



**Next-generation monitoring
& mapping tools
to assess marine
ecosystems & biodiversity**

Milestone M2.1

Completion of sampling (means of verification: Water samples)

Greece 2.0
NATIONAL RECOVERY AND RESILIENCE PLAN



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M2.1 COMPLETION OF SAMPLING (MEANS OF VERIFICATION: WATER SAMPLES)

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DOCUMENT INFORMATION AND VERSION CONTROL

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VERSION CONTROL

Revision-N°	Date	Description	Prepared By	Reviewed By
1 st	8/11/2024	1 st Draft	S. Genitsaris	C. Gubili
2 nd	12/11/2024	Final Draft	C. Gubili S. Genitsaris	A. Mazaris

Executive Summary

Milestone 2.1 (Completion of sampling) is connected to Task 2.1 of WP2. This milestone verifies the successful completion of water samplings at different marine sites, covering variable representative hydrological conditions, stratification patterns and trophic states (i.e., eutrophication levels). The sampling sites were selected in coastal areas of Thermaikos Gulf, Kavala Gulf, and Saronikos Gulf, as well as offshore sites in the Aegean Sea, as proposed in the Technical Document. Underwater samples were collected with Niskin-type samplers, and a submersible standalone pumping device. Furthermore, a newly designed low-cost 3D-printed probe which is equipped with the appropriate filter was successfully tested. For each sampling site three replicates were collected, corresponding to > 100 filters for the WP, plus four negative controls. Water samples were filtered through variable filter types targeting different biocommunities. Filters are preserved in ~95% ethanol in 2-ml tubes or placed directly at -20 °C and are undergoing DNA extraction and sequencing.

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CONTRIBUTORS

TABLE 1 NAMES AND ROLES OF CONTRIBUTORS TO THIS DELIVERABLE.

Name	Affiliation	WP Lead	Task Lead
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1. Introduction

As described in Deliverable D2.1, several methods of eDNA sampling from aquatic systems are now implemented aiming at capturing multiple levels of biodiversity of different biocommunities. After a systematic review of the modern literature, and personal expertise of the scientists involved in WP2, we concluded that among these sampling techniques, vacuum pump filtering, inline filtering, and passive samplers stand out for their complementary strengths in different research contexts (Deliverable D2.1). Vacuum pump filtering offers unmatched efficiency for processing large water volumes, crucial for capturing eDNA in diverse and expansive marine environments. Its ability to handle substantial sample sizes enhances detection probabilities, making it ideal for comprehensive biodiversity assessments. Inline filtering, on the other hand, provides a streamlined and contamination-resistant option for fieldwork, preserving eDNA integrity from collection to laboratory analysis. Its portability and ease of use make it a practical choice for studies conducted in remote or resource-limited settings. Lastly, passive samplers introduce a low-cost, low-labor alternative that captures eDNA over time, offering valuable temporal resolution and reducing the need for extensive field equipment. Together, these three methods provide a versatile toolkit that can be tailored to various marine research needs and was applied in the context of the NEMO-Tools project.

2. Samplings

We performed three daily sampling campaigns in a number of marine sites covering variable representative hydrological conditions, stratification patterns and trophic states (i.e. eutrophication levels) in the coastal areas of Thermaikos Gulf, Kavala Gulf, and Saronikos Gulf, as well as offshore sites in the Aegean Sea, as per the Technical Document. In each Gulf, six sampling sites representing variable degrees of eutrophication, were selected, and two depths per site (surface and end of the euphotic zone as calculated by the transparency based on Secchi Disk throws, or bottom in shallow waters) were sampled. For each sampling site and depth, three replicates were collected per sampling approach, corresponding to > 100 filters for WP2, plus four negative controls. Key abiotic variables (T, S, pH) were measured *in situ* with portable instruments (Figure 1). Samplings took place between April and July 2024 using boats that were provided by UoA and INALE institutions. All necessary equipment was also provided by the laboratories participating in the project.

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Figure 1. Snapshots from the sampling expeditions, including onboard filtering for eDNA capture and abiotic measurements.

Saronikos Gulf samplings were performed on 22nd April 2024 around Salamina island (Figure 2), where pronounced nutrient and waste inputs are recorded from Elefsina's industrial zone indicating eutrophication pressures. Further offshore samples were taken during the campaign.



Figure 2. Coastal sampling sites in Saronikos Gulf.

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Details of the Saronikos Gulf sampling sites' location, and *in situ* environmental measurements are presented in Table 1.

Table 1. Location of sampling sites in Saronikos Gulf, transparency, temperature (T), salinity (psu) and dissolved oxygen saturation (DO %). N/A denotes not available value.

Sampling codes	Coordinates	Transparency (m)	Depth (m)	T (°C)	Salinity (psu)	pH	DO (% saturation)
NTS1_s	37.992861N	8	Surface	18.4	38.2	8.1	N/A
NTS1_d	23.548194 E		20	16.3	38.2	8.07	10
NTS2_s	37.8979 N	12	Surface	18.1	39	8.05	11.5
NTS2_d	23.5471 E		30	17.9	39.4	8.03	11.5
NTS3_s	37.85025 N	12	Surface	18.3	39	8.1	7
NTS3_d	23.43295 E		30	16.2	38.4	8	11
NTS4_s	37.9028 N	12	Surface	18.9	38.5	8.1	9
NTS4_d	23.38031 E		30	17.6	38.4	8.2	8.5
NTS5_s	37.981033 N	8	Surface	19.1	38.2	8.2	25.7
NTS5_d	23.413883 E		10	18.6	38.2	8.22	28.3
NTS6_s	38.013217 N	5.5	Surface	19.6	37.8	8.25	35
NTS6_d	23.495516 E		14	17.3	38	8.2	35.5

Kavala Gulf samplings were performed on 11th July 2024 in coastal sites (Figure 3), where pronounced nutrient and waste inputs are recorded in the literature indicating eutrophication pressures.



Figure 3. Coastal sampling sites in Kavala Gulf.

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Details of the Kavala Gulf sampling sites' location, and *in situ* environmental measurements are presented in Table 2.

Table 2. Location of sampling sites in Kavala Gulf, transparency, temperature (T), salinity (psu) and dissolved oxygen saturation (DO %). N/A denotes not available value.

Sampling codes	Coordinates	Transparency (m)	Depth (m)	T (°C)	Salinity (psu)	pH	DO (mg L ⁻¹)
NTK1_s	40.72905 N	16	Surface	28.2	33.2	8.15	6.23
NTK1_d	24.25467 E		40	18.5	36.6	8.17	7.06
NTK2_s	40.73095 N	16	Surface	28.3	32.9	8.09	6.24
NTK2_d	24.21155 E		40	17.8	37.8	8.24	6.95
NTK3_s	40.77222 N	8	Surface	28.6	33	8.1	6.19
NTK3_d	24.35214 E		20	20.8	37	8.22	7.14
NTK4_s	40.81696 N	8	Surface	28.4	33.1	8.11	6.2
NTK4_d	24.41260 E		20	21.2	37.1	8.2	6.9
NTK5_s	40.83599 N	5	Surface	28.6	32.6	8.12	6.41
NTK5_d	24.35594 E		12	23	35.1	8.2	6.8
NTK6_s	40.83800 N	5	Surface	28.1	32.7	8.14	6.48
NTK6_d	24.32123 E		11	23.8	32.3	8.2	7.16

Thermaikos Gulf samplings were performed on 16th July 2024 in coastal sites (Figure 4), where pronounced nutrient and waste inputs are recorded in the literature indicating eutrophication pressures.

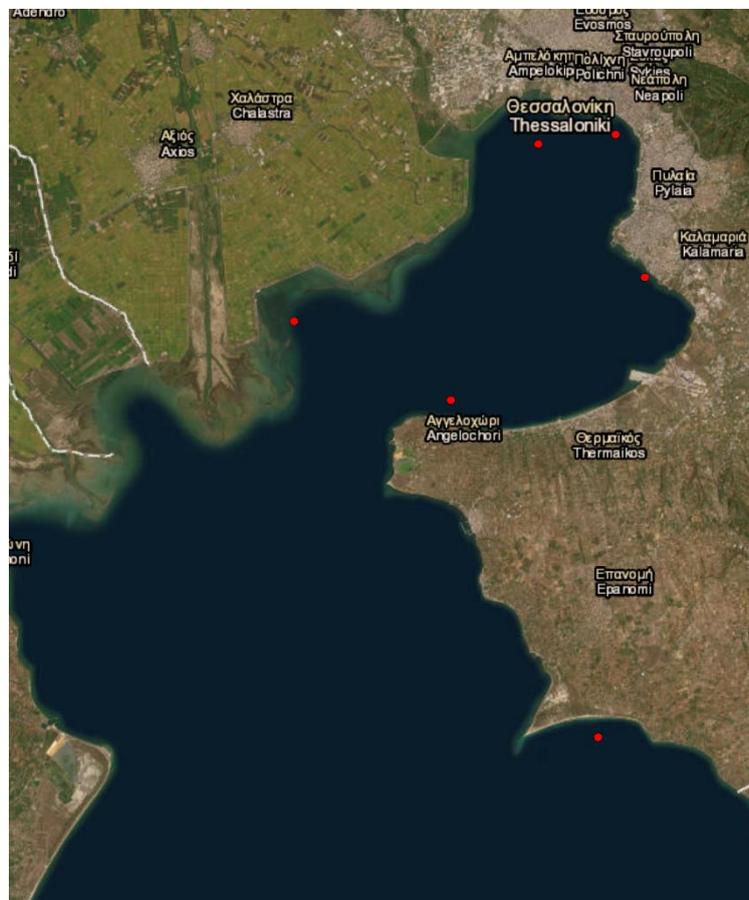


Figure 4. Coastal sampling sites in Thermaikos Gulf.

Details of the Thermaikos Gulf sampling sites' location, and *in situ* environmental measurements are presented in Table 3.

Table 3. Location of sampling sites in Thermaikos Gulf, transparency, temperature (T), salinity (psu) and dissolved oxygen saturation (DO %). N/A denotes not available value.

Sampling codes	Coordinates	Transparency (m)	Depth (m)	T (°C)	Salinity (psu)	pH	DO (mg L ⁻¹)
NTT1_s	40.54690 N	7	Surface	29.2	33.9	8.21	6.53
NTT1_d	22.76250 E		18	22.8	35.9	7.97	3.96
NTT2_s	40.62085 N	7	Surface	30.9	33.3	8.33	10.06
NTT2_d	22.89578 E		12	27.1	34.3	8.15	4.54
NTT3_s	40.62475 N	7	Surface	30.6	33.6	8.32	8.65
NTT3_d	22.93801 E		11	26.8	34.4	8.07	8.79
NTT4_s	40.56521 N	7	Surface	29.8	34	8.27	7.77
NTT4_d	22.95401 E		7	29.3	33.9	7.97	7.59
NTT5_s	40.51391 N	8	Surface	29.1	33.4	8.03	6.48
NTT5_d	22.84810 E		20	22	35	8.04	5.35
NTT6_s	40.37286 N	8	Surface	28.6	34.4	8.02	6.05
NTT6_d	22.92852 E		20	20	36.9	8.13	6.87

3. Results/Validation process

All water samplings and eDNA capture with the three selected approaches were completed successfully. All filters were stored in -20 °C for further analysis.

4. Next steps

All eDNA samples are undergoing DNA extraction, which is projected to be completed in the next weeks. When completed, metabarcoding sequencing will be implemented in all DNA extracts using multiple genetic markers towards revealing the deep microbial (prokaryotic and eukaryotic) and fish diversity and inferring functional patterns.

5. References

Deliverable D2.1. (2024). Innovative Sampling Pipelines. Chrysoula Gubili, Savvas Genitsaris, Panagiota Xanthopoulou, Antonios Mazaris. pp 13.