIDAE	Mullus	*Mullus barbatus (12S)*
POMACENTRIDAE	Chromis	*Chromis chromis (12S)*
POMATOMIDAE	Pomatomus	*Pomatomus saltatrix (12S)*
SCIAENIDAE	Argyrosomus	*Argyrosomus regius [?]*
SCOMBRIDAE	Sarda	*Sarda sarda (12S)*
SCOMBRIDAE	Scomber	*Scomber scombrus (12S)*
SERRANIDAE	Serranus	*Serranus cabrilla (12S)*
SPARIDAE	Boops	*Boops boops (cytb)*
SPARIDAE	Diplodus	*Diplodus sargus (12S)*
SPARIDAE	Diplodus	*Diplodus vulgaris (12S)*
SPARIDAE	NA	SPARIDAE *(12S)*
SPARIDAE	Dentex	*Dentex dentex (12S)*
SPARIDAE	Pagellus	*Pagellus acarne (12S)*
SPARIDAE	Pagellus	*Pagellus erythrinus (12S)*
SPARIDAE	Sparus	*Sparus aurata (12S)*
SPARIDAE	Spicara	*Spicara flexuosa or S. smaris (12S)*
SPARIDAE	Spicara	*Spicara maena (12S)*
SPARIDAE	Spondyliosoma	*Spondyliosoma cantharus (12S)*

Table 1. Taxonomic assignment to fish species from eDNA based on 12S and cyb molecular markers. Marked in yellow are the species assigned with a singleton, while the unsure correct assignments is marked with [?]

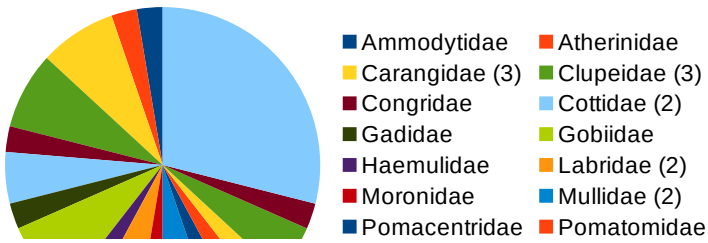

Figure 4. Proportion of specie of fish, grouped by the respective families, retrieved by eDNA approach combining sterivex and autonomous samplers.

The small volume sampling (sterivex, 1L each with 3 replicates at every morning and evenings) performed more efficiently than the large filters mounted on the autonomous samplers (50L each) in retrieving eDNA to trace fish

at OBSEA (Fig. 5A). And while the autonomous sampler collected eDNA in time-laps over 24 hours, the sampling with the sterivex allowed to analyse separately the detection of fish species in the morning and evenings separately (Fig. 5B).

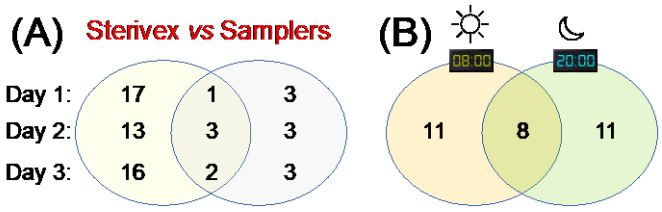

Figure 5. Proportion of species of fish retrieved by eDNA approach by: (A) the two sampling methods (sterivex vs autonomous samplers) and (B) sterivex sampling in the mornings vs evenings.

[Venice], [Date (24/05/2022]

Location and date

Signature of principal investigator