NEMO TOOLS

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

Milestone M2.2

DNA extractions for high-throughput sequencing (means of verification: Qubit measurements and PCR)

Document information and version control

Project Acronym	NEMO-Tools
Project Title	Next-generation monitoring and mapping tools to assess marine ecosystems and biodiversity
Project Number	016035
Work Package	WP2
Related Task(s)	T2.2
Deliverable Number	Milestone M2.2
Deliverable Name	DNA extractions for high-throughput sequencing (means of verification: Qubit measurements and PCR)
Due Date	14 May 2025
Date Delivered	14 May 2025
Dissemination Level	Sensitive — limited under the conditions of the Grant Agreement (Confidential, only for members of the consortium including the Commission Services and their appointed reviewers)

Version control

Revision-N°	Date	Description	Prepared By	Reviewed By
1st	28/04/2025	1st Draft	S. Genitsaris	C. Gubili

Executive Summary

Milestone 2.2 (DNA extractions) is connected to Task 2.2 of WP2. This milestone verifies the successful DNA extractions from all samples. The extraction protocol followed is described analytically in D2.2. The databases of DNA concentrations shows that few gauzes taken with the passive sampler did not capture sufficient material for high-concentration DNA products, however a large part of those were successfully amplified through PCR.

TABLE OF CONTENTS

CONTRIBUTORS

Table 1 Names and roles of contributors to this deliverable.

Name	Affiliation	WP Lead	Task Lead
Savvas Genitsaris	National and Kapodistrian University of Athens		2.2
Chrysoula Gubili	Hellenic Agricultural Organization – DIMITRA - Fisheries Research Institute	2	
Panagiota Xanthopoulou	Hellenic Agricultural Organization – DIMITRA - Fisheries Research Institute		
Antonios Mazaris	Aristotle University of Thessaloniki		

M2.2 DNA Extractions for High-Throughput Sequencing (Means of Verification: Qubit Measurements and PCR)

Database 1. DNA concentrations (ng µL-1) per coastal sample taken with inline filtering, as indicated by Qubit measurements.

Sampling codes	Coordinates	Depth (m)	Means of sampling	DNA concentrations (ng µL^{-1})
NTK1_s	40.72905 N	Surface	Inline filtering	8.64
NTK1_d	24.25467 E	40	Inline filtering	6.74
NTK2_s	40.73095 N	Surface	Inline filtering	7.7
NTK2_d	24.21155 E	40	Inline filtering	4.12
NTK3_s	40.77222 N	Surface	Inline filtering	5.9
NTK3_d	24.35214 E	20	Inline filtering	12.6
NTK4_s	40.81696 N	Surface	Inline filtering	7.76
NTK4_d	24.41260 E	20	Inline filtering	11.4
NTK5_s	40.83599 N	Surface	Inline filtering	9.66
NTK5_d	24.35594 E	12	Inline filtering	12.7
NTK6_s	40.83800 N	Surface	Inline filtering	11
NTK6_d	24.32123 E	11	Inline filtering	50.6
NTT1_s	40.54690 N	Surface	Inline filtering	16.4
NTT1_d	22.76250 E	18	Inline filtering	81
NTT2_s	40.62085 N	Surface	Inline filtering	71
NTT2_d	22.89578 E	12	Inline filtering	74
NTT3_s	40.62475 N	Surface	Inline filtering	24
NTT3_d	22.93801 E	11	Inline filtering	34.4
NTT4_s	40.56521 N	Surface	Inline filtering	35.2
NTT4_d	22.95401 E	7	Inline filtering	14.7
NTT5_s	40.51391 N	Surface	Inline filtering	8.44
NTT5_d	22.84810 E	20	Inline filtering	9.66
NTT6_s	40.37286 N	Surface	Inline filtering	6.24
NTT6_d	22.92852 E	20	Inline filtering	50.6
NTS1_s	37.992861N	Surface	Inline filtering	2.34
NTS1_d	23.548194 E	20	Inline filtering	2.58
NTS2_s	37.8979 N	Surface	Inline filtering	0.876
NTS2_d	23.5471 E	30	Inline filtering	3.32
NTS3_s	37.85025 N	Surface	Inline filtering	3.72
NTS3_d	23.43295 E	30	Inline filtering	5.82
NTS4_s	37.9028 N	Surface	Inline filtering	3.68
NTS4_d	23.38031 E	30	Inline filtering	5.88
NTS5_s	37.981033 N	Surface	Inline filtering	7.12
NTS5_d	23.413883 E	10	Inline filtering	5.1
NTS6_s	38.013217 N	Surface	Inline filtering	6.6
NTS6_d	23.495516 E	14	Inline filtering	1.67

M2.2 DNA Extractions for High-Throughput Sequencing (Means of Verification: Qubit Measurements and PCR)

Database 2. DNA concentrations (ng µL-1) per coastal sample taken with passive gauze sampler, as indicated by Qubit measurements.

Sampling codes	Coordinates	Depth (m)	Means of sampling	DNA concentrations (ng µL^{-1})
NTK1_s	40.72905 N	Surface	Gauze	0
NTK1_d	24.25467 E	40	Gauze	0
NTK2_s	40.73095 N	Surface	Gauze	0
NTK2_d	24.21155 E	40	Gauze	0.106
NTK3_s	40.77222 N	Surface	Gauze	0
NTK3_d	24.35214 E	20	Gauze	0
NTK4_s	40.81696 N	Surface	Gauze	0
NTK4_d	24.41260 E	20	Gauze	0
NTK5_s	40.83599 N	Surface	Gauze	0
NTK5_d	24.35594 E	12	Gauze	0
NTK6_s	40.83800 N	Surface	Gauze	0
NTK6_d	24.32123 E	11	Gauze	0
NTT1_s	40.54690 N	Surface	Gauze	0.474
NTT1_d	22.76250 E	18	Gauze	0.984
NTT2_s	40.62085 N	Surface	Gauze	2.8
NTT2_d	22.89578 E	12	Gauze	0.904
NTT3_s	40.62475 N	Surface	Gauze	0.888
NTT3_d	22.93801 E	11	Gauze	0.738
NTT4_s	40.56521 N	Surface	Gauze	0.954
NTT4_d	22.95401 E	7	Gauze	5.9
NTT5_s	40.51391 N	Surface	Gauze	0.128
NTT5_d	22.84810 E	20	Gauze	0.474
NTT6_s	40.37286 N	Surface	Gauze	0.126
NTT6_d	22.92852 E	20	Gauze	0.17
NTS1_s	37.992861N	Surface	Gauze	0
NTS1_d	23.548194 E	20	Gauze	0.242
NTS2_s	37.8979 N	Surface	Gauze	0.216
NTS2_d	23.5471 E	30	Gauze	0.172
NTS3_s	37.85025 N	Surface	Gauze	0.104
NTS3_d	23.43295 E	30	Gauze	0
NTS4_s	37.9028 N	Surface	Gauze	0.1
NTS4_d	23.38031 E	30	Gauze	0
NTS5_s	37.981033 N	Surface	Gauze	0
NTS5_d	23.413883 E	10	Gauze	0
NTS6_s	38.013217 N	Surface	Gauze	0.104
NTS6_d	23.495516 E	14	Gauze	0.374

M2.2 DNA EXTRACTIONS FOR HIGH-THROUGHPUT SEQUENCING (MEANS OF VERIFICATION: QUBIT MEASUREMENTS AND PCR)

Database 3. DNA concentrations (ng µL-1) per coastal sample taken with vacuum filtering, as indicated by Qubit measurements.

Sampling codes	Coordinates	Depth (m)	Means of sampling	DNA concentrations (ng µL^{-1})
NTK1_s	40.72905 N	Surface	Filtering	2.92
NTK1_d	24.25467 E	40	Filtering	3.68
NTK2_s	40.73095 N	Surface	Filtering	4.58
NTK2_d	24.21155 E	40	Filtering	4.56
NTK3_s	40.77222 N	Surface	Filtering	6.66
NTK3_d	24.35214 E	20	Filtering	3.42
NTK4_s	40.81696 N	Surface	Filtering	3.38
NTK4_d	24.41260 E	20	Filtering	9
NTK5_s	40.83599 N	Surface	Filtering	15.1
NTK5_d	24.35594 E	12	Filtering	13.8
NTK6_s	40.83800 N	Surface	Filtering	10.2
NTK6_d	24.32123 E	11	Filtering	13
NTT1_s	40.54690 N	Surface	Filtering	20
NTT1_d	22.76250 E	18	Filtering	17.6
NTT2_s	40.62085 N	Surface	Filtering	23.4
NTT2_d	22.89578 E	12	Filtering	28.2
NTT3_s	40.62475 N	Surface	Filtering	21.8
NTT3_d	22.93801 E	11	Filtering	21.4
NTT4_s	40.56521 N	Surface	Filtering	16.9
NTT4_d	22.95401 E	7	Filtering	24
NTT5_s	40.51391 N	Surface	Filtering	20.6
NTT5_d	22.84810 E	20	Filtering	37.8
NTT6_s	40.37286 N	Surface	Filtering	4.34
NTT6_d	22.92852 E	20	Filtering	25.6
NTS1_s	37.992861N	Surface	Filtering	17.9
NTS1_d	23.548194 E	20	Filtering	58.6
NTS2_s	37.8979 N	Surface	Filtering	9.56
NTS2_d	23.5471 E	30	Filtering	3.78
NTS3_s	37.85025 N	Surface	Filtering	13.7
NTS3_d	23.43295 E	30	Filtering	4.62
NTS4_s	37.9028 N	Surface	Filtering	15.9
NTS4_d	23.38031 E	30	Filtering	21
NTS5_s	37.981033 N	Surface	Filtering	15
NTS5_d	23.413883 E	10	Filtering	2.98
NTS6_s	38.013217 N	Surface	Filtering	10.36
NTS6_d	23.495516 E	14	Filtering	7.5

M2.2 DNA Extractions for High-Throughput Sequencing (Means of Verification: Qubit Measurements and PCR)

Database 4. DNA concentrations (ng µL-1) per offshore sample taken with vacuum filtering, as indicated by Qubit measurements.

Sampling codes	Coordinates	Depth (m)	Means of sampling	DNA concentrations (ng µL^{-1})
DK3s	36.65681 N 27.13926 E	Surface	Filtering	0.28
DK4s	36.55826 N 27.17592 E	Surface	Filtering	0.28
DS3s	36.456274 N 25.410807 E	Surface	Filtering	0.74
A1	37.594635 N 23.528138 E	Surface	Filtering	3.88

References

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

Deliverable D2.2. (2024). Protocol of the taxonomic and functional identification of species of the different biocommunities examined. Chrysoula Gubili, Savvas Genitsaris, Panagiota Xanthopoulou, Antonios Mazaris. pp 21.