NEMO
TOOLS

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

Milestone M1.2

Study visits for method testing

DOCUMENT INFORMATION AND VERSION CONTROL

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Executive Summary

Milestone 1.2 presents field study reports documenting on-site data collection, method and equipment testing, and calibration activities conducted throughout the project by the expert research teams of the NEMO-Tools project.

Executive Summary

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CONTRIBUTORS

TABLE 1 NAMES AND ROLES OF CONTRIBUTORS TO THIS MILESTONE.

Name	Affiliation	WP Lead	Task Lead
Antonios Mazaris	Aristotle University of Thessaloniki		
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Field study report sheets

I.

Date: 22/04/2024

Location: Saronic Gulf, Greece

Duration: Single sampling cruise with a sailing boat

Scope: Collection of seawater samples for environmental DNA (eDNA) analysis targeting fish, bacterial and protist communities. The primary objective of this field survey was the recording and characterisation of the taxonomic and functional diversity of microbial communities in the study area. Sampling aimed also to compare the performance of three eDNA collection methods (active filtration, Sterivex, and passive gauze filtering). The survey also contributes to the broader comparative framework of microbial biodiversity assessment across Greek coastal systems.

Summary of activities: Water samples were collected at six stations representing different parts of the Saronic Gulf. At each station, two depths were sampled (surface and lower euphotic zone). All three filtration methods were applied at each sampling point to evaluate potential methodological differences in eDNA recovery. All filters and gauze units were stored at –20°C immediately after collection for downstream molecular analysis. Environmental parameters were recorded with a CTD probe. Sailor citizens were involved in the activities.

Results/findings: Preliminary analysis of the collected water samples confirmed successful recovery of environmental DNA from all three sampling methods. Anchovies and sardines dominated. Species of the families Sparidae, Scombridae, Mugilidae, Gobidae, and Callionymidae were also identified. Microbial communities (bacteria and protists) were detected across all stations and depths, with clear differences in diversity and relative abundance patterns among sampling sites and methods. Dinoflagellates, diatoms, and Syndiniales were the dominant unicellular eukaryotes, while bacterial assemblages were consistently structured by major coastal groups such as Alphaproteobacteria, Gammaproteobacteria, Cyanobacteria, and Bacteroidia. Methodological differences were evident, particularly for Gauze samples, which showed higher variability in community structure. *Detailed presentation of the samplings are presented in D2.1.*

Link to Deliverable(s): The activities of the samplings, and the data collected during this cruise contribute directly to the project deliverables D2.1, D2.2 and the upcoming D2.4. The outcomes of this field survey, including microbial community profiles, diversity assessments and methodological comparison, are fully analyzed and discussed in the associated deliverable report.

Equipment used/tested:

- Active filtration using a vacuum pump (polycarbonate filters, 0.2 μm)

- Sterivex syringe filtration (0.2 μm)

- Passive gauze filtration (immersed for ~10 min per station)
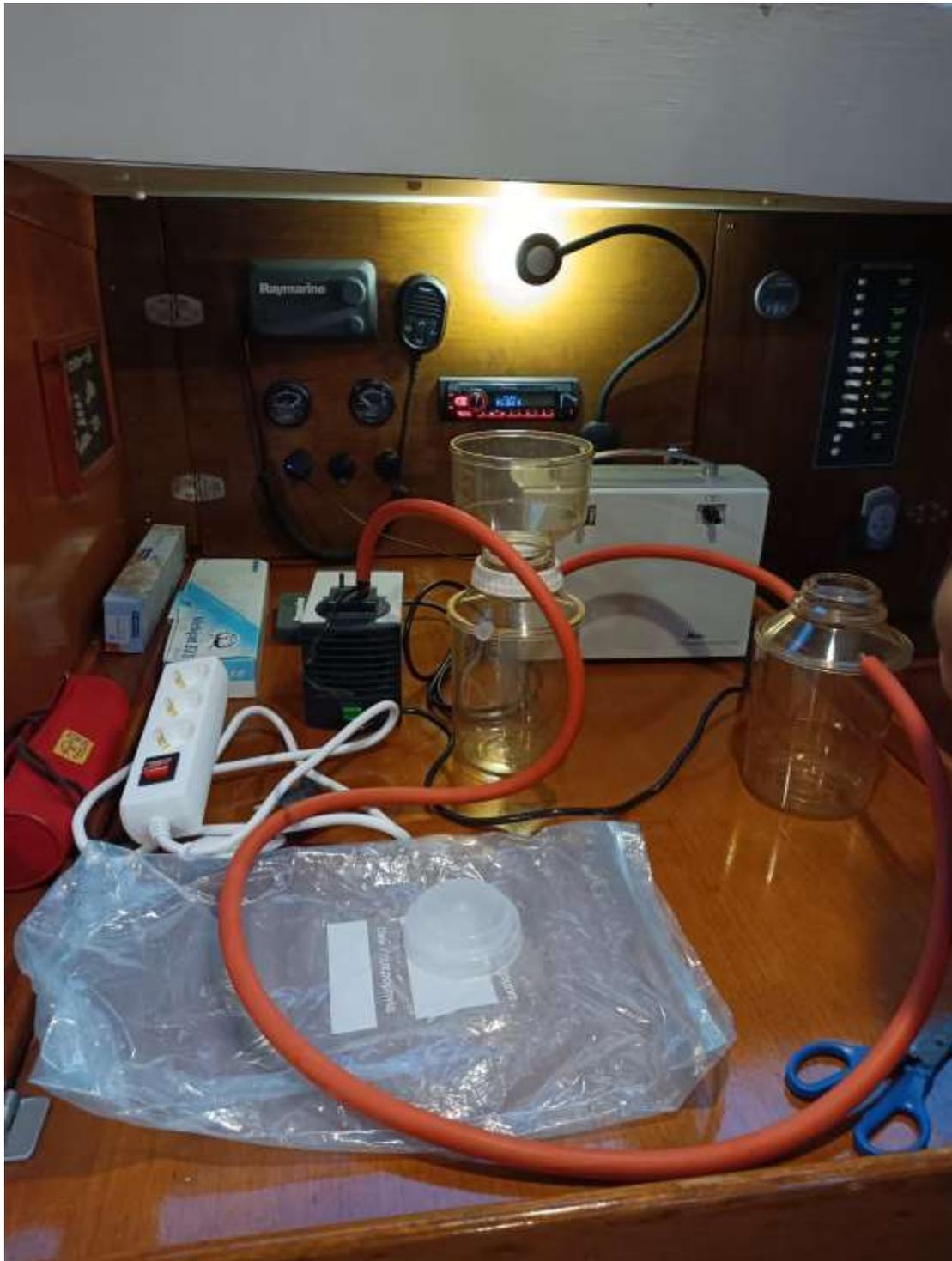
Photographs:

II.

Date: 16/07/2024

Location: Thermaikos Gulf, Greece

Duration: Day sampling survey with the research vessel of the Fisheries Research Institute (ELGO-DIMITRA/INALE)

Scope: Collection of seawater samples for environmental DNA (eDNA) analysis targeting fish, bacteria, as well as protist communities. The initial aim of the survey was to collect water samples in order to record and characterize the taxonomic and functional diversity of the aforementioned communities in the study area. Moreover, sampling aimed to compare the performance of three eDNA collection methods (two active filtration and one passive gauze filtering). The survey will contribute to the broader comparative framework of biodiversity monitoring across Greek coastal systems.

Summary of activities: Water samples were collected at six stations of the Thermaikos Gulf. Two depths were sampled (surface and lower euphotic zone) at each station. Water samples were collected with a Niskin bottle and were stored at 4 °C until filtration. All three filtration methods were applied at each sampling point to evaluate potential methodological differences in eDNA recovery. All filters and gauze units were stored at –20 °C immediately after filtration for downstream molecular analysis. Environmental parameters were recorded with a CTD probe.

Results/findings: Preliminary analysis of the collected water samples confirmed successful recovery of environmental DNA from all three sampling methods. Fish assemblages, microbial communities (bacteria and protists) were detected across all stations and depths, with differences in diversity and relative abundance patterns among sampling sites and methods. Anchovies and sardines dominated all stations. Species of the families Sparidae, Scombridae, Mugilidae, Gobidae, and Callionymidae were also detected. Gauzes were less successful in detecting fishes compared to active filtering. Dinoflagellates, diatoms, and Syndiniales were the dominant unicellular eukaryotes, while bacterial assemblages were consistently structured by major coastal groups such as Alphaproteobacteria, Gammaproteobacteria, Cyanobacteria, and Bacteroidia. Methodological differences were evident, particularly for Gauze samples, which showed higher variability in community structure. *Detailed presentation of the samplings are presented on D2.1.*

Link to Deliverable(s): The activities of the samplings, and the data collected during this cruise contribute directly to the project deliverables D2.1, D2.2 and the upcoming D2.4. The outcomes of this field survey, including microbial community profiles, monitoring of fish assemblages, diversity assessments and methodological comparison, are fully analyzed and discussed in the associated deliverable report.

Equipment used/tested:

- Active filtration using a vacuum pump (polycarbonate filters, 0.2 μm)

- Sterivex syringe filtration (0.2 μm)

- Passive gauze filtration (immersed for ~10 min per station)

Photographs from fieldwork activities:

III.

Date: 18/07/2024

Location: Kavala Gulf, Greece

Duration: Day sampling survey with the research vessel of the Fisheries Research Institute (ELGO-DIMITRA/INALE)

Scope: Collection of seawater samples for environmental DNA (eDNA) analysis targeting fish, bacteria, as well as protist communities. The initial aim of the survey was to collect water samples in order to record and characterize the taxonomic and functional diversity of the aforementioned communities in the study area. Moreover, sampling aimed to compare the performance of three eDNA collection methods (two active filtration and one passive gauze filtering). The survey will contribute to the broader comparative framework of biodiversity monitoring across Greek coastal systems.

Summary of activities: Water samples were collected at six stations of the Thermaikos Gulf. Two depths were sampled (surface and lower euphotic zone) at each station. Water samples were collected with a Niskin bottle and were stored at 4 °C until filtration. All three filtration methods were applied at each sampling point to evaluate potential methodological differences in eDNA recovery. All filters and gauze units were stored at –20 °C immediately after filtration for downstream molecular analysis. Environmental parameters were recorded with a CTD probe.

Results/findings: Preliminary analysis of the collected water samples confirmed successful recovery of environmental DNA from all three sampling methods. Fish assemblages, microbial communities (bacteria and protists) were detected across all stations and depths, with differences in diversity and relative abundance patterns among sampling sites and methods. Anchovies dominated all stations. Species of the families Sparidae, Scombridae, Mugilidae, Gobidae, Alosidae, and Callionymidae were also detected. Gauzes were less successful in detecting fishes compared to active filtering. Dinoflagellates, diatoms, and Syndiniales were the dominant unicellular eukaryotes, while bacterial assemblages were consistently structured by major coastal groups such as Alphaproteobacteria, Gammaproteobacteria, Cyanobacteria, and Bacteroidia. Methodological differences were evident, particularly for Gauze samples, which showed higher variability in community structure. *Detailed presentation of the samplings are presented on D2.1.*

Link to Deliverable(s): The activities of the samplings, and the data collected during this cruise contribute directly to the project deliverables D2.1, D2.2 and the upcoming D2.4. The outcomes of this field survey, including microbial community profiles, monitoring of fish assemblages, diversity assessments and methodological comparison, are fully analyzed and discussed in the associated deliverable report.

Equipment used/tested:

- Active filtration using a vacuum pump (polycarbonate filters, 0.2 μm)

- Sterivex syringe filtration (0.2 μm)

- Passive gauze filtration (immersed for ~10 min per station)

Photographs from fieldwork activities:

IV.

Date: 22/09/2024

Location: Skyros

Duration: 3 days

Scope: Field data collection on the occurrence of *Cystoseira* spp at Skyros for the development of the task on mapping macroalgae using Earth Observation.

Summary of activities: Elusive macroalgae forests have been recorded in the 3 target areas. Geolocation data have been collected using GARMIN GPSMAP 67 and imagine data using GoPro camera system.

Results/findings: The observations have been geolocated using GARMIN Basecamp software application and used in the development of the machine learning workflow. The delineation of macroalgae forests from the bare rocky surfaces have been achieved with high accuracy. The scalability and the development of monitoring processes is promising and challenging as the validation data needed for that have to follow specific requirements that are not widely available in the coastal seascsapes.

Link to Deliverable(s): [Mapping Subtidal Marine Forests in the Mediterranean Sea Using Copernicus Contributing Mission](#)

Equipment used/tested: Snorkeling tools, GoPro camera and waterproof GPS

Photographs:
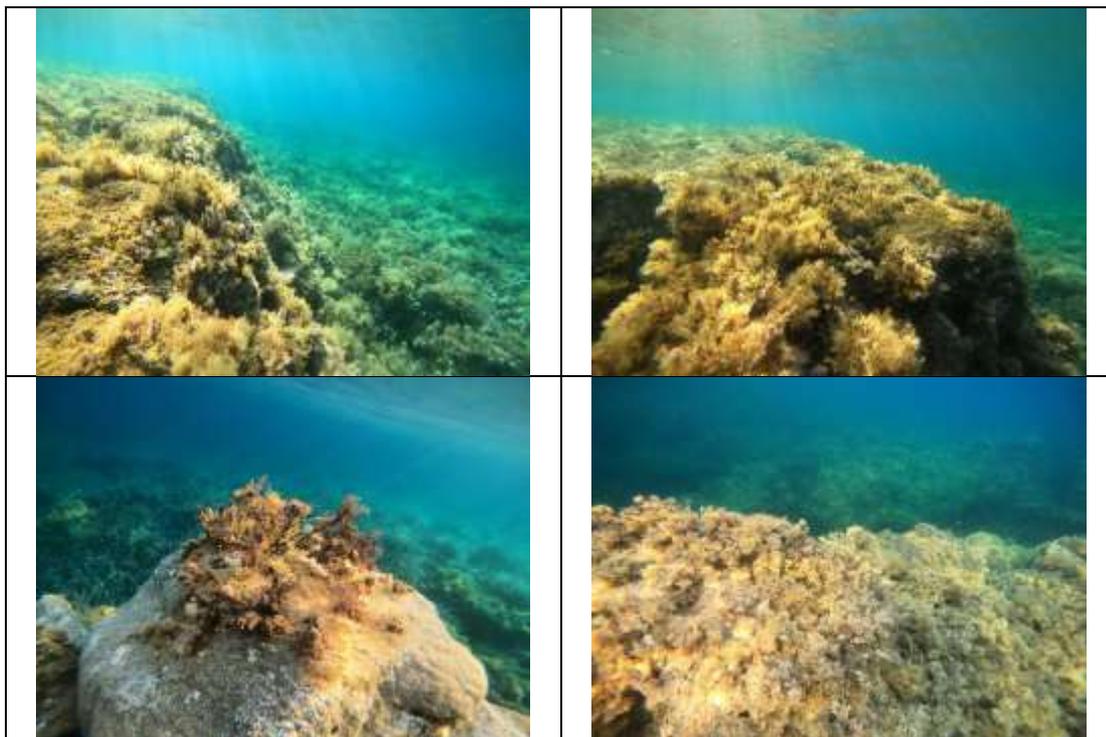
V.

Date: 08-08-2025

Location: Cape Agrilia, Lesvos

Duration: 1 day

Scope: Experimental deployment of low-cost NEMOTools hydrophones in the vicinity of an aquaculture facility at Agrilia, Southeast Lesvos, in an effort to record the sounds produced by bottlenose dolphins (Tursiops truncatus) that frequent these waters.

Summary of activities: Two NEMOTools hydrophones were mounted on a metallic custom-made base, along with a scientific hydrophone (SNAP model from Loggerhead Instruments) which served as a reference, and a commercially available low-cost hydrophone (Hydromoth). The survey boat was anchored 200 m east of the aquaculture site, and the acoustic station was lowered off the stationary boat to a depth of 7 m. The recording started at 07:20 local time and was stopped after one hour.

Results/findings: Although dolphins were neither sighted nor recorded, this fieldwork session captured the passage of a 195 m long ferry boat, northbound to Mytilene port, over 2 km in distance from our hydrophones. This find gave insight into the low-frequency capabilities of our acoustic toolkit over large spatial scales.

Link to Deliverable(s): 1.3 One (1) peer-reviewed article on the demonstration/application of prototype, cost effective hydrophones

Equipment used/tested: Two NEMO-Tool hydrophones, one SNAP hydrophone, custom-made metallic base, two handheld recorders (Philips DVT 1120 and Tascam DR-05X)

Photographs:

VI.

Date: 26-06-2025

Location: Marathonisi island, Zakynthos

Duration: 1 day

Scope: Experimental deployment of low-cost NEMOTools hydrophones in order to assess their performance in recording the traffic density of the Marine Protected Area of Marathonisi island (mostly frequented by recreational speedboats) using a high-end scientific counterpart as reference.

Summary of activities: Two NEMOTools hydrophones were mounted on a commonly used umbrella stand, along with a high-end scientific hydrophone (SNAP model from Loggerhead Instruments) and a commercially available low-cost hydrophone (Hydromoth). The custom-made hydrophone station was subsequently submerged at approximately 3 meters depth and recorded the traffic density of the day for 3 full hours (11:20 – 14:20). The recordings were accompanied by on-site manual annotations which were noted down during the 3-hour recording session.

Results/findings: The NEMOTools hydrophones were able to record with very low margins of error (174 out of 182 vessel instances) the traffic density of the day. These recordings provided very useful, quantitative data with which we later calculated sound metrics in order to further assess the performance of our low-cost acoustic toolkits.
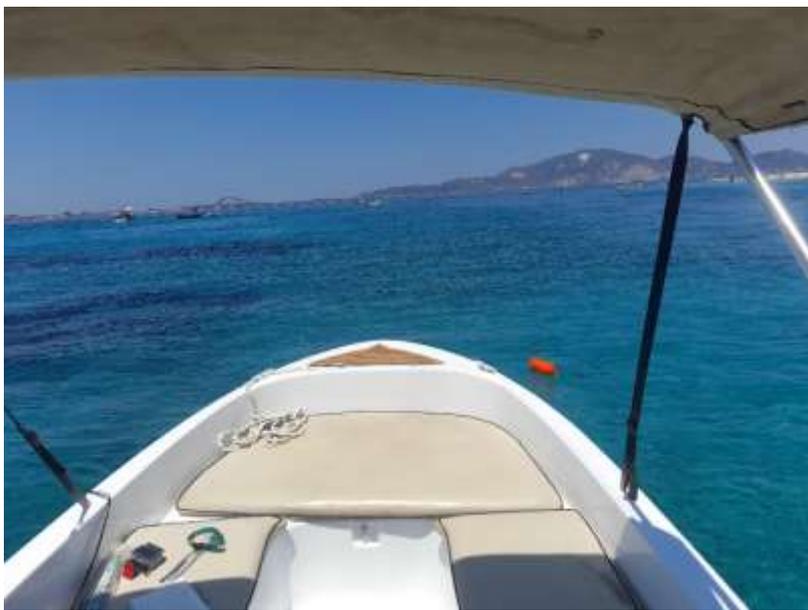
Link to Deliverable(s): 1.3 One (1) peer-reviewed article on the demonstration/ application of prototype, cost effective hydrophones

Equipment used/tested: Two NEMO-Tool hydrophones, one SNAP hydrophone, one Hydromoth hydrophone, umbrella stand, two handheld recorders (Philips DVT 1120 and Tascam DR-05X)

Photographs:

M1.2 Study Visits for Method Testing

VII.

Date: 10-06-2025

Location: Port of Mytilene, Lesvos

Duration: 1 day

Scope: Experimental deployment of low-cost NEMOTools hydrophones in order to assess their performance in recording inboard vessels.

Summary of activities: Two NEMOTools hydrophones were mounted on a metallic custom-made base, along with a scientific hydrophone (SNAP model from Loggerhead Instruments) which served as a reference, and a commercially available low-cost hydrophone (Hydromoth). The recording session lasted from 17:40 to 18:10 local time. The acoustic station was deployed off the pier at 4 m depth (resting on the seafloor) and recorded the scheduled departure of a passenger boat (40 m in length) that travels daily between Mytilene and Ayvalik (Turkey).

Results/findings: The NEMOTools hydrophones adequately recorded the noise emitted from the 3 ferryboat passings of the day. These recordings provided very useful, quantitative data with which we later calculated various sound metrics in order to further assess the performance of our low-cost acoustic toolkits.

Link to Deliverable(s): 1.3 One (1) peer-reviewed article on the demonstration/ application of prototype, cost effective hydrophones

Equipment used/tested: Two NEMO-Tool hydrophones, one SNAP hydrophone, one Hydromoth hydrophone, custom-made metallic base, two handheld recorders (Philips DVT 1120 and Tascam DR-05X)

Photographs: No photos were taken on that day.

VIII.

Date: 14/08/2025 – 15/08/2025

Location: Villa site, Lesvos

Duration: 2 day

Scope: Experimental deployment of the low-cost NEMOTools hydrophones in order to capture the coastal underwater soundscape of Villa site, southern Lesvos, which is known to host rich soniferous fish activity.

Summary of activities: Two NEMOTools hydrophones were mounted on a metallic custom-made base, along with a scientific hydrophone (SNAP model from Loggerhead Instruments) which served as a reference and a commercially available low-cost hydrophone (Hydromoth). Starting at 13:10 on the 14th of June, the acoustic station was fixed onto the rocky substrate at a depth of 6 m, while the handheld recorders were placed in a weatherproof case secured outside the seawater and recorded for 34.3 hours in total. The hydrophones were retrieved the following day.

Results/findings: The custom-made acoustic station recorded without problems the daily cycle of our study area and provided insights into the capabilities of our acoustic toolkit in monitoring long-term areas of interest.

Link to Deliverable(s): 1.3 One (1) peer-reviewed article on the demonstration/ application of prototype, cost effective hydrophones

Equipment used/tested: Two NEMO-Tool hydrophones, one SNAP hydrophone, custom-made metallic base, two handheld recorders (Philips DVT 1120 and Tascam DR-05X), one plastic storage box.

Photographs:

IX.

Date: 8th, 17th, 22nd March, 6th, 8th, 9th, 21st May and 28-30th July 2025

Location: Tokmakia islets plateau, NE Lesvos – rocky drop-off of the "Thalassia Periochi Nisidon Tokmakia" NATURA 2000 site (GR4110015)

Duration: 10 Days of field work

Scope:

Multimodal field data collection (acoustic + optical) in the wider Tokmakia area to:

1. Map seabed morphology and identify coralligenous formations using side-scan sonar.

2. Develop and test Task 1.2 ROV-based methods for quantitative monitoring of benthopelagic communities.

3. Map and characterise gorgonian populations and assess marine litter impacts along the 25–70 m depth range, beyond safe SCUBA limits, within NEMO-Tools WP1.

Summary of activities:

1. Side-scan sonar (SSS) mapping

- Seafloor mapping NE of Tokmakia using a **CM2 (C-MAX) side-scan sonar**.

- Survey covered ~**4.2 km²** with overlapping lines to achieve full bottom coverage.

- SSS mosaics and habitat interpretation were used **primarily to select and prioritise ROV dive locations** and to delineate likely coralligenous outcrops and surrounding seabed types.

2. ROV-based surveys

ROV surveys were carried out along the northern rocky arc of the Tokmakia plateau, focusing mainly on 40–60 m depth (average ~50 m), following the T1.2 methodological protocol.

- **Phase 1 – Scouting the area:**
  - Systematic ROV reconnaissance runs were conducted in a vertical zig-zag pattern along the drop-off, maintaining ~ 1 m altitude from the seabed to ensure clear visual coverage of substrate and fauna.
  - Start/end ROV coordinates and depth limits were logged for:
    - coralligenous formations,
    - gorgonian facies,
    - areas with marine litter (including type and interaction with benthic habitats).

- **Phase 2a – Area samples for photoquadrat image analysis:**

  o In coralligenous sectors, [~XX] high-resolution images/photoquadrats were collected per site (sites ≤100 m extent), at 0.5–1 m distance from the substrate, using a vertical camera orientation.

  o For coralligenous belts >100 m, additional sets of photoquadrats were taken approximately every 100 m of along-slope extent.

  o ROV track and bounding coordinates were recorded for each photoquadrat "site". Gorgonians were intentionally excluded from the frame in these samples to focus on the coralligenous matrix.

- **Phase 2b – Fixed-width strip transects in gorgonian forests:**

  o Once gorgonian facies were located, fixed-width strip transects were run with the ROV facing the gorgonian fans (concave side where possible), at 0.5–1 m distance from the colony plane.

  o Transects were performed from top-to-bottom or bottom-to-top of the forest and repeated at 5 m depth intervals (10 m where 5 m spacing was not feasible) to cover the full depth range of each forest.

  o Start/end coordinates, depth, ROV–substrate distance and approximate speed were recorded for each transect to support later density estimation and image analysis.

Results/findings:

- **The SSS survey produced a seabed mosaic** and broad habitat interpretation that identified rocky and coralligenous sectors and guided ROV deployment.
- Combined SSS-ROV data confirmed:
  o **a continuous to semi-continuous coralligenous belt** along the surveyed section of the drop-off (~35-60 m, locally outside this range depending on microtopography),
  o **several gorgonian forests** with mapped upper and lower depth limits.
- ROV-based distance sampling and imagery provide quantitative data on *Eunicella cavolini* and *Paramuricea clavata (*density, size structure, injury types), and systematic documentation of marine litter in these habitats.
- Marine litter is dominated by **fishing lines and ropes** entangled with gorgonians and sponges, georeferenced for potential management/mitigation actions.
- The integrated **acoustic-optical approach** proved effective for non-destructive coralligenous mapping and produced a quantitative baseline on gorgonian populations and litter pressures under NEMO-Tools.

Link to Deliverable(s):

D1.2 – Peer-reviewed article on the development and use of ROV methods for monitoring benthopelagic communities:

Sini, M.; Pistevos, J.C.A.; Bosmali, A.; Manoliou, A.; Nikolaou, A.; Pitarra, G.; Petsimeris, I.T.; Andreadis, O.; Hasiotis, T.; Mazaris, A.D.; et al. Multimodal Underwater Sensing of Octocoral Populations and Anthropogenic Impacts in

a Conservation-Priority Area (NE Aegean Sea, Greece). *J. Mar. Sci. Eng.* **2025**, *13*,2237. https://doi.org/10.3390/jmse13122237

Incidental paper from discovered breeding aggregation:

Pistevos, J.C.A., Nikolaou, A., Petsimeris, I.T., Pitarra, G., Greggio, N., Andreadis, O., Hasiotis, T., Mazaris, A., Katsanevakis., S. (In Press) First documented black seabream (*Spondyliosoma cantharus*) nesting aggregation in the Mediterranean Sea. Journal of Environmental Biology of Fishes,

Equipment used/tested:

- **QYSEA E-Master ROV** with:
  - calibration laser system,
  - forward DVL,
  - dual camera setup (primary + secondary).
- **CM2 (C-MAX) side-scan sonar** system.
  - GNSS/RTK GNSS for navigation and positioning.
  - ROV umbilical and surface control console.
- **Research vessels *Okeanis* and *Amfitriti*** (University of the Aegean) for SSS and ROV operations.
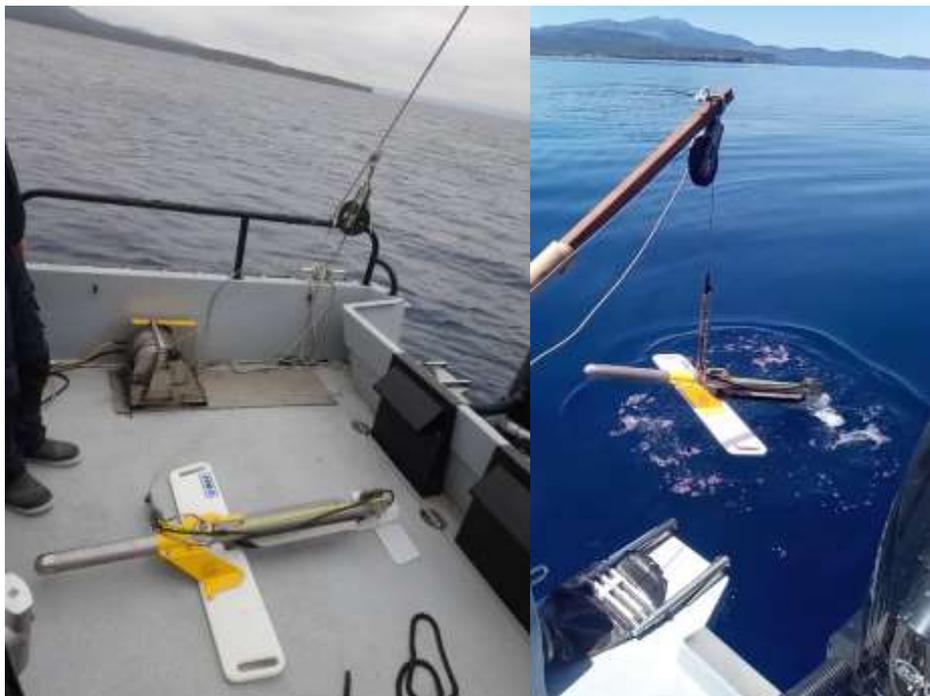
Photographs:

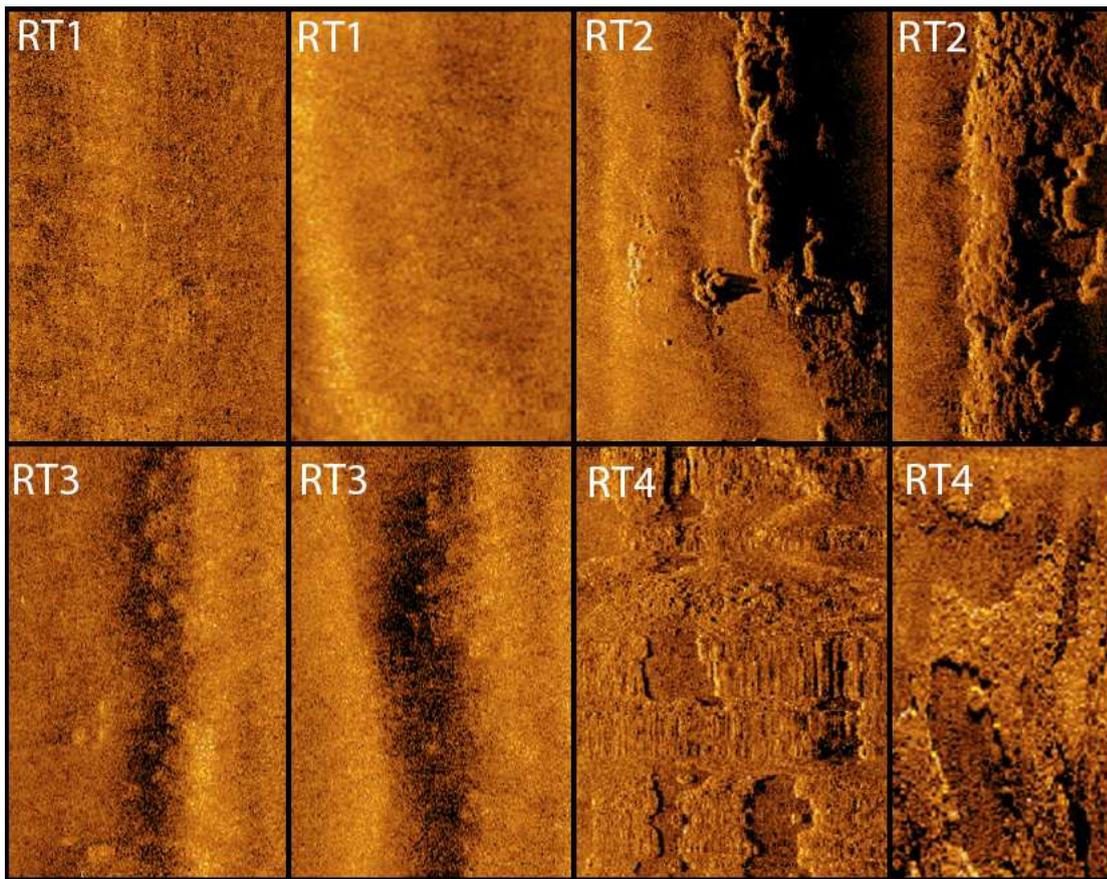**Figure 1**. Side-scan sonar deployment in the field

Figure 2. Side-scan sonar images showing the reflectivity type (RT) of key habitat types RT3 – biogenic formations (a1, a2), RT2 – rocky outcrops (b1, b2), RT1-soft bottom (c1, c2), RT4 - seagrass (d1,d2).

Figure 3. Deployment of the ROV in the field

Figures below are the examples of the gorgonian forests and coralligenous formations discovered by the ROV as well as examples of marine litter damage across the site.

M1.2 Study Visits for Method Testing