1. Project Information

Proposal reference number[1]	JS3_CALL_3_ 4060_SEASAM
Project Acronym (ID)[2]	SEASAM
Title of the project[3]	Simulating an automated environmental DNA sampler/analyser for *in situ* metabarcoding
Host Research Infrastructure[4]	ISMAR - Venezia
Starting date - End date[5]	21/05/2023 – 17/06/2023
Name of Principal Investigator[6] **Home Laboratory** **Address** **E-mail address** **Telephone**	Maddalena Tibone Atlantic Technological University, Old Dublin Road, Galway, Ireland maddalena.tibone@research.atu.ie +39 3459684975

2. Project objectives[7] (250 words max.)

The objectives stated in the project proposal were to (i) simulate an automated environmental DNA (eDNA) sampler/analyser and (ii) cross-reference of eDNA with imaging data. However, during the project planning, these objectives were modified to accommodate field and laboratory logistic possibilities. The focus was shifted towards comparing active and passive eDNA sampling methods to compare their effectiveness in capturing fish DNA from the environment.
Short-term objectives were identified as active sample collection through filtration of seawater, and passive sample collection through deployment and retrieval of passive samplers, both published and "home-made" devices. To compare the methodologies, eDNA was extracted from the samples and amplified using a real-time quantitative PCR (qPCR) assay to verify the presence and quantity of fish DNA.
The medium and long-term objectives of the project include the definition of an effective sampling protocol for both active and passive eDNA sampling, and the provision of recommendations on which sampling method is more effective in describing the local species richness/biodiversity. These

[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] Specify starting and end date of the project (including eventual preparatory phase before the access).

[6] Fill in with the full contact of the Principal Investigator (user group leader).

[7] Write the short-term, medium and long-term objectives of the project. Use no more than 250 words.

objectives will allow a better understanding of the ideal sampling methodology to be applied in the context of an automated sampler/analyser. Lastly, the data collected from eDNA samples will be compared to the data obtained through analysis of the underwater camera images. This will help to further validate the eDNA workflow by providing evidence in support to methodological recommendations.

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

The main goal of this project (i.e. targeted field sampling and processing of eDNA at the Acqua Alta oceanographic platform) was achieved.
Regarding field work, due to technical/logistic constraints it was not possible to remain overnight aboard the Aqua Alta platform (as previously envisaged), thus the sampling design had to be adapted to regular daily visits, although adverse weather conditions prevented access to the platform on multiple occasions. Nonetheless, sampling took place over the four weeks of access, with a total of 8 daily missions to the infrastructure. During this process, samples were collected with one active technique and with two or more passive methods. Secondly, a subset of the samples collected (50% of the active samples and 20% of the passive samples) was processed in the molecular laboratory at ISMAR. Processing included eDNA extraction and amplification by means of qPCR with a universal assay targeting fish. Results were collected and analysed on site, and protocol optimization was successfully carried out when necessary. This included resolving issues related to presence of PCR inhibitors in the samples, which resulted in false negatives in early qPCR trials. This issue was successfully tackled with strategies to reduce inhibition.

4. Dissemination of the results[9]

The dissemination of the results obtained will be carried out after further analysis of the samples, as some of the issues with the amplification output still need to be clarified.
Dissemination of results will include the present report, and the presentation of the project at internal ATU seminars and international conferences, as well as the data set publication in peer reviewed ISI Journals as part of the PhD thesis work in which the present activities are framed. In addition, the protocols and methodological developments defined during the project will be shared with the scientific community through online platforms (e.g. protocol.io).
Data has not yet been delivered on the date of the report submission. The metadata of the samples collected, and the qPCR output data are available upon request. Any further data produced from these samples will be made openly available as soon as possible.

[8] 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.

[9] 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.**

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

Sampling was carried out successfully at the Acqua Alta platform, using both active and passive sampling techniques. Active filtration samples (hereinafter CN) were collected on Cellulose-Nitrate membranes (0.45 µM porosity, 47 mm diameter). Passive samples were collected on different substrates: (i) glass fibre filters (1.2 µM porosity, 47 mm diameter) tied to a grid (hereinafter GF/C), (ii) rolls of gauze inside a Metaprobe support (Maiello *et al*. 2022) (hereinafter M) and (iii) Free Gauze spread out on a grid to maximise the sampling surface (hereinafter FG). CN samples were collected each day of platform access collecting water with a Niskin bottle at -13 m of depth. GF/C and M samples were collected after 3 hours, 24 hours, 48 hours, 1 week and 2 weeks of deployment under the platform at -13 m. The time of deployment was variable, depending on the possibility to access the platform (see section 3). For each timepoint, three replicate samples were collected, for a total of 36 CN samples, 42 GF/C samples and 42 M samples. Only 5 FG samples were collected in total, after 3 hours, 24 hours, 48 hours, 72 hours and 1 week of deployment at -13 m. In addition, negative samples were collected from both the Niskin and the pumping system, after decontaminating them, by filtering 2 L of milliQ water on cellulose nitrate filters. Negatives were collected only on 5 of the 8 days on the platform, due to logistic/timing difficulties.

A subset of the samples (50% of CN, 20% of GF/C, 20% of M and 80% of FG) were processed with eDNA extraction and qPCR amplification in the laboratories at ISMAR. A universal qPCR assay targeting fish was used to verify the presence of fish DNA. As many amplifications were unsuccessful on first try, the presence of PCR inhibitors was hypothesized, and a 5-fold dilution was applied to the extracts to reduce inhibition.

Figure 1 shows an overview of the preliminary outcomes of the project. Firstly, the success rate of amplification increased for all sampling methods, when the raw extracts were diluted 1:5 in water. This confirms the presence of PCR inhibitors in the samples. Overall, the active filtration method was more successful than the passive methods. Among the latter, the free gauze showed 100% amplification after dilution, while no amplification with the raw extracts. The amplification success of both GF/C and M samples increased after dilution but did not reach 100%. This indicates that both substrates capture very low quantities of eDNA.

All the negative control samples (both from the Niskin and the pump system) amplified after the 5-fold dilution. Although most samples showed very late amplification, indicating only traces amounts of fish DNA in the negative controls, the decontamination process should be reviewed and improved.

When analysing the qPCR results, a puzzling aspect was the presence of multiple peaks in the melt curve profiles. For positive amplification of genomic fish DNA (from a tissue sample) an individual peak would be expected. Instead, the presence of multiple peaks could suggest non-specific amplification of DNA from other organisms present in the sample, such as bacteria. When separating the PCR products by means of gel electrophoresis, two bands were present (when only one band would have been expected) further confirming the possibility that, after over 30 PCR cycles the selected primers may produce non-specific amplification with microbial DNA fragments.

[10] Describe in detail results and main findings of your experiment at the present stage.

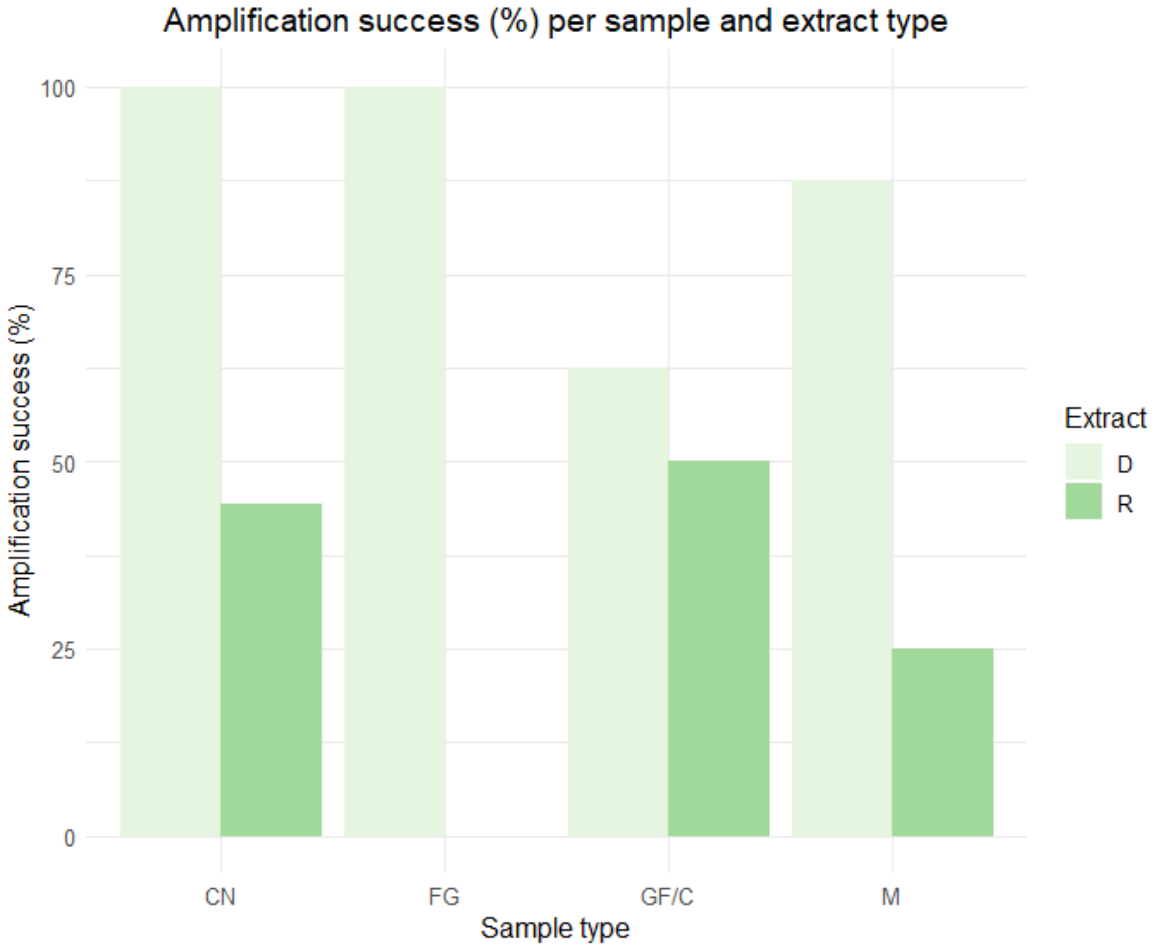

Figure 1. Bar plot indicating the amplification success (shown as a percentage) divided by sample type (CN for active sampling; FG, GF/C and M for passive sampling) and type of extract used for the amplifications (D = diluted 1:5 in water, R = raw extract).

Overall, over the course of this project we carried out successful sampling, extractions, amplifications and troubleshooting. Considering the results obtained up to now, the active sampling method is the most effective one in terms of amplification success. Of the passive methods, the FG samples were the most effective after extract dilution, indicating that gauze is a better substrate than glass fibre filters and that a higher sampling surface is an advantage.

Further work on these samples will include metabarcoding analysis to identify the composition of the local fish community. This profile will be compared with the imaging data from the underwater cameras to verify the effectiveness of eDNA sampling as a biodiversity monitoring tool. In addition, further tests will be run to explain the presence of multiple peaks in the melt curves.

References

Maiello, G., Talarico, L., Carpentieri, P., De Angelis, F., Franceschini, S., Harper, L. R., Neave, E. F., Rickards, O., Sbrana, A., Shum, P., Veltre, V., Mariani, S., & Russo, T. (2022). Little samplers, big fleet: eDNA metabarcoding from commercial trawlers enhances ocean monitoring. Fisheries Research, 249(July 2021), 106259. https://doi.org/10.1016/j.fishres.2022.106259

_Galway, 30/06/2023_____
Location and date

Signature of principal investigator