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D4.1 Evaluating Operation Protocols of the MMLs

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PROJECT INFORMATION

Project Acronym	EcoMarine
Project title	Building a Comprehensive Mechanism for Preserving Marine Ecosystems and Life from the negative consequences of Climate Change and the disposal of Plastic Debris
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Project website	www.ecomarine-project.eu

PREPARED BY

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1. Executive Summary

The ECOMARINE project intended to create 4 fully operational marine conservation monitoring labs (MMLs) in Malaysia and India. **Work Package 4** aims to ensure the high-standard and long-term operation of the 4 new MMLs, where there will be regular collection and interpretation of various datasets related with marine ecosystem monitoring.

After the installation and first pilot operations of the 4 MMLs, it was expected that each new MML deliver their own Operation Protocols in deliverable 3.2. Here it's intended that each MMLs make a preliminary evaluation and projection of operational protocols functioning after their first operations and visits from the European consortium members to Malaysia and India.

In the **4.1. ECOMARINE Deliverable** reported here the Asian partners summarizes a **Description of MMLs activities and links to scientific reports**, a brief **Self-evaluation of the protocols efficiencies and possible limitations**, **Comments about the upcoming research plans** in the short/mid-term using the MMLs and **Possible further research application**s using the new MML infrastructures for widening the research capacity of the new MMLs.

The **sampling activity** by the four new MMLs in coastal areas of India and Malaysia has been periodic (monthly in some cases), intense and fruitful. Moreover, each HEI made a very good adaptation of the **proposed protocols** and **applied** them for the three targets included in ECOMARINE: Monitoring of the microplastic debris, Monitoring of productive protected ecosystems, Monitoring climate change, ocean acidification and temperature rise, blue carbon and related ecosystem responses. The scientific results related with these activities are already compiled and detailed presented in the ECOMARINE deliverable 4.3. The global view **on self-evaluations of the protocols used by the Asian partners is clearly positive being able to detect limitations** on which the four MMLs can work in the short term to improve its efficiency. The best part is that **the four new MMLs have a plan for new sampling campaigns and research**, including application for **official accreditations** as reference labs in some of the topics covered by the ECOMARINE project. There are also plans from **improve marine literacy and education** of citizens and other stakeholders, including **E-learning,** and a **strong will to continue international cooperation within and among Asian and European partners**.

The ECOMARINE project has achieved its main goal: there are **four new MMLs that comprise an important advancement in infrastructure for research and education in the 4 HEIs in India and Malaysia.**

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D4.1.1 **Evaluating Operation Protocols of the MMLs in Malaysia (Universiti Malaysia Terengganu).**

D4.1.2 **Evaluating Operation Protocols of the MMLs in Malaysia (Universiti Kebangsaan Malaysia).**

D4.1.3 **Evaluating Operation Protocols of the MMLs in India (Andhra University).**

D4.1.4. **Evaluating Operation Protocols of the MMLs in India (University of Kerala).**

ECOMARINE

Building a Comprehensive Mechanism for Preserving Marine Ecosystems and Life from the negative consequences of Climate Change and the disposal of Plastic Debris

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D 4.1. Evaluation of Operation protocols

MARINE MONITORING LAB

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I.	**Monitoring Microplastic Debris: 28/08/2023**
II.	**Monitoring Productive Ecosystems: 28/08/2023**
III.	**Monitoring Climate Change and Blue Carbon Responses: 28/08/2023**

2. Description of MMLs activities.

Research Objective	Dates of data collection	Sampling location	Type of samples collected	Research protocol used	List of Equipment used	Scientific Reports delivered and proof of evidence (links) (Scientific papers, reports, other,)
I. Monitoring Microplastic Debris.						
Microplastic sampling and detection	25 Apr 2024	Terengganu river, Terengganu, Malaysia	Water samples (Surface water)	Microplastic Sampling Protocol for Surface Water (Towing) Microplastic Analysis Protocol	Microplastic Net (Neuston), Flowmeter, YSI Hydrolab, Sieve Stereomicroscope with Axiocam Digital Camera	Data was presented at the final Ecomarine Seminar (Jun 2024), subjected to paper submission by Dec 2024.
II. Monitoring Productive Ecosystems.						
Mangrove habitat mapping	17 – 20 Aug 2022	Pulau Ketam, Selangor, Malaysia	Images from drone	UAV/DRONE Remote Sensing Protocol	Multirotor Drone	Report will be submitted to Department of Fisheries Malaysia. Expected to be published by 2025.
Mapping of polycheate reef	7 Jan 2023, 11 Sept 2023	Jeram, Selangor, Malaysia	Images from drone	UAV/DRONE Remote Sensing Protocol	Multirotor Drone	Report will be submitted to Department of Fisheries Malaysia. Expected to be published by 2025.
Coral reefs habitat mapping	6-9 Sept 2023	Jeram, Selangor, Malaysia	Images from camera and video	Mapping Coastal Habitats	Camera underwater, Video-camera	Report will be submitted to Department of Fisheries Malaysia. Expected to be published by 2025.
Coral reefs habitat mapping	17-18 Jan 2024	Pangkor and Jarak, Perak, Malaysia	Images from camera and video	Mapping Coastal Habitats	Camera underwater, Video-camera	Report will be submitted to Department of Fisheries Malaysia. Expected to be published by 2025.
Coral reefs habitat	25 Feb 2024	Pulau Bidong,	Bathymetry of	Acoustic (SONAR)	Sonar, Software Reef	Still in progress.

mapping		Terengganu, Malaysia	reefs	Remote Sensing Protocol	Master, iPad, Laptop, Fish Finder Mounting,	
III. Monitoring Climate Change and Blue Carbon Responses.						
Mangrove and seagrass sampling	1 March 2024- 31 Dec 2025	Peninsular Malaysia	Sediment cores	Blue carbon protocol	Push pull sediment corer	Still in progress

3. Brief self-evaluation of the protocols efficiencies and possible limitations.

I.	**Monitoring Microplastic Debris.**

Microplastic Sampling Protocol for Surface Water (Towing):

1. Advantages:
    - This method is effective in collecting a wide range of microplastic sizes. This protocol also could provide a comprehensive sample or large sample volume from different water bodies, thus will avoid the sample discrimination. For instance, in our study, we use this protocol to monitor the microplastics distribution in three zonation of water (three water bodies) comprised of seawater, estuary and fresh/river water.
2. Possible Limitations:
    - We notice that there will be potential airborne contamination or field contamination from the towing activities, thus the blank control and field blank is highly required during the sampling activity. Towing speed and duration may also influence the quantity and type of microplastics collected, therefore it is recommended to use double towing/double nets at both sides of the boat for the towing activities (replicates). The surface water towing is also limited to surface water (1-50cm from the surface), thus not accounting for deeper water microplastics.

Microplastic Analysis Protocol:

1. Advantages:
    - The physical observation and analysis using stereomicroscope coupled with Axiocam Digital camera is the first step of analysis/characterization that is carried out after the digestion, extraction and filtration. This technique will provide accurate identification and quantification of microplastics based on size, shape and color.
    - Microplastics could be observed up to size of 10 um (>10 um), while size measurement of microplastics could be carried out with the lowest limit of measurement of size 30 um (>30 um). Higher resolution of images could be captured, that highly useful especially for publication purpose. This technique is very important especially when considering size for analysis, for example in understanding the potential risk/hazard of microplastics based on physical features.
2. Possible Limitations:
    - This technique is time-consuming and resource intensive. It also requires specialized equipment and trained personnel that can performed the physical

sorting persistently. There will be potential for misidentification of microplastics, especially at smaller sizes or by untrained personnel. Therefore, it is highly recommended the use of advanced analytical techniques such as FTIR, micro-FTIR, or Raman spectroscopy to ensure precise results.

II. Monitoring Productive Ecosystems

UAV/DRONE Remote Sensing Protocol

1. Advantages:
    - Drones can capture high-resolution images and generate accurate maps. Few cycles of practices and trial of polycheate reefs in Jeram, Malaysia provided us a proper map of the polycheate reef. Drones mapping also can perform repeated flights over the same area to monitor changes over time, making them ideal for tracking progress or changes in landscapes. We are now planning to do seasonal changes of polychaete reef at Jeram.
    - Compared to traditional aerial surveys (e.g., manned aircraft), drones are relatively inexpensive to operate. However, it also depends on the tides and weather conditions. Ray of lights can limit the usability dan data quality of the images. One of the efforts of drone mapping at Jeram for 2 days was unusable due to images error from sun glare. The volume of data collected by drones can be substantial, require significant processing power and time to generate maps.
    - Drones can cover large areas quickly and provide near-real-time data, which is crucial for time-sensitive projects. Drone operations are subject to aviation regulations regulated by Civil Aviation Authority of Malaysia (CAAM) and Department of Survey and Mapping Malaysia (JUPEM).
    - Drones can access hard-to-reach or hazardous areas that may be challenging or dangerous for humans. It was very functional for drone mapping due to the muddy ecosystems of polycheate reef and hard for researchers to access. However, due to the large area of reefs, the battery life (3 units) have restricts the duration of flights. Both university (UMT and UKM) have combined both resources (batteries set) and still not enough for the flights of whole area.
2. Possible Limitations:
    - It is suggested to purchase portable power stations for future work.

Acoustic (SONAR) Remote Sensing Protocol

1. Advantages:
    - Sonar systems can produce highly detailed maps of underwater features, including the seafloor, wrecks, and other submerged objects. Sonar remote sensing protocol have been tested at Pulau Bidong, Terengganu, Malaysia. Sonar practical mapping are sometimes affected by high wave action (1-1.5m) due to

many boats and ferries traffic at Pulau Bidong and monsoonal season.

- Sonar systems can cover wide swaths of the seafloor, allowing for efficient mapping of large areas. The systems can provide real-time data, which is useful for navigation, immediate analysis, and decision-making. The surveys and monitoring from this project is going to be compared with drone images from training programs.

2. Possible Limitations:
   - Our current challenges are the data analyses from the sonar since no training have been provided for the purchases of software ReefMaster.

III. Monitoring Climate Change and Blue Carbon Responses.

Vacuum Type Push Pull Sediment Corer

1. Advantage:
   - The equipment is suitable for blue carbon ecosystems like seagrass. Sampling can be achieved on the boat using the extension pole sampling technique, which allows you to cover more sampling sites compared to conventional SCUBA diver sampling
2. Possible Limitations:
   - It seems like there are some issues with the sediment not being suitable for blue carbon ecosystems like mangroves. The complex root substrate can make it challenging for the push-pull type corer, and in extreme cases, the PVC corer tube may break or crack during the extraction exercise. It's important to consider alternative sampling methods or equipment in such situations.

Perspex Chamber and Loggers:

1. Advantage:
   - The equipment is suitable for blue carbon ecosystems like seagrass. Sampling can be achieved by conventional SCUBA diver technique. The temperature, dissolved oxygen and salinity loggers attached to the chamber are easy to operate and maintain.
2. Possible Limitations:
   - The chamber is too light and may drift away if sea conditions are wavy. Use more weight for countermeasures.

4. Comments about the upcoming research plans in the short/mid-term using the MMLs and possible further research applications using the new MML infrastructures for widening the research capacity of the new MML.

- MML for Monitoring Productive Ecosystems is now successfully enquire 3 additional grants from the Department of Fisheries Malaysia to map the habitat at different states in Malaysia. It is suggested to continue and expand the research to seasonal and yearly monitoring at some specific area. Collaboration between Universiti Malaysia Terengganu (Jennie Lee), Universiti Kebangsaan Malaysia (Asamuddin Abd Hasan) dan Universiti Malaysia (Lee Soon Loong) to do seasonal patterns of polycheate reefs. Sonar mapping will also be embedded in habitat mapping project with Department of Fisheries Malaysia. All research activities are engaged with undergraduate students, post-graduate students, researchers, academic, governmental department and local communities.
- Besides, MML facility for final year students for their research projects/final year dissertation, which is a compulsory subject to be completed. They are being exposed to topics from all the three main components of the Ecomarine project.
- MML for Monitoring Climate Change and Blue Carbon Responses engaged with local stakeholders (Department of Forestry Malaysia) and Edith Cowan University Australia to continue and expand the field works on the blue carbon ecosystem within Peninsular Malaysia.

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2. Description of MMLs activities.

Research Objetive	Dates of data collection	Sampling location	Type of samples collected	Research protocol used	List of Equipment used	Scientific Reports delivered and proof of evidence (links) (Scientific papers, reports, other,)
I. Monitoring Microplastic Debris. a. To characterise microplastic types in Melaka river sediment b. To quantify the abundance and concentration of microplastic in Melaka river sediment	June & December 2022	Sungai Melaka, Melaka, Malaysia	Microplastic in river sediments.	D4.1.1; Chapt. 1; pp 4-19	YSI DSS Pro multiparameter probe Stereomicroscope: Olympus SZ51, Nylon 6 (Polyamide), Polypropylene (PP), Polystyrene (PS), Low-Density Polyethylene (LDPE), High Density Polyethylene (HDPE) Lab oven, Filtration set	Hafiz et. al., 2024. Time- and Space-Varying Microplastic Contributions from a Tourism Area to River Sediments. ECOMARINE Project Final Conference 2024. Putrajaya, Malaysia. 10 June 2024. Link: ..\..\FINAL CONFERENCE\SCIENTIFIC COM\EPFC 2024 Programme and Abstract Book_FINAL_30May24_LATEST.pdf
a. To characterize microplastic types in marine fishes landed in Mersing. b. To quantify abundance and concentration of microplastic in marine fishes. water/sediment/biota c. To evaluate the impact of microplastic on marine fishes.	March-October 2022	Mersing, Johor, Malaysia	Microplastic in marine fishes.	D4.1.1; Chapt. 1; pp 4-19	Stereomicroscope: Olympus SZ51, Lab oven, Filtration set	Noor Alia et al., 2024. Microplastics Prevalence in Marine Fish Species from Mersing, Johor, Malaysia. ECOMARINE Project Final Conference 2024. Putrajaya, Malaysia. 10 June 2024. Link: ..\..\FINAL CONFERENCE\SCIENTIFIC COM\EPFC 2024 Programme and Abstract Book_FINAL_30May24_LATEST.pdf

a. To characterize microplastic types in selected mollusk. b. To quantify abundance and concentration of microplastic in selected mollusk. water/sediment/biota c. To evaluate the impact of microplastic on marine mollusk.	March-June 2022	Sekinchan, Selangor, Malaysia.	Microplastic in marine mollusk.	D4.1.1; Chapt. 1; pp 4-19	USB microscope Dinolite + stand RK10, Lab oven, Filtration set	Mardiana-Jansar et al., 2024. Microplastic abundance on selected mollusk species. ECOMARINE Project Final Conference 2024. Putrajaya, Malaysia. 10 June 2024. Link: ..\..\FINAL CONFERENCE\SCIENTIFIC COM\EPFC 2024 Programme and Abstract Book_FINAL_30May24_LATEST.pdf
a. To characterise microplastic types in Melaka river water b. To quantify the abundance and concentration of microplastic in Melaka river water.	June & December 2023	Malacca River, Melaka, Malaysia	Microplastic in river water.	D4.1.1; Chapt. 1; pp 4-19	Stereomicroscope: Olympus SZ51, Filtration set	Arunggani et al., 2024. Preliminary Assessment for Microplastic Pollution in the Malacca River. ECOMARINE Project Final Conference 2024. Putrajaya, Malaysia. 10 June 2024 Link: ..\..\FINAL CONFERENCE\SCIENTIFIC COM\EPFC 2024 Programme and Abstract Book_FINAL_30May24_LATEST.pdf
a. To characterise microplastic types in Melaka river water b. To quantify the abundance and concentration of microplastic in Melaka river water.	October 2023	Mersing, Johor, Malaysia.	Microplastic in seawater.	D4.1.1; Chapt. 1; pp 4-19	YSI DSS Pro multiparameter probe Stereomicroscope: Olympus SZ51, Filtration set	Nur-Farahin et al., 2024. Microplastic Abundance in the Seawaters of Tanjung Resang Beach, Mersing, Johor ECOMARINE Project Final Conference 2024. Putrajaya, Malaysia. 10 June 2024 Link: ..\..\FINAL CONFERENCE\SCIENTIFIC COM\EPFC 2024 Programme and Abstract Book_FINAL_30May24_LATEST.pdf
a. To characterise microplastic types in marine sediments around Mersing, Johor.	October 2023	Mersing, Johor, Malaysia	Microplastic in marine sediment.	D4.1.1; Chapt. 1; pp 4-19	Stereomicroscope: Olympus SZ51, Metal sieve (5mm, 1mm, 0,5mm, 0.125mm,	Hana-Humaira et al., 2024. Abundance of Microplastics in Beach Sediment at the Tanjung Resang Coast, Mersing, Johor ECOMARINE Project Final Conference 2024. Putrajaya, Malaysia. 10 June 2024

b. To quantify the abundance and concentration of microplastic in marine sediments around Mersing, Johor.					0.063mm), Lab oven, Filtration set	Link: ..\..\FINAL CONFERENCE\SCIENTIFIC COM\EPFC 2024 Programme and Abstract Book_FINAL_30May24_LATEST.pdf
a. To identify the diversity of diatom on microplastic microplastic of contact lense origin. b. To quantify abundance of diatom present on microplastic (contact lense)			Diatom on marine microplastic of contact lense origin.		usb microscope Dinolite + stand RK10	Nurdini et al., 2024. Growth of Marine Diatom in Silica Source from Disposable Contact Lens ECOMARINE Project Final Conference 2024. Putrajaya, Malaysia. 10 June 2024 Link: ..\..\FINAL CONFERENCE\SCIENTIFIC COM\EPFC 2024 Programme and Abstract Book_FINAL_30May24_LATEST.pdf
II. Monitoring Productive Ecosystems. a. To map the bottom bathymetry of selected locations with potential marine habitat areas b. To map the spatial distribution of marine habitat types at selected locations	23-28 April 2024	Pulau Mertang, Johor	Geo-referenced Water depth; Sea bed photos; Sonar data (.SL3)	D4.1.1; Chapt. 3.4; pp 83-90	#1-Olympus TG6 Camera #4- Life jackets #26- Lowrance Elite FS7	Ghazali et al, 2024. Sonar-based Mapping of Subtidal Marine Habitat around Pulau Mertang, Johor. ECOMARINE Project Final Conference 2024. Putrajaya, Malaysia. 10 June 2024. Link: ..\..\FINAL CONFERENCE\SCIENTIFIC COM\EPFC 2024 Programme and Abstract Book_FINAL_30May24_LATEST.pdf

a. To map mangrove forest area using satellite imagery b. To capture temporal changes in mangrove forest area	Feb 2022	Matang Mangrove Forest Reserve, Perak, Malaysia	9 years Satellite Imagery; Rapid Eye and Landsat	D4.1.1; Chapt. 3.3; pp 51-73	Existing ArcGIS software	Nurul Asyikin and Muzzneena, 2024. Mangrove Dynamics Mapping: An Analysis of Matang Mangrove Forest Reserve Using Multi-Temporal Satellite Imagery. ECOMARINE Project Final Conference 2024. Putrajaya, Malaysia. 10 June 2024 Link: ..\..\FINAL CONFERENCE\SCIENTIFIC COM\EPFC 2024 Programme and Abstract Book_FINAL_30May24_LATEST.pdf
III. Monitoring Climate Change and Blue Carbon Responses. a. To estimate soil carbon stocks, carbon burial rate and ecosystem carbon stocks of mangrove forest	24 July 2024	Kuala Selangor Nature Park, Selangor, Malaysia	Mangrove forest soil core	D4.1.1; Chapt. 2.3.1; 2.3.3;2.3.4; 2.3.4; pp 24-32	#4-life jackets; #5-radios/walkie-talkie; #8- GPS; #12-Analytical balance; #19- Semi Micro balance; #27- Metal sieve; #15- Freezer; #17- Lab oven #33-Heavy duty core sampler	Rozaimi et al., 2024. Framing Blue Carbon Additionality of Mangrove Soils from Kuala Selangor Nature Park (Selangor, Malaysia). ECOMARINE Project Final Conference 2024. Putrajaya, Malaysia. 10 June 2024. Link: ..\..\FINAL CONFERENCE\SCIENTIFIC COM\EPFC 2024 Programme and Abstract Book_FINAL_30May24_LATEST.pdf
a. To investigate microbial communities in the mangrove and seagrass areas	March 2022	Merambong shoals and Sungai Pulai, Johor and Pulau Seribuat, Pahang, Malaysia	Microbial communities on mangrove and seagrass	D4.1.1; Chapt. 2.3.3; 2.3.4; 2.3.6; pp 27-35	#8- GPS; #27- Metal sieve; #15- Freezer; #17- Lab oven	Aqeela et al, 2024. Taxonomic Diversity and Metabolic Dynamics of Sediment Bacterial Communities in Blue Carbon Ecosystems. ECOMARINE Project Final Conference 2024. Putrajaya, Malaysia. 10 June 2024. Link: ..\..\FINAL CONFERENCE\SCIENTIFIC COM\EPFC 2024 Programme and Abstract Book_FINAL_30May24_LATEST.pdf
a. To quantify the contribution of macroalgal biomass that supplements blue carbon stocks of a seagrass meadow	February 2021	Merambong shoal, Johor, Malaysia	Macroalgae on seagrass	D4.1.1; Chapt. 2.3.6; pp 34-35	#4-life jackets; #8-GPS; #27- Metal sieve; #15- Freezer; #17- Lab oven	Rozaimi et al., 2024. Carbon and nitrogen deposits of macroalgal origin on a tropical seagrass meadow. *Ecosystem Health and Sustainability* (In press). Link: https://spj.science.org/doi/10.34133/ehs.0157

a. To		South East Asia and Malaysia	High resolution regional climate models	E-Course Module (M3)	Existing Computing/Server network	Noor Amalina et al., 2024. Climate downscaling. ECOMARINE Project Final Conference 2024. Putrajaya, Malaysia. 10 June 2024. Link: ..\..\FINAL CONFERENCE\SCIENTIFIC COM\EPFC 2024 Programme and Abstract Book_FINAL_30May24_LATEST.pdf

3. Brief self-evaluation of the protocols efficiencies and possible limitations.

I. Monitoring Microplastic Debris

The proposed ECOMARINE protocol successfully determines the microplastics' color, form, and quantity. However, it is unable to detect the plastic compounds and nanoparticle-sized plastics that we have discovered, which calls more sophisticated equipment like uFTIR or gas chromatography-pyrolysis-MS. With the additional equipment we independently acquired, like the water sampler, the microplastics in water sampling procedure is both feasible and efficient. To further improve our abilities to model the distribution of microplastics, we might consider obtaining hydrological equipment such as RTK-GPS for shallow water bathymetric study.

II. Monitoring Productive Ecosystems

The proposed ECOMARINE protocol of using kayak to undertake the sonar survey of marine habitats is not practical nor efficient in the warm tropical environment of Malaysia. Instead, a small boat or dinghy is preferred and used with promising results. Preliminary results showed that sonar-based mapping of patchy coral reefs is acceptable and with some optimization of field data collection protocol e.g. echosounder setup, speed, survey track etc, the accuracy of the mapping can be improved. However, for seagrass habitat mapping, refinement of the protocol is needed to capture the acoustic signal of the smaller species and low density seagrass cover in Malaysia in comparison to those *Posidonia oceanica* meadows which are found in Europe. In terms of sonar data analysis, while the ReefMaster v.2 software is able to analyze bottom composition, its ability to discriminate bottom composition may be limited which lead to the moderate precision and accuracy of bottom type identification. Alternative bottom analysis software may also be used e.g. BioBase.

III. Monitoring Climate Change and Blue Carbon Responses

The proposed ECOMARINE protocol for carbon stocks in sediment pools of blue carbon ecosystem is versatile and applicable for soft-bottom sites (seagrass sediments and mangrove soils) in Malaysia. The outlined methods can be used with minor to no modifications and ongoing work are employing the same described techniques. For measuring blue carbon productivity, the outlined method is applicable for seagrass meadows and moderate modifications are needed. This is to suit the seagrass species found in Malaysia, of which are different in morphology compared to ***Posidonia*** meadows found in Europe. The protocol of habitat mapping can be modified to include monitoring of blue carbon vegetation and this has been successfully used to complete a research component in investigating the link between macroalgal stocks in existing blue carbon systems.

4. Comments about the upcoming research plans in the short/mid-term using the MMLs and possible further research applications using the new MML infrastructures for widening the research capacity of the new MML.

The UKM-ECOMARINE MML in Mersing, Johor will regularly;

1) Conduct Basic National Training (BNT) programmes every academic semester in conjunction with UKM's Marine Science Programme field courses. The aim is to train at least 50 students / semester. Selected methods from the ECOMARINE research protocols will be chosen to complement relevant marine science courses. There are at least eight relevant undergraduate courses that will benefit from the MML and its protocol. Some data will also be collected during the training sessions.

2) Collect data in all three habitat monitoring aspects (MP, HM and BC) as part of UKMs marine science programme undergraduate final year project (FYP) thesies. The aim is to regularly produce 3-6 FYP thesis /year which may ultimately be published as journal articles. At every opportunity, we will strive to enroll postgraduate students (MSc and PhD) who will utilize the MML, collect data and contribute to the monitoring of marine habitat.

3) Collaborate with UKM's Marine Ecosystem Research Center (EKOMAR) in engaging with local communities in Mersing e.g. schools, fishermen, ecotourism operator to participate in citizen science activities. This may require some adaptation to the ECOMARINE work protocols to suit the knowledge level of the respective communities.

4) Acquire research grants to compliment and extend the marine habitat monitoring efforts of the MML.

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2. Description of MMLs activities.

Research Objetive	Dates of data collection	Sampling location	Type of samples collected	Research protocol used	List of Equipment used	Scientific Reports delivered and proof of evidence (links) (Scientific papers, reports, other)
I.1. Monitoring Microplastic Debris in the sediments	27/11/2021, 29/12/2021, 24-01-2022, 30-03-2022, 02-07-2022, 29-08-2022, 27-09-2022, 31-10-2022, 28-11-2022, 20-12-2022, 20-01-2023, 25-02-2023, 20-03-2023, 22-04-2023, 11-05-2023, 14-06-2023	Dumping Yard, Palm beach, YMCA, Yoga Dept., RK beach, Pondurangapuram, NCB	Sediment sample collection for analysis of microplastic debris	**The Andhra University (AU) protocol developed in ECOMARINE** and based on Masura, J., et al. 2015. Laboratory methods for the analysis of microplastics in the marine environment: Recommendations for quantifying synthetic particles in watersand sediments. *NOAA Technical Memorandum* NOS-OR&R-48.	**Soil coring device, quadrates. Sieve shakers, sieves, stereo microscope,** Magnetic stirrer, hot air oven, Rotating stirrers, water baths, hot plates, homogenizer, FTIR spectrometer	Presentations made in the EPFC 2024. Microplastics in the beach sediments along the Visakhapatnam coast: seasonal and spatial variations. Janakiram P, Umadevi K, Chandrasekhar D, Geetha S. Paper for publication is under progress
I.2. Monitoring Microplastic Debris in the sea bed (10m depth) sediments	31-12-2023	NCCR: Visakhapatnam Port (NS1-NS9)	Sediment sample collection for analysis of microplastic debris	**The AU developed in ECOMARINE** based on Masura, J., et al. 2015.	**Soil coring device, quadrates. Sieve shakers, sieves, stereo microscope,** FTIR spectrometer	
I.3. Monitoring Microplastic Debris and plastic chelates in the water samples	06-03-2022 Pre monsoon, 12-08-2022 Monsoon,	Kailasagiri, Rushikonda, Peddarushikonda, Bheemili, Pandurangapura	Collection of water samples from surface and at 10m depth, for analysis of	**The AU developed in ECOMARINE** based on Masura, J., et al. 2015.	**Plankton net, Safety jackets, Vacuum pump filter holder set, Oil free vacuum pump, Sieve shakers,**	Presentations made in the EPFC 2024. Dissolved plastic polymer chelates in the coastal water of Visakhapatnam

	31-12-2022 Post monsoon	m, Inner harbour, Jetty no.11	microplastic debris and plastic chelates present in the water (hiring a boat)		**sieves, stereo microscope,** Niskin's water bottles**,** FTIR spectrometer	Pasupuleti Janakiram et al. Paper for publication is under progress
I.4. Monitoring Microplastic Debris and plastic chelates in biota	18-12-2022, 06-03-2022, 23-06-2023, 9-11-2023, 14-12-2023, 20-02-2024, 04-03-2024, 20-03-2024	*Rastrelliger kanagurtha & Carcharhinus hemiodon, Lates calcarifer, Sepia , Rastrelliger kanagurtha, Ariomma indicum-mettapara , Rastrelliger kanagurtha , Sardinella gibbosa, Sardinella sirm, , Metapenaeus monoceros, Nemipterus peronii, Saurida tumbli, Hilsa kelee, Euthynnus affinus (Meckaral tuna), scomberomorus guttatus (Seer fish),*	Collection of these fishes from traditional landing centers and fishing harbors of Visakhapatnam and Bheemili in the respective fishing seasons. Analysis of each fish species was repeated 5 times.	**The AU developed in ECOMARINE** and based on Method adopted by Garneau D, 2016 was followed to extract microplastic chelating compounds present in the various tissues present in the fish.	**Sieve shaker, sieves, stereo microscope, glass ware and chemicals,** Dissecting apparatus, tissue homogenizer, rotating stirrer, magnetic stirrer, hot air oven, hot plate, water bath, FTIR spectrometer	Presentations made in the EPFC 2024. Bioaccumulation of plastic chemicals in commercially important marine organisms of Visakhapatnam coast Umadevi K, Janakiram P, Geetha S, Chandra sekhar D. Paper for publication is under progress
II.1. Monitoring Productive Ecosystems (sea grass ecosystem)	05-02-2022, 09-02-2022, 15-02-2022, 17-02-2022, 15-03-2022, 18-04-2022,	Chepaluppada bridge, Gudivada Gosthani river creek, Chirrayanam , East Godavari	Water samples, sediment samples, samples of seagrass, recorded *in situ*	**The AU Ecomarine protocol**s Adopting standard methods given by Strickland and Parson (1972).	**Photo quadrates, iron quadrates, underwater camera (GoPro), GPS, Salino meter, pH meter, temperature pH**	Presentations made in the EPFC 2024. **1**.Mapping of sea grass habitats in Andhra Pradesh state, east coast of India

	12-05-2022, 16-06-2022, 15-07-2022, 18-08-2022, 16-09-2022, 15-10-2022, 20-11-2022, 22-12-2022, 26-01-2023, 25-02-2023, 01-04-2023, 07-05-2023, 18-06-2023, 17-07-2023, 15-08-2023 & 31-08-2023, 17-09-2023, 16-10-2023, 17-11-2023, 22-12-2023, 20-01-2024 & 26-01-2024, 11-02-2024, 14-03-2024		Temperature, salinity, pH, transparency of water, nutrients (NO_2, PO_4), Dissolved oxygen (DO), counting the number of leaves in a quadrate for biomass estimations, picking out the plants in 25^2cm quadrate were chlorophyll estimations.	Eaton A et al, 2005, Standard Methods for the Examination of Water and Waste Water	**logger, UV-VIS spectro Photometer, Stereo microscope. Vacuum pump filter holder set, Oil free vacuum pump, safety jackets, Fish finder (Sonar Lowarance Elite make), soil coring device, sieves, sieve shaker,** **Other instruments, used :** Secchi disc, boat hiring, hot air oven, water bath, hot plate, wet lab facility for maintaining sea grass in the tanks	Umadevi K, Janakiram P, Chandrasekhar D, Geetha S. 2. First report of seagrass (*Halophila ovalis*) from a brackish water lake, chirrayanam, Katrenukona mandal, Dr.BRAK district Andhra Pradesh, India. Chandrasekkhar D, Umadevi K, Janakiram P, Geetha S. Paper for publication is under progress
III.1 Monitoring Blue Carbon Responses (CO_2 absorption by sea grass)	26-01-2023, 25-02-2023, 01-04-2023, 07-05-2023, 18-06-2023, 17-07-2023, 15-08-2023 & 31-08-2023, 17-09-2023, 16-10-2023, 17-11-2023, 22-12-2023, 26-01-2023,	Chirrayanam, East Godavari	Sea grass present in 25^2cm quadrate	**The AU Ecomarine Blue carbon protocol:** The carbon stocks in the seagrass biomass were calculated from the carbon content and biomass of seagrass species as given by Armitage and Fourqurean (2016). Sediment carbon stocks were estimated from the sediment	**Photo quadrates, iorn quadrates, underwater camera (GoPro), safety jackets, GPS, stereo microscope (Nikon),** **Other instruments, used :** Secchi disc, boat hiring, , wet lab facility for maintaining sea grass in the tanks	Acclimatizing the sea grass to the laboratory conditions for experimental work is under progress.

	25-02-2023, 01-04-2023, 07-05-2023, 18-06-2023, 17-07-2023, 15-08-2023 & 31-08-2023, 17-09-2023,			organic carbon following Ganguly et al. (2017)		

3. Brief self-evaluation of the protocols efficiencies and possible limitations.

I. Monitoring Microplastic Debris.

Masura J, et al., 2015 was adopted for the analysis of microplastic debris throughout the period of study in the Ecomarine project. The samples analyzed adopting this method yielded good results when the results obtained were compared with the results already published on sediment and water microplastics. When the sediment samples are digested in sodium chloride and zinc chloride after gravimetric analysis, the samples digested in zinc chloride yielded better results (the number of peaks occurred in the FTIR analysis were more prominent). Method adopted following Garneau D, 2016 yielded good FTIR results.

Limitation:

1. For FTIR analysis we have to go to college of Pharmaceutical sciences, Andhra University every time.
2. To collect the offshore water samples hiring boat was very expensive, so we are able to do seasonal sampling instead of monthly sampling.

II. Monitoring Productive Ecosystems

1. The brackish water lake at Chirrayanam was situated 250km away from Visakhapatnam. Monthly samples were collected for biomass, chlorophyll estimation and for hydrography. Although the methods adopted for the above research are old, the results obtained are accurate.
2. The sea grass mapping has been undertaken by remote sensing, approaching the department of Geo-Engineering, AU college of Engineering. The result obtained was coincided with the ground data collected.

The limitations are

1. Distance
2. Carrying the equipment & bringing back to the laboratory became highly difficult
3. In-situ fixation of samples for DO and nutrients has become a limitation because of frequent breakage of glassware in the travel, as there is no proper road service from Chirrayanam village to highway.

III. Monitoring Climate Change and Blue Carbon Responses.

1. Live samples of sea grasses brought to the laboratory and maintained in the aquarium. But carbon dioxide sequestration experiments became failed because of the poor adaptation of sea grass to the laboratory conditions

2. Equipment purchase time for carbon dioxide sequestration was not extended, so availability of instruments has become a limitation.

4. Comments about the upcoming research plans in the short/mid-term using the MMLs and possible further research applications using the new MML infrastructures for widening the research capacity of the new MML.

Upcoming research plans using MML:

The sea grass along with native sediments present in the brackish water lake at Chirrayanam has been maintaining in the MML. Experiments on the carbon dioxide sequestration adapting method of Ganguly et.al (2017) were already designed, will be repeated in future until satisfactory results obtained.

Experiments with seeds of *Halophila ovalis* will be undertaken in the MML to germinate in the laboratory and seeding the seedlings in the areas the sea grass meadows were recorded earlier and disappeared now.

Short/Mid-term use of MML infrastructure:

The MML will be used by the PG students and also undergraduates for their short term research leading to the submission of dissertation for their Undergraduate and Post-Graduate degrees.

Research fellows working for PhD degree can also use MML for hydrography, Microplastic analysis in sediment, water and biota. Stereomicroscope is useful for researchers working on diverse research areas for analysis of their samples.

Sieves, Sieve shakers, UV Visible spectrophotometer, GPS, Soil coring device, Temperature and pH logger, under-water camera, fish finder, quadrates and photo-quadrates are useful not only for microplastic research but also for other research topics involved with the use of such instruments which may widen the research capacity of the MML and the Andhra University at large.

ECOMARINE

Building a Comprehensive Mechanism for Preserving Marine Ecosystems and Life from the negative consequences of Climate Change and the disposal of Plastic Debris

Agreement number	619158-EPP-1-2020-1-CY-EPPKA2-CBHE-JP
EU programme	Capacity Building for Higher Education (EAC/A02-2019-CBHE)
Project website	www.ecomarine-project.eu

D 4.1. Evaluation of Operation protocols

MARINE MONITORING LAB

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2. Description of MMLs activities.

Research Objetive	Dates of data collection	Sampling location	Type of samples collected	Research protocol used	List of Equipment used	Scientific Reports delivered and proof of evidence (links) (Scientific papers, reports, other,)
I. Monitoring Microplastic Debris.	Feb 26 2023 – June 18 2023	All coastal districts of Kerala; Gujurat, Maharastra, Goa, Karnataka and Kerala (west coast of India)	Water, sediment, biota, fishfeed	Dehaut et al., 2016 and Karami et al., 2017 (fish); Masura et al 2015 (sediment and density separation); Wang et al., 2021 (water); Karami et al., 2017; Thiele et al 2021 and Karabalaei et al., 2020(fishfeed)	Seive shaker, incubator,vaccum pump, Leica stereozoom microscope EZ4D, ATR-FTIR (NICOLET iS50FTIR), µRaman spectroscopy (LabRAM HR Evolution)	**BOOKS** 1. Kumar A.B. and Devi S.S. 2023. Marine Plastic Pollution; sources, impacts and solution. University of Kerala, Dept of Aquatic Biology & Fisheries, Trivandrum, India. ISBN: 978-93-5913-343-0. (https://ecomarine-project.eu/wp-content/uploads/2023/06/MARINE-PLASTIC-POLLUTION-Ecomarine-Book.pdf) 2. Kumar, A.B. and Devi, S.S. 2024. Comprehensive Guide to Monitoring Marine Litter and Analysing Microplastics in Aquatic Ecosystems. University of Kerala, Dept. of Aquatic Biology & Fisheries, Thiruvananthapuram, India. ISBN: 978-93-340-6521-3 (https://ecomarine-project.eu/wp-content/uploads/2024/06/Monitoring-Marine-Litterd-small-size.pdf) **BOOK CHAPTERS** Devi, S.D. and Kumar, A.B. 2024. Impacts of mesoplastics and microplastics on marine biodiversity. In: Meso and Microplastic Risk Assessment in Marine Environments: New Threats and Challenges (Sekar, S., Venkataraman, S., Sabarathinam, C., Viswanathan, P.M. Eds.). Elsevier, Amsterdam, pp. 309-34P. ISBN 978-0-323-90980-8

						https://doi.org/10.1016/B978-0-323-90980-8.00014-5 **RESEARCH PAPERS** 1. Devi et al., 2024. MP contamination in Ashtamudi Lake, India: Insights from a Ramsar wetland. J. Contam. Hydro. 264. 104367. https://doi.org/10.1016/j.jconhyd.2024.104367. 2. Devi et al., 2024 Does the MPs ingestion patterns and polymer composition vary across the oceanic zones? A study from the Indian coast. Mar Pollut Bull. 204, 116532. https://doi.org/10.1016/j.marpolbul.2024.116532.
II. Monitoring Productive Ecosystems.	May 22 2023– June 13 2024	Rocky reef areas and ship wreck areas of Kerala coast	Water, sediment, flora and fauna			**BOOK** 1. Nisanth, H.P., Vishnu, H., Chinnu, V. & Biju Kumar, A. 2024. Communicating Biological Research Through Photos - A Photography Manual. Department of Aquatic Biology and Fisheries, University of Kerala. ISBN: 978-93-340-2050-2 (https://ecomarine-project.eu/wp-content/uploads/2024/02/Photography-book-social-media.pdf
III. Monitoring Climate Change and Blue Carbon Responses.	July 02 2023- Dec 20 2023	Kannur, Kasargode and Kollam districts of Kerala State	Mangrove, sediment, water	Chave et al., 2009 (wood density); Komiyama et al., 2005(AGB); Komiyama et al., 2008,	Laser Range Finder+ Hypsometer, Water quality multiparameter equipment	1. Sibin et al. 2024. Exploring blue carbon research in India: Current landscapes and future avenues. (Submitted to the journal Ecological Indicators)

				Kauffman and Donato 2012 (BGB)		

3. Brief self-evaluation of the protocols efficiencies and possible limitations.

I. Monitoring Microplastic Debris.

The research team in University of Kerala followed the following protocols for extraction of microplastics (MPs).

We conducted the following studies during the project period: (i) Marine debris monitoring in the beaches of all along the west coast of India; (ii) Microplastics monitoring in the beaches of all along the west coast of India; (iii) Microplastics in the beaches of selected islands of Lakshadweep; (iv) Microplastics in the sediment, water and fauna of Ashtamudi Ramsar Lake, Kerala; (v) Microplastics in the deepsea fishes off southwest coast of India; (vi) Microplastics in the commercial aquaculture feeds of Kerala coast.

For microplastic extraction, we sticked to the following protocols: Dehaut et al., 2016 and Karami et al., 2017 (fish); Masura et al 2015 (sediment and density separation); Wang et al., 2021 (water); Karami et al., 2017; Thiele et al 2021 and Karabalaei et al., 2020 (fishfeed). Except for MPs in fish feed, other protocols were found to be very efficient. For fish feed we underwent slight modification with that of Karami et al., 2017 to get proper digestion.

With the support of the Ecomarine project, MML has intensified its microplastics research, leading to the improvement of previously suggested methods. These enhanced methods have been compiled and published as a handbook.

Limitation: One limitation of the protocol is its ability to monitor nanoplastics, especially within the tissue samples of organisms, and to assess the impact of chemicals on living systems, including humans. Standardization of the existing protocols is needed to address these issues.

II. Monitoring Productive Ecosystems

As part of the Ecomarine project, we initiated the monitoring of biodiversity associated with rocky reefs and shipwreck sites along the Kerala coast of India, focusing on areas subjected to upwelling. This effort utilized Remotely Operated Vehicles (ROVs).

We were unable to use drones due to equipment shortages and restrictions on drone usage in Indian coastal areas. Instead, biodiversity assessments were conducted using deep-sea scuba diving and ROVs. During scuba dives, photoquadrats were employed along with additional instruments such as GPS and depth loggers. Documentation and analysis followed automated or semi-automated photoquadrat analysis protocols (Beijbom et al., 2015; González-Rivero et al., 2016; Gormley et al., 2018; Williams et al., 2019). Video documentation with ROVs was conducted using equipment and services provided by eyeROV Technologies, Cochin, a start-up under the Government of Kerala. The Ecomarine project achieved digital documentation of marine biodiversity and invasive species in the region, marking the first study of its kind along the Indian coast. The research team also published a manual on preparing digital photos for biological research.

Limitation: Due to budget constraints and procurement timelines, drones and ROVs could not be purchased under the Ecomarine project. High-quality instruments exceeded the project's budget allocation. Consequently, ROVs were hired, and we utilized paid services of deep-sea divers to document the biodiversity of rocky reefs and shipwreck sites, which are vital fishing stations for traditional fishers in Kerala.

III. Monitoring Climate Change and Blue Carbon Responses.

Blue carbon estimation in the current study followed the following methods in the protocol.

Mangroves: Sample collection and methodology (Donato et al., 2011).

Estimation Above ground mass- Calculation of the vegetation biomass (Chidumayo, 1990). The stem biomass of the plant can be calculated by using the allometric equation (Kauffman and Donato, 2011; Komiyama et al., 2000).

For determining organic carbon content (% Corg), the following methods were adopted:

1) Using an automated elemental analyser (called a CHN analyser since many elemental analyzers are configured to simultaneously measure carbon (C), hydrogen (H), and nitrogen (N) content (Sollins et al. 1999)
2) Using combustion and empirical relationships between organic carbon and organic matter (known as Loss on Ignition, LOI
3) Using wet chemistry techniques such as the Walkley-Black method (H2O2 and Dichromate Digestion (Walkley-Black method)
4) Carbon Pool estimation of above Ground biomass: Kauffaman and Donato, 2012.
5) Estimation of Plant litter biomass: Kauffman et al., 1995

Soil carbon can be determined by the Walkley-Black chromic acid wet oxidation method (Jackson and Barak, 2005).

Carbon Estimation: The total carbon stock (t ha-1) of the sample was computed by adding the carbon stored in the above-ground carbon in tree species with the carbon stored in the soil (Kauffman and Donato 2012)

In general, the protocols listed above provided satisfactory results.

Limitations: Estimation of blue carbon in sea grass ecosystem could not be estimated because we could not purchase the benthic chamber due to time restrictions set for the purchase of equipment, even though University of Kerala joined late in the Ecomarine project.

In the future phase of research the MML would take up remote sensing studies to estimate the blue carbon.

4. Comments about the upcoming research plans in the short/mid-term using the MMLs and possible further research applications using the new MML infrastructures for widening the research capacity of the new MML.

The MML in the University of Kerala shall aim to develop strategies and measures for environmental protection and preservation of marine ecosystems aligned with the ones developed by the Government of India. The new monitoring programmers and practices developed by the lab, augmented through the training offered by the European institutions/Universities/Research centers, will assist in this direction. In addition, The MML will help spread awareness among the people related to environmental protection and the preservation of marine ecosystems.

Major Objectives of the MML at the University of Kerala
A new collaborative research grant was approved on the research project titled "The ecology and evolution of cultural and cooperative behavior among dolphins and humans", in collaboration with Oregon State University, USA, and the project is supported by National Geographic.
Amount: USD 127,006
Period: 05/01/2024 to 05/01/2028

Applied for Indo-Norwegian Cooperation Programme in Higher Education and Research - INCP2 - Call for applications 2024.

Develop Operational Protocols: Establish comprehensive operational protocols for MML. Already a protocol for microplastic monitoring of aquatic ecosystems has been published.

Support Research in Thematic Domains:

(i) Monitoring climate change by leveraging the blue carbon potential of coastal/marine ecosystems

Continue the work on blue carbon ecosystems; One post-doctoral fellow has joined the MML for continue the work, and the research is supported by the Directorate of Environment and Climate Change, Government of Kerala. The MML will open up the facilities for dissertations and project work for graduate, post-graduate and PhD research programs from outside.

(ii) Monitoring marine biodiversity

The MML is continuing the marine biodiversity documentation activities. Marine mammal monitoring has already been initiated with Nat Geo project. One PhD student has joined for PhD program to carry out the work on intertidal biodiversity of Kerala coast, using the facilities of MML, University of Kerala.

The University of Kerala would further seek CSR funding to document biodiversity using ROVs, and drones.

MML would also try to bring out a few manuals for marine biodiversity of selected fauna of Indian coast, which may help young researchers to take up taxonomy and biodiversity research.

(iii) **Monitoring microplastic debris in marine ecosystems.**

MML is active in marine debris research and already many academic institutions are using the facilities of the lab for their research. Further, the MML is also into outreach activities such as beach clean-up campaigns, marine debris awareness programs, and this will be further strengthened in the future.

For furthering research, the reseach programs of graduate and post-graduate and PhD students can be linked with the topic. Already four post-graduate students are using the MML for their project work.

In future, the areas identified for the work include research on 'plastisphere' associated with marine debris; Neopelagic community rafting on floating plastic debris in ocean surface waters; and health impacts of chemicals associated with microplastics.

Capacity-Building and Skilling Activities: Organize regional and national capacity-building activities with trained MML staff and external experts as and when needed. Enhance marine field research skills and competencies of students and educational staff through various capacity development programs.

Promote E-Learning: Enable students and researchers to participate in the international e-learning course developed by the ECOMARINE project. Develop MOOC programs on ocean literacy by the training staff of MML.

Outreach Programs: Organize outreach programs to raise awareness about ocean literacy and climate change, fostering a knowledge society.

Continue Partnerships: Maintain collaborations with institutions involved in the ECOMARINE project, supporting innovative research and social services for coastal and marine environment protection.

Achieve Accreditation: Attain accreditation for the MML by adhering to quality standards.