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MARBEFES Project

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Project General Coordinator: Jan Marcin Węsławski
Project Scientific Manager: Julie Bremner
Project Manager: Joanna Potrykus

Task Leader(s)
Marie C. Nordström (University of Helsinki) and Clement Garcia (Cefas)

Author(s)
Marie C. Nordström, John Aldridge, Francisco R. Barboza, Pierre Blévin, Julie Bremner, Jeanette Carlsson, Jens Carlsson, Eva Chatzinikolaou, Sabine Cochrane, Thanos Dailianis, Silvia de Juan, Mihhail Fetissov, Costas Frangoulis, Jacopo Giampaoletti, Hilmar Hinz, Panagiotis Kasapidis, Burak Kaynaroglu, Kleoniki Keklikoglou, Jonne Kotta, Paolo Magni, Nils Nitov, Iara Diamela Rodriguez, Tiina Salo, Robert Szava-Kovats, Anna Törnroos-Remes, Riikka Puntila-Dodd, Emmanouela Vernadou & Clement Garcia

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Summary

European Member States, like other countries, need to understand and sustain biodiversity and ecosystem functions to ensure continued supply of ecosystem services on which human well-being relies. Effective environmental management depends on valuing coastal and marine biodiversity and their benefits through ecological and societal measures. MARBEFES aims to improve understanding of biodiversity and develop methods for ecological, economic and cultural valuation. Specifically, MARBEFES WP3 provides the structure for the project work on biodiversity and ecosystem tools. It develops and appraises a suite of tools for assessing ecosystems - physical conditions, biogeochemical cycles, habitats, biodiversity, ecosystem function and ecosystem services - from primary producers to higher tropic levels. It aims to:

- Develop analytical and conceptual tools to better understand rules on biodiversity and ecosystem functioning across seascapes and the physical-chemical factors that shape them, in order to clarify patterns and processes across taxa and environments and through time, spanning surface, water column and seafloor
- Develop tools to enable prediction of the impacts of multiple human activities, future scenarios and management options on ecosystem biodiversity, structure, function and production of ecosystem services
- Develop new methods and a toolbox to better inventory and monitor seascape biodiversity, ecological functioning and ecosystem services

WP3 developed and tested 16 different tools and knowledge-generation initiatives, of which the majority are operational tools for practitioners to use in the context of their specific environment-related problems (biodiversity links to ecosystems services and options to manage them).

The Handbook on Seascape Biodiversity, Function and Ecosystem Services presents the suite of tools developed in MARBEFES for assessing marine geophysics, ecological phenomena and their connections to function and services. The handbook is designed to ensure clarity and usability and is organised for easy navigation by tool category or remit. Each tool section gives a concise overview and is accessible to non-specialists. As a technical handbook for assessments of biodiversity, functional biodiversity, ecological processes, connectivity, ecosystem functions and ecosystem services, it delivers expert-reviewed, reliable information. Each tool is covered in approximately two pages, offering consistency, practical examples, and standardised content throughout.

The Handbook is structured along a gradient of tool focus from biodiversity through to ecosystem services. Out of the 16 different tools and knowledge-generation initiatives developed by WP3, 10 were specifically developed with the aim to describe biodiversity, function and ecosystem services. The tool section of this report presents the 10 tools, which address all or a subset of these themes (see the main introduction of this report and the Handbook on Assessing Human Multi-impacts on Seascape Ecosystems for details regarding the other tools). The tools were further categorised and

colour-coded by approach (molecular, trait-based, network-based, image-based or biogeochemical approaches), as well as star-coded by the anticipated level of practitioner usability (from direct to indirect). In the tool section, each tool is presented succinctly, and a worked example is provided to illustrate the type of outputs expected. The final section provides an overview of how to use the tools in an integrated manner to complement their strengths.

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1 Project Background

MARBEFES: European Member States, as with countries worldwide, have a fundamental need to understand how biodiversity and ecosystem functioning must be maintained to ensure the delivery of ecosystem services and societal goods and benefits, which must in turn be sustainably utilised by humankind. Critically, this calls for valuation of coastal and marine biodiversity and the ecosystem services they provide, as a basis for cost-effective environmental management. Above all, this requires ecological, economic, and cultural valuation to fully capture the diversity of ways in which biodiversity contributes to the healthy functioning of ecosystems and the supply of ecosystem services. MARBEFES set itself the objectives to ensure an increase in understanding of biodiversity, and the delivery of methods for ecological, cultural and economic valuation. As such, it dedicates several work packages (WPs) to deliver fully developed methods and tools, and in parallel test and validate them in diverse environmental settings across Europe (from the Arctic to semi-tropical areas) through a series of case study areas stretching from the coast to the open sea, termed Broad Belt Transects (BBTs). In this way, MARBEFES aims at showing the way to describe, assess, and value different nature components (habitats, species, ecosystems), to explore different alternative scenarios for policymakers.

Work package 3 (WP3): In this project context, MARBEFES WP3 provides the structure for the project work on biodiversity and ecosystem tools. It develops and appraises a suite of tools for assessing ecosystems - physical conditions, biogeochemical cycles, habitats, biodiversity, ecosystem function and ecosystem services - from primary producers to higher trophic levels. In practice, WP3 is built upon four main tasks:

- **Geo-physical drivers of biodiversity across seascapes** focusing on developing and validating regional models of ecosystem dynamics and biodiversity drivers, linking riverine, estuarine and coastal processes to shelf sea environments;
- **Tools for exploring ecological phenomena** developing tools to explore ecological connectivity and the links between biodiversity and function across seascapes;
- **Impacts of multi-pressures on ecological systems** developing tools for integrated impact assessment, considering the biodiversity, ecological processes and function and how these translate to ecosystem services;
- **Innovation in biodiversity inventory and monitoring** focusing on improved inventory and monitoring of marine and coastal biodiversity, the use of the latest remote monitoring methods and developing new tools for characterising biodiversity, collecting functional data and detecting the ecological effects of environmental change.

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Collectively, these four tasks from WP3 aim at:

- Developing analytical and conceptual tools to better understand processes underlying biodiversity patterns and ecosystem functioning across seascapes and the physical-chemical factors that shape them
- Developing tools to enable prediction of the impacts of multiple human activities, future scenarios and management options on ecosystem biodiversity, structure, function and supply of ecosystem services
- Developing new methods and toolboxes to better inventory and monitor seascape biodiversity, ecological functioning and ecosystem services

Biodiversity and ecosystem tools: MARBEFES WP3 presided over the development and testing of sixteen (16) different tools or knowledge generation initiatives, the majority of which are presented as operational tools for practitioners to use in the context of their specific environment-related problems (biodiversity links to ecosystems services and options to manage them) within WP3 deliverable reports (see below). In parallel, WP3 initiatives also aimed at advancing the capabilities of its tools by increasing the quality and the diversity of available evidence that the tools build on. In the activity called *"The viability of indicators of functional change"*, it sought to comprehensively describe the state-of-the-art of established, empirically measured links between functional identity (functional trait expression) and ecosystem functioning (flow of energy and matter). In the activity called "*Exploring the potential of citizen science to support BBTs"*, with respect to identifying under-used, potentially difficult, sources of evidence, WP3 also endeavoured to explore pathways for the uptake of citizen science programmes that monitor biodiversity by reviewing existing projects and developing approaches to integrate the information generated by the projects to support decision making . Another pathway for new sources of evidence was explored by WP3 through non-traditional methods to generate trait data by developing a framework for collecting so-called informal trait information from *e.g.* researchers or student lab or field projects that are only recorded in difficult-to-access grey literature in multiple languages (activity "*New tools for the inventory of ecological function"*). These initiatives are not included in here as they are knowledge-generation activities and not suited for a Handbook on Seascape Biodiversity, Function and Ecosystem Services, and are, as such, reported as part of other outputs within MARBEFES.

D3.1 Deliverable: **Handbook on Seascape Biodiversity, Function and Ecosystem Services**. The handbook presents the suite of tools developed in MARBEFES for assessing marine geophysics, ecological phenomena and their connections to function and services. Together with the **Handbook on Assessing Multi-impacts on Seascapes ecosystems**, this deliverable comprises the final MARBEFES reports on biodiversity and ecosystem tools, providing first-level guidelines. The purpose of this report is to provide an overview of the tools developed in WP3, allowing the reader to conduct a quick assessment as to whether any of the tools on the list could be of help for their specific objectives before delving deeper into the tool's functioning and methodology, and the report hence aims at being a "first port of call" in guiding this choice. Each tool is presented with a short case study to provide an example of outcomes and help with the decision. The two WP3 Handbooks (D3.1 and D3.2) should be considered together with the WP4 Deliverable **Handbook on the Ecological, Economic and Socio-Cultural Valuation of Biodiversity** (D4.1) for a full suite of MARBEFES tools. The full testing and validation of each tool within MARBEFES case-studies (BBTs), alongside recommendations, are described and reported within the WP2 Deliverable **Final Assessment Report from Tests** (D2.2). Finally, the most up-to-date material and methods for applying each of the tools presented here are stored in the WP6 Deliverable **Marine Biodiversity and Valuation Toolbox** (D6.1), a web-based collection of the tools developed within MARBEFES.

2 Purpose of the Handbook on Seascape Biodiversity, Function and Ecosystem Services

2.1 Seascape Biodiversity, Function and Ecosystem Services

In our quest to safeguard and sustainably use marine ecosystems and the societal benefits they provide, it is imperative to understand components of ecosystem structure as well as functioning. **Marine biodiversity**, the ecological processes this diversity maintains, as well as the diverse linkages within marine ecosystems are all realised in the **seascape.** Seascapes are spatially heterogenous and dynamic marine spaces that host diverse species assemblages and habitats, forming the abiotic and biotic drivers shaping community composition and diversity. Approaches to acquire, understand, and use **ecosystem functioning** estimates are presented, spanning methods relying on proxies (*e.g.*, functional groups, traits) to direct quantification of processes (*e.g.*, primary productivity, nutrient fluxes) underpinning seascape **ecosystem services** and status. The Handbook presents tool options to understand physical, chemical and biological processes, and to describe marine biodiversity and ecosystem structure at selected areas in Europe across biological organizational levels from the molecular, individual and population levels to communities, food webs and ecosystems, as well as the services they deliver.

2.2 Handbook Objectives and Organisation

The handbook was designed around seven (7) principles. In order to (i) **provide a concise overview of the tools**, each tool section focusses on a few key characteristics, which are (ii) **organised for easy navigation** either by tool broad category, remit or ease of access to non-specialists. The handbook is defined as a (iii) **technical handbook** for the assessment of seascape biodiversity, function and ecosystem services and each tool description contained therein is (iv) **authoritative and reliable** having been developed, written and reviewed by experts in the field. This handbook is meant to be a concise overview of each tool (one page with a fundamental tool description followed by short examples of tool implementation) so that it is (v) **easy to dip in and out of**, each tool section is (vi) **consistent and standardised** with the same type of information provided for each and all contain (vii) **practical examples** for a tangible application and example outcome of respective tool.

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3 Tool Overview

WP3 developed 16 tools or knowledge generation initiatives, which all can be used for describing seascape structure, function and services. Applied in different settings and for different purposes, the tools can also be useful for assessing human impact. In the interest for each of the handbooks to guide the user to the most relevant tool, tools are here listed and divided according to their primary focus under the MARBEFES project. Therefore, out of the 16 tools or knowledge generation initiatives that WP3 developed, 3 constitute activities outside the scope of the handbooks (an overview of citizen science, informal trait approaches and a literature review of indicators of functional change) and will be reported elsewhere, 7 were primarily developed to describe seascape structure, function and services and are therefore presented in the present report (D3.1) and 3 were primarily developed to assess human impacts and are presented in the other handbook (D3.2). Finally, 3 of the tools (‘Multi-Scale Trait-Based Approaches Linking Biological Structure to Ecosystem Function’, ‘Assessing Functional Trait-Based Species Vulnerability Against Environmental and Human Drivers’, and ‘Metric of Habitat Function’) were developed for both objectives and are therefore presented in both handbooks with the example of application in each tailored to the purpose of respective handbook. For example, the “Assessing Functional Trait-Based Species Vulnerability Against Environmental and Human Drivers” tool presents an application describing the change of functional trait structure according to an environmental gradient in the present handbook (D3.1) and focuses on vulnerability assessment from anthropogenic impacts in the other handbook (D3.2). Overall, the present Handbook on Seascape Biodiversity, Function and Ecosystem Services (D3.1) presents 10 tools (7 specific, 3 in common with D3.2) and the Handbook on Assessing Multi-impacts on Seascapes ecosystems present 6 tools (3 specific, 3 in common with D3.1) (Table 1).

For ease of cross-reference, each of the WP3 tools has been classified according to the tool’s main categories or themes (first principle upon which the tool is based), the relevance of each tool for research questions aimed at assessing biodiversity, and whether the tool focuses on ecosystem components or assesses ecosystem functioning or ecosystem services. Tools were assigned a colour-coded category (common across D3.1 and D3.2):

- Molecular approaches [fuchsia] are techniques using sub-organism methods (e.g. DNA/RNA) as a primary assessment method.
- Trait-based approaches [green] are tools built upon the use of functional traits to inform either a response of the biological community to the environment or an effect on ecosystem functioning.
- Imaging approaches [coral] rely on methodologies based on deriving information out of images.
- Physical-chemical approaches [blue] are hydrodynamic or biogeochemical models.
- Network approaches [orange] are tools that use a diagram or a network-based approach as a basis for their implementation (e.g., food web, path diagram).
- Decision-support tools [grey] (not presented in D3.1) are integrated tools with an interface for entering scenario parameters.

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***Table 1** Classification of the 13 WP3 tools (excluding the 3 knowledge generation initiatives – see text) across the two handbooks, based on whether they are used for describing seascape structure, function and services (D3.1), for assessing human impacts (D3.2), or both. The tools that are not described in this handbook are greyed and in italics.*

Tool name	**D3.1**	**D3.2**
Genetic Tools for Environmental Monitoring	✓	
Assessing Functional Trait-Based Species Vulnerability Against Environmental and Human Drivers	✓	✓
Methodological Framework for Seascape Scale Benthic Ecosystem Functioning Research: Integrating Current Practices and Emerging Technologies	✓	
Deriving Faunal Function from Benthic Samples Using the ZooScan - an Automated Imaging System	✓	
Multi-Scale Trait-Based Approaches Linking Biological Structure to Ecosystem Function	✓	✓
Assessing Morphological Traits of Marine Invertebrates Related to Ocean Acidification and Climate Change Using Micro-Computed Tomography (Micro-CT)	✓	
Metric of Habitat Function	✓	✓
Biogeochemical Modelling: Linking Marine Physics, Chemistry and Biology	✓	
Modelling Ecosystem Service Dynamics Using Structural Equation Modelling	✓	
Assessing Food Web Structure and Function Using Ecological Interaction Network Approaches and Bioenergetic Modelling	✓	
TeloStress – Telomere Length as an Indicator of Physiological Stress		✓
A Bayesian Belief Network Framework for Testing Management Scenario Effects on Ecosystem Services		✓
Food-Web–Informed Cumulative Effects Assessment to Evaluate Ecosystem Functioning Across Human-Use Scenarios		✓

Finally, each tool has also been categorised for whether non-specialist practitioners are likely to engage with or use the tool in a direct, semi-direct, or indirect way. One star (*) indicates a direct level of engagement, the tool is interactive or partially interactive and allows for current and future scenarios that can be directly implemented and tested by stakeholders. Two stars (**) indicates a semi-direct level of engagement, the tool is highly specialised but does benefit from non-specialist engagement in setting the right questions and identifying suitable outputs. Finally, three stars (***) indicates an indirect level engagement, where the tool is still at a research and development stage and is likely to benefit non-specialists only indirectly (for example, by exploring new sources of data that could be used through other tools).

Table 2 *Tools and their assigned category (molecular [fuchsia], trait-based approaches [green], imagery [coral], physical-biogeochemical [blue] and network approaches [orange]). Note that tools are not restricted to a single category but, here, the most representative for the implementation was chosen. Green checkmarks, red crosses and amber question marks, respectively, indicate whether the tool addresses, does not address or could address the category with a little amendment. Stars indicate the level of non-specialist engagement, * = direct; ** = semi-direct, and *** = indirect.*

Tool name	**Category**	**Biodiversity**	**Ecosystem components**	**Ecosystem function**	**Ecosystem services**
Genetic Tools for Environmental Monitoring ***	Molecular	✓	✓	✗	✗
Assessing Functional Trait-Based Species Vulnerability Against Environmental and Human Drivers **	Traits	✓	✓	✓	✗
Methodological Framework for Seascape Scale Benthic Ecosystem Functioning Research ***	Traits	✓	✓	✓	✗
Deriving Faunal Function from Benthic Samples Using the ZooScan – an Automated Imaging System ***	Imagery	✓	✓	✓	✗
Multi-Scale Trait-Based Approaches Linking Biological Structure to Ecosystem Function **	Traits	✓	✓	✓	?
Assessing Morphological Traits of Marine Invertebrates Related to Ocean Acidification and Climate Change Using Micro-Computed Tomography (Micro-CT) ***	Imagery	✗	✓	✓	✗
Metric of Habitat Function *	Traits	✗	✓	✓	✓
Biogeochemical Modelling: Linking Marine Physics, Chemistry and Biology **	Physical-biogeochemical	✗	✓	✓	✓
Modelling Ecosystem Service Dynamics Using Structural Equation Modelling **	Network	✗	✓	✓	✓
Assessing Food Web Structure and Function Using Ecological Interaction Network Approaches and Bioenergetic Modelling **	Network	✓	✓	✓	✓

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4 Tool List

Genetic Tools for Environmental Monitoring - eDNA-Based Tools for Assessing and Monitoring Biodiversity in Marine Ecosystems	***
Tool focus:	Biodiversity, Ecosystem components
Tool type:	Molecular
Tool category:	Molecular approaches
Main users:	Scientists and Practitioners

Tool description: Environmental DNA (eDNA) is a molecular tool used to detect and monitor organisms by analysing genetic material shed into the environment through skin cells, mucus, faeces, or gametes. These DNA traces accumulate in water, soil, air, or sediment and can be collected, extracted, and analysed to identify species or entire biological communities. Using molecular methods such as PCR and metabarcoding with next-generation sequencing (NGS), eDNA allows for sensitive, non-invasive biodiversity surveys without capturing or observing organisms directly. This approach provides an efficient, scalable, and low-impact means of assessing biodiversity across ecosystems.

Data needs: Reliable eDNA analysis requires well-documented environmental, molecular, and bioinformatic data. Each step, from sampling to interpretation, must ensure traceability, reproducibility, and quality control. **Environmental data**: Each sample should include metadata such as GPS location, date, time, water temperature, pH, turbidity, conductivity, and dissolved oxygen. Sample type, volume, depth, and habitat details are essential. Field blanks and replicates help detect contamination and improve confidence in results. **Molecular data**: Laboratory records should document DNA extraction methods, kit types, yields, and purity. PCR details, including primer sequences, target genes (*e.g.*, COI, 12S, 16S, ITS), and conditions, must be reported. Sequencing data (*e.g.*, from Illumina or Nanopore platforms) include raw reads, quality scores, and barcode assignments for downstream processing. **Bioinformatic and reference data**: High-quality databases (GenBank, BOLD, SILVA) enable taxonomic assignment of sequences. Custom regional libraries improve identification where public data are incomplete. Pipelines should include quality filtering, chimera removal, OTU/ASV clustering, and taxonomic classification. **Ecological data**: To interpret results, eDNA outputs are linked to species occurrence, read abundances, and environmental variables such as habitat, land use, or pollution levels. Validation against traditional surveys (*e.g.*, netting, trapping, cores or trawls) strengthens confidence in detections.

Key references:

Deiner *et al.* (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular Ecology*, 26: 5872–5895.

Goldberg *et al.* (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods in Ecology and Evolution*, 7: 1299–1307.

Kelly *et al.* (2014). Using environmental DNA to census marine fishes in a large mesocosm. *PLoS ONE*, 9: e86175.

Porter & Hajibabaei (2018). Scaling up: A guide to high-throughput genomic approaches for biodiversity analysis. *Molecular Ecology*, 27: 313–338.

Thomsen & Willerslev (2015). Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity. *Biological Conservation*, 183: 4–18.

Valentini *et al.* (2016). Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Molecular Ecology*, 25: 929–942.

Genetic Tools for Environmental Monitoring - eDNA-Based Tools for Assessing and Monitoring Biodiversity in Marine Ecosystems

Example of implementation: As a research and monitoring tool, eDNA enhances precision, reproducibility, and cost-effectiveness while reducing ecological disturbance. It provides a standardised framework for species detection and biodiversity evaluation, supporting evidence-based conservation, environmental policy, and sustainable management.

- Biodiversity monitoring: Detecting species across habitats and over time.
- Invasive species detection: Enabling early identification and management of non-native taxa.
- Endangered species monitoring: Revealing rare or elusive species with minimal disturbance.
- Community-level studies (metabarcoding): Describing whole assemblages from environmental samples.
- Ecosystem health assessments: Linking biological indicators to environmental change.

Ongoing specific implementation in MARBEFES:

- Water sampling for biodiversity assessment in deep and shallow Arctic waters using samples from Porsanger Fjord in northern Norway and from east of Greenland, north of Svalbard. This work highlights how eDNA can effectively characterise marine biodiversity across contrasting Arctic habitats, providing comprehensive species inventories without the need for extensive physical sampling. The focus is on demonstrating the capability of eDNA to support marine biodiversity research in remote and logistically challenging environments.
- Integration of eDNA water-sampling capacity on Autonomous Underwater Vehicles (AUVs), in collaboration with Akvaplan Niva (Norway). Deploying AUVs for in situ water sampling and filtration significantly reduces reliance on large research vessels, improving the cost-efficiency and scalability of biodiversity monitoring. This approach demonstrates how AUV-based eDNA sampling can expand spatial coverage, support repeated sampling campaigns, and free vessel time for other research activities.
- Detection of sharks, skates, and rays off the west coast of Ireland using elasmobranch-specific metabarcoding. Because universal fish primers can overwhelm sequencing output with non-target species, targeted elasmobranch primers were used to enhance detection of this low-abundance group. This work illustrates how eDNA assays can be customised for taxa of interest and improve detection sensitivity in otherwise “noisy” marine environments.
- Sediment sampling from the Irish Sea to assess benthic biodiversity. These samples highlight the capacity of sedimentary eDNA to reveal benthic invertebrate communities, providing an efficient complement to traditional grab-sample identification and enabling finer-scale mapping of benthic habitats.
- Water and sediment sampling from Sardinian lagoons with contrasting nutrient and salinity regimes, along with marine control sites. Benthic samples were collected at each sediment site for morphological identification, enabling direct comparison with eDNA results. This work showcases the ability of eDNA to track morphology-based biodiversity patterns and evaluate how environmental gradients structure biological communities.
- Preservation of Sardinian water and benthic samples in RNAlater for eRNA analysis. By enabling the detection of expressed genes, eRNA provides insights into active biological processes and local ecosystem functions. These analyses offer a promising approach for assessing ecosystem health, detecting stress responses, and identifying functional shifts linked to environmental conditions.

Assessing Functional Trait-Based Species Vulnerability Against Environmental and Human Drivers **

Tool focus:	Biodiversity, Ecosystem components, Ecosystem functioning
Tool type:	Statistical analysis process, Correlative model, Multivariate Analysis, R Environment
Tool category:	Trait-based approaches
Main users:	Scientists (or Experts in trait-based approaches)

Tool description: The vulnerability of individual species to natural or human-driven changes in their environment is dictated by the traits they possess. This tool uses traits as indicators of the vulnerability of species across communities and of the functional implications of species' responses to environmental change. The tool uses a method based on 3-tables ordination: the RLQ. The RLQ is a multivariate analysis used to study the relationships between environmental data (table R), species abundance (or biomass or presence/absence) (table L), and species traits (table Q). The analysis provides ordination scores that summarise the joint structure among the three tables, thereby identifying how environmental gradients relate to specific traits. The RLQ starts with the independent ordination of each table. The table R (environment – sites) is generally ordinated using a Principal Component Analysis (PCA), a dimensionality reduction method that reduces large data sets into fewer variables while preserving key data trends. The table L (sites – species) is generally ordinated using a correspondence analysis (CA/COA), a similar dimension-reducing method suited to frequency data, such as count, presence-absence, or biomass data. It seeks 'correspondence', *i.e.* highlights which sites correspond to which species (while PCA maximizes variance explained, CA maximizes the inertia explained, the 'correspondence' between the rows and columns of the table). The method used for table Q (species – traits) is strongly linked to the type of trait data: PCA (continuous), Multiple Correspondence Analysis (MCA) (categorical), Hill & Smith Analysis (HillSmith) (mix) or fuzzy-ordination (Fuzzy – PCA or Fuzzy – CA) (fuzzy coded). The RLQ combines the three separate analyses and identifies the main relationships between environmental gradients and trait attributes through species abundance or biomass distribution. It reveals how functional traits are selected for (or against) by environmental drivers subsequently influencing species distribution and community structure and thereby informing on the environmental factors they might be vulnerable to (selecting against) and which traits are driving this vulnerability.

Data needs: The RLQ analysis requires three interrelated data matrices. R: a matrix of environmental variables at each site (*e.g.*, temperature, sediment type, primary production), L: a matrix relating species distribution across sites (*e.g.,* abundance, count, biomass, presence/absence), Q: a matrix of species traits (*e.g.,* body size, larval dispersal, bioturbation, mobility). Most trait data types can be accommodated by the analysis (continuous, categorical, fuzzy-coded).

Key references: MARBEFES R tutorial and example data: https://github.com/Clem2012/MARBEFESWP3_VA
Borcard *et al.* (2018). *Numerical Ecology with R* (2nd ed.).; Dray *et al.* (2014). Combining the fourth-corner and the RLQ methods for assessing trait responses to environmental variation. *Ecology* 95:, 14-21. DOI 10.1890/13-0196.1
Gusmao *et al.* (2022). Oceanographic gradients explain changes in the biological traits of nesting seabird assemblages across the south-eastern Pacific. *Frontiers in Marine Science* 9:897947. doi: 10.3389/fmars.2022.897947; Zhu *et al.* (2024). Quantifying the effects of landscape and habitat characteristics on structuring bird assemblages in urban habitat patches. *Scientific Reports* 14, 12707. https://doi.org/10.1038/s41598-024-63333-z

Assessing Functional Trait-Based Species Vulnerability Against Environmental and Human Drivers

Example of implementation: In MARBEFES, the trait-based vulnerability appraisal through RLQ analysis was developed and applied within the Irish Sea over a transect spanning from Ireland (Dublin Bay) to the UK (Liverpool Bay), with the Isle of Man to the north and Wales to the south. The tool used a data set of benthic invertebrate abundance containing 885 sites and 786 taxa, each taxon had 10 (fuzzy-coded) traits with 47 attributes, and each site had 9 environmental variables (*e.g.*, bathymetry, sediment type).

The RLQ methods revealed three groups of trait-environment relationships (Figure **1**). The red group comprises traits associated with attached epifauna, crustose or tunicate morphology with tube-building capabilities, linked to large distance to the coast and high current speed. The green group is composed of large species with asexual reproduction, linked to deep habitat with high primary production and suspended particles. The amber group contains long-lived medium-sized species living in crevices, associated with high latitude. Geographically (Figure 2), the red group is associated with the most central part of the case study area (green) (Figure 2a), the green group is mostly located inshore close to Liverpool Bay and North Wales (Figure 2b) while the amber group is associated with the most northerly parts of the area (Figure 2b).

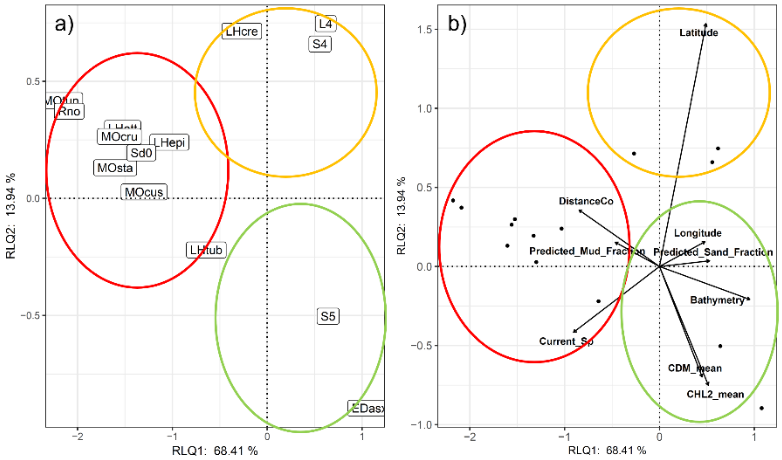

***Figure 1** The two main axes of the trait vulnerability RLQ tool, representing the relationships between (a) functional traits and (b) environmental variables. Three groups of trait-environment relationships were identified by the analysis: red, amber and green.*

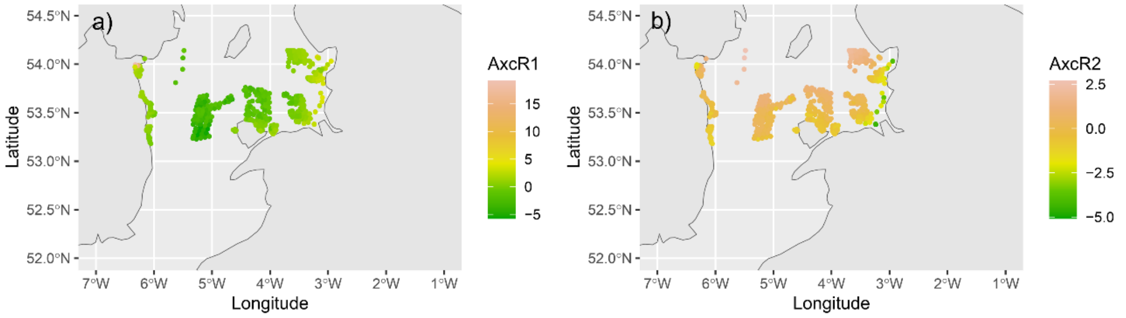

Figure 2

Geographical representation of the RLQ outputs, map a) corresponds to the scores along the first axis (red group against amber and yellow) and map b) represent the score along the second axis (red and green group against amber).

Methodological Framework for Seascape-Scale Benthic Ecosystem Functioning Research: Integrating Current Practices and Emerging Technologies

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Tool focus:	Biodiversity, Ecosystem components, Ecosystem function
Tool type:	Framework aiding in method selection considering strategic research priorities
Tool category:	Trait-based approaches
Main users:	Scientists, Environmental managers and Funding agencies

Tool description: The tool presents a framework for selecting methods to estimate benthic ecosystem function. The tool builds on a qualitative review backed up by a user-based questionnaire survey of experts working in the field of ecosystem functioning assessments. This work presents a comprehensive framework for assessing benthic ecosystem functions at seascape scales, integrating structural, process-based, and technology-driven approaches. A central component of the review is the categorisation of methods based on how functional measurements are obtained — whether inferred from community structure, derived from observed ecological processes, or directly quantified as ecosystem-level outcomes (see Figure 3). This classification helps scientists identify the most appropriate methodologies based on the ecological questions, spatial scale, and the type and quality of available data. It also assists in aligning functional assessments with logistical and financial constraints as these are evaluated alongside the methodologies.

By clarifying what each method measures and how, the framework supports more consistent, transparent, and comparable assessments across regions. For environmental managers, it offers guidance for designing monitoring programmes that are scientifically robust and policy relevant. Funders benefit from a clearer understanding of where strategic investment in methods or emerging technologies can address key knowledge gaps. Ultimately, this work advances the integration of functional ecology into marine management, conservation planning, and environmental policy.

Data needs: While the framework does not prescribe specific datasets, its effective application depends on a clear understanding of existing data availability, resolution, and quality within a given area. Users should evaluate both current and legacy datasets—including habitat maps, species inventories, or biogeochemical measurements—to determine which methodological approaches are feasible and most informative. The framework encourages users to align method selection with both the ecological characteristics of the region and their specific assessment objectives. Additionally, identifying data gaps early can help guide future sampling efforts and support strategic planning for long-term ecosystem function assessments.

Key references: There are some key references that this work builds on that identified the needs for a more integrated assessment framework on a seascape level.

Bremner *et al.* (2023). Seagrass Mapping Toolbox for South Pacific Environments. *Remote Sensing,* 15: 834. https://doi.org/10.3390/rs15030834

de Juan *et al.* (2022). Biological traits approaches in benthic marine ecology: Dead ends and new paths. *Ecology and Evolution*, 12. ISSN 2045-7758

de Juan *et al.* (2023). The continental shelf seascape: a network of species and habitats. *Biodiversity and Conservation* 32: 1271–1290 https://doi.org/10.1007/s10531-023-02

Methodological Framework for Seascape Scale Benthic Ecosystem Functioning Research: Integrating Current Practices and Emerging Technologies

Example of implementation: This example provides a description of how the framework can be implemented in a theoretical but commonly encountered situation. A coastal marine management team is tasked with assessing benthic ecosystem functions in a mixed-habitat bay to support conservation planning and meet regional policy requirements. The area includes soft sediments, seagrass beds, and scattered rocky reefs. The team begins by evaluating existing data: habitat maps are available at high resolution, and species occurrence data have been collected through previous monitoring programs, but no direct measurements of ecological processes or ecosystem functions exist.

Using the framework, the team identifies that the current data support a Type 1 (structure-based) approach. They apply trait-based analyses to infer potential functions such as bioturbation, filtration, and carbon storage, based on community composition and functional traits. Recognizing that these are proxy estimates, they use the framework's classification system to highlight key knowledge gaps—particularly the absence of direct process or outcome-based measurements—and identify Type 2 (process-based) and Type 3 (integrated function-based) methodologies for future incorporation. For example, they propose using benthic flux chambers in representative habitats to quantify oxygen and nutrient fluxes, providing more robust, ecosystem-level indicators.

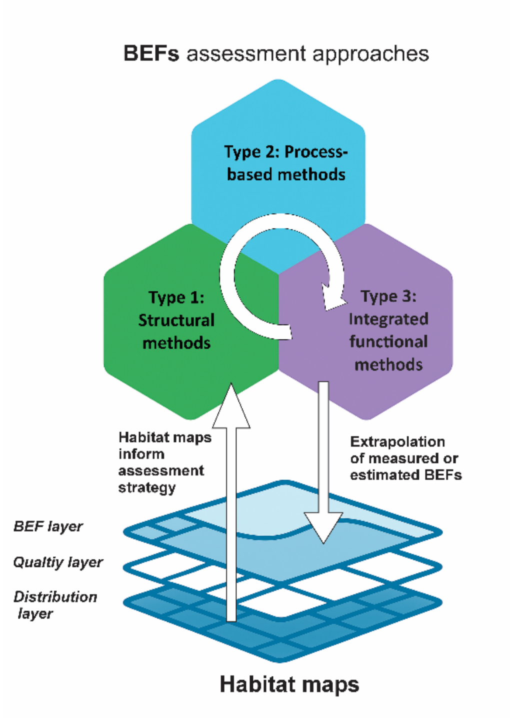

Figure 3 *Schematic drawing of Benthic Ecosystem Function (BEFs) assessment framework*

The framework also supports the team in developing a tiered, adaptive assessment strategy. Structure-based methods are used to provide broad spatial coverage and baseline understanding, while more resource-intensive functional measurements are prioritized for critical or vulnerable habitats. This integrated approach ensures the assessment remains ecologically meaningful, scalable, and cost-effective over time.

From a funder's perspective, the framework offers a strategic tool to identify where investments are most needed—whether to strengthen basic data coverage in poorly surveyed areas, or to support the adoption of innovative technologies that enhance functional resolution. For instance, by clarifying the strengths and limitations of emerging tools like eDNA, the framework helps funders evaluate how such technologies can contribute to functional assessments—*e.g.*, by expanding species detection in hard-to-sample habitats or improving trait-based models where traditional sampling is limited.

Moreover, the framework's emphasis on standardization and cross-regional comparability allows funding bodies to align their calls with international ecological and policy goals. This ensures that research efforts and technological development contribute meaningfully to large-scale, integrative ecosystem assessments—supporting long-term monitoring, conservation, and sustainable management of benthic environments.

Deriving Faunal Function from Benthic Samples using the ZooScan - an Automated Imaging System ***

Tool focus:	Biodiversity, Ecosystem components, Ecosystem function
Tool type:	Equipment (ZooScan), Proprietary software (Ecotaxa)
Tool category:	Image-based approaches
Main users:	Scientists

Tool description: The traditional approach for assessing biomass of benthic macrofauna in marine seabed monitoring is to identify taxa and record taxa-level biomass as blotted wet weight. However, this method fails to incorporate individual-based measurements, leading to a lack of insight into size structure variability within species. We adapted and tested a method to derive individual-based measurement for benthic invertebrate using a method developed for automated zooplankton assessment, the wet bed scanner (Hydroptic© ZooScan), associated with the processing software, and routines within the Ecotaxa online database for tidying and storing the images. The approach is best suited to small, robust taxa which can readily be scanned but traditional methods of measurement for larger individual or species are compatible with outputs of the tool. MARBEFES has defined a protocol that 1) demonstrated the utility of the combination of ZooScan and Ecotaxa in collecting individual based size measures from benthic macrofaunal samples to allow individual biomass measures to be determined and 2) collated information on size and biomass measures from intact specimens to aid the development of appropriate ‘size’ - biomass relationships for benthic taxa (*e.g.*, by species, by major taxonomic group or by generalised body shape). Body-mass (by identified taxon) is apportioned to each scanned individual relative to its ‘size’ (as described by the 2D surface area in the scanned image). Individual biomass is determined by calculating a value for biomass per pixel (with the known total taxa-level biomass) and then apportioning the recorded biomass relative to body size - by multiplying the biomass per pixel value by the individual total pixel area. In addition, due to the physiological link between body-mass or length and various metabolic rates (*e.g.*, respiration, feeding), the information collated will be more easily translatable into estimates of processes and functions using allometric relationships published in the literature.

Data needs: The ZooScan tool generates data and so has no data pre-requisites. **The use of the** ZooScan and Ecotaxa require training and expertise in the instrument and imagery analysis. The tool uses standard benthic samples collected from monitoring or research surveys, preserved and then sorted, identified, counted and weighed at species/taxon level. Benthic macroinvertebrates are routinely stored for several years, preserved in an ethanol mixture, following the completion of reports and outputs associated with the original purpose for their collection. These faunal residues are ideally suited for application of the ZooScan protocol allowing for collection of additional information (at the individual level for each taxon). This tool is best suited to small, robust taxa as those taxa generally remain intact during the extraction and identification process. However, the tool allows for definition of size-to-biomass relationships and “reconstruction” individuals offering promising avenue for accurately estimating fragile taxa.

Key references: Cranford *et al.* (2011). Bivalve filter feeding: variability and limits of the aquaculture biofilter. In Shumway, S.E. ed. *Shellfish aquaculture and the environment*. John Wiley & Sons. Gorsky *et al.* 2010. Digital zooplankton image analysis using the ZooScan integrated system. *Journal of Plankton Research* 32: 285-303. Jones *et al.* (1992). Gill dimensions, water pumping rate and body size in the mussel *Mytilus edulis*. *Journal of Experimental Marine Biology and Ecology* 155: 213 – 237. Picheral *et al.* (2017). EcoTaxa, a tool for the taxonomic classification of images: http://ecotaxa.obs-vlfr.fr

MARBEFES Project has received funding from the European Union’s Horizon Europe research and innovation programme under grant agreement no 101060937 and UKRI under Grant Agreements 10040216, 10048815 and 10041354’

Deriving Faunal Function from Benthic Samples using the ZooScan - an Automated Imaging System

Example of implementation: The protocol was tested for deriving faunal function with the ZooScan from benthic infaunal data and specimens from two study sites (Norfolk banks, North Sea, and Cornish Peninsula, Celtic Sea). Specimens from both surveys had been separated by taxon and location, were stored in vials following sample processing and were of known biomass (wet weight in grammes). The protocol was employed to collect individual size-based measurements from a range of benthic macroinvertebrates by estimating biomass records from the 2D surface area of the scanned individuals (Figure 4a). Taking amphipods as an example, biomass distributions were assessed in samples from different sediment types to investigate associations between body size and habitat type (Figure 4b). Amphipod body-mass from the two study sites tended to be slightly right skewed across all habitats (mean values were greater than median values), this meant that the average was influenced by a few large individuals rather than an equal contribution across the size spectrum. The biomass distribution of amphipods within the mud/sandy mud had a bimodal distribution, with the mud containing the largest individuals (by weight). This would not have been detected with standard benthic samples. These measures were further used to estimate metabolic rates such as respiration or bivalve filtration rate allowing for turning strictly structural metrics into more meaningful metabolic values more appropriate to evaluate ecosystem processes allowing for an overall more accurate estimate of seabed functioning.

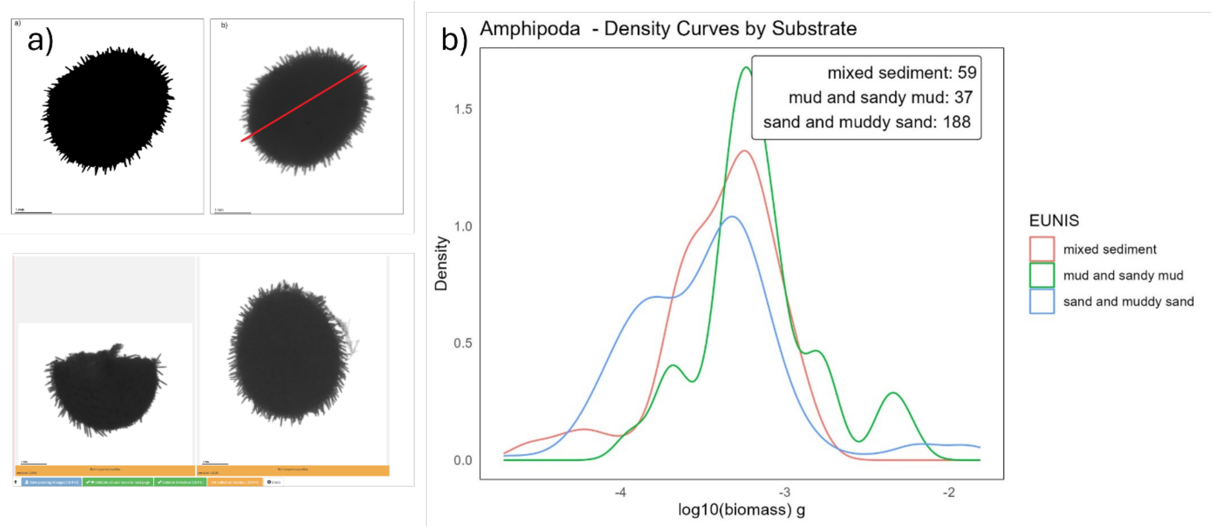

Figure 4 *a) scanned individual of the pea urchin* Echinocyamus pusillus *showing the silhouette used to determine pixel area, and the maximum length of the same individual. Scale bar = 1 mm. Ecotaxa allows the marking of incomplete or damaged individuals. b) density plots of body size for Amphipoda across three substrates showing the potential for assessing variation across different benthic habitats.*

Multi-Scale Trait-Based Approaches Linking Biological Structure to Ecosystem Function	**
Tool focus:	Biodiversity, Ecosystem components, Ecosystem function
Tool type:	Stepwise data analysis guidance, primer on trait-based approaches
Tool category:	Trait-based approaches
Main users:	Scientists, planners (with more advanced knowledge of data analysis)

Tool description: Biodiversity is often assessed by the number of species and their abundance or biomass, using indices of *e.g.* species richness or diversity. However, in recent decades a need for an improved insight on how the ecosystem is functioning, or the functional roles of species, have led to the development of different types of trait-based approaches (TBAs). Common for all of these are the use of traits, or characteristics of an organism. A trait is defined here as any morphological, physiological, life-history or behavioural characteristic measurable on an individual level, such as size, longevity, feeding or movement type. In trait-based approaches that focus on assessing biodiversity patterns in space and time, the trait(s) of an organism is(are) often combined with the density of the organism, with assessments often conducted on a community level. The “Multi-scale trait-based approach to link biological structure to ecosystem function” tool (Multi-scale TBA tool) was developed to (i) advance such trait-based approaches across taxonomic (*e.g.*, benthic organisms, fish, phytoplankton) and organisational scales (*e.g.*, species, communities, habitats, seascapes), and to (ii) provide step-wise guidance, concrete examples and code of analysis, as well as a primer on trait-based assessments. It was also developed with the potential to support, for example, identification of functionally important areas, and informing marine spatial planning and management in coastal areas regarding functional patterns.

Data needs: Biological community data (abundance or biomass or presence/absence) for an ecosystem component, such as invertebrates, fish, macrophytes, or phytoplankton, trait data for the included organisms, and depending on the analysis of interest, habitat data in the study area (*e.g.*, EUNIS habitat information). The level of expertise needed to apply the tool includes knowledge in preparation and data analysis of marine biological community data, basic knowledge of GIS (*e.g.*, ArcGIS or QGIS), and basic knowledge in the R programming language. These are skills primarily possessed by scientists, experts or marine planners. However, the accompanying primer on trait-based approaches also provides guidance on why and how to apply trait-based approaches on a more general level.

Key references:

de Juan *et al.* (2022). Biological traits approaches in benthic marine ecology: Dead ends and new paths. *Ecology and Evolution* 12:e9001.

Frelat *et al.* (2022). Tutorial for analysing trait-environment relationships, Zenodo, 10.5281/zenodo.6712534 and https://github.com/rfrelat/TraitEnvironment;

MARBEFES Project has received funding from the European Union’s Horizon Europe research and innovation programme under grant agreement no 101060937 and UKRI under Grant Agreements 10040216, 10048815 and 10041354’

Multi-Scale Trait-Based Approaches Linking Biological Structure to Ecosystem Function

Example of implementation - Assessment of functional differences of EUNIS habitats: In this example of the Multi-scale TBA tool, the aim is to assesses macrofaunal functional (trait) diversity across habitats in the Archipelago Sea in the Northern Baltic Sea (Figure 5). The widely used pan-European EUNIS https://eunis.eea.europa.eu/ habitat classification system was used, but any habitat information or classification can be applied. The assessment allows for a spatial representation of functional (trait) diversity (Rao's Q diversity index) (Figure 5A). In this case, there is a gradient from inner to the outer archipelago, with differences in functional diversity between habitats in both shallow (infralittoral) and deeper (circalittoral) areas and highest functional diversity in hard substrates (such as coarse sediments and rock) (Figure 5B). Such insights could be informative for assessing or monitoring changes in biodiversity on a larger seascape scale, or when *e.g.* planning marine protected areas.

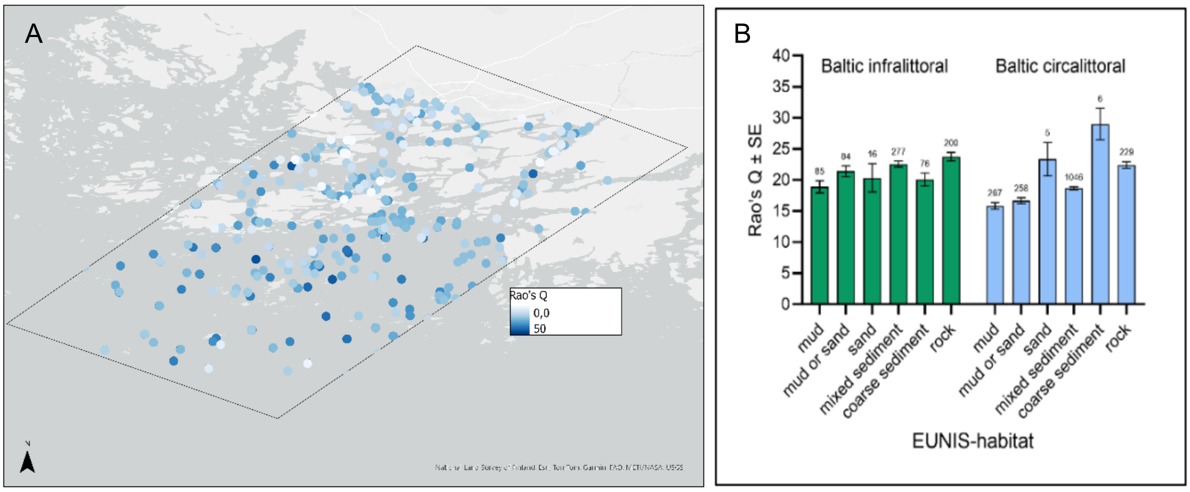

Figure 5 *Differences in functional diversity (Rao's Q) between EUNIS-level 3 habitats in an area in the Archipelago Sea, Northern Baltic Sea. A spatial representation of Rao's Q diversity index in the seascape is presented in A), with darker colour indicating higher mean values. Light grey denotes land areas. Rao's Q values across habitats and depth (infra- and circalittoral) are presented in B), with the number of data points per habitat above bars. Note that data are unbalanced across habitats.*

Example of implementation - Assessment of trait biogeographies across seascapes: Mapping out spatial patterns of individual functional traits across different taxonomic groups provides a powerful way to both investigate trait-environment relationships as well as to predict outcomes of how communities will respond to *e.g.,* climate or other environmental change. In this example of how the Multi-trait TBA tool can be implemented, a single trait within (or across) taxonomic groups is mapped in space, producing trait biogeographic maps across the seascape. Here, three key traits: size (small-large, cm), longevity (short-long, weeks-years) and mobility (no movement-movement >1km), with relevance to (i) ecosystem functioning (*e.g.*, production) and general ecological processes (*e.g.*, size structure, turnover, connectivity), and (ii) availability of trait information across several taxonomic groups (*e.g.*, benthos, fish, macrophytes, phytoplankton). In brief, the tool provides a stepwise analysis guidance on mapping out individual traits based on community data (community weighted means). The assessment advances trait-based approaches in that it allows for cross-taxonomic assessment as well as enables spatial visualisation of trait values by combining categorical information into a single, informative, value, after which spatial interpolations or more advanced spatial modelling can be applied.

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The outcome from this example of the Multi-trait TBA tool are biogeographic trait maps, illustrated here for macrofauna in a seascape in the Archipelago Sea in the Northern Baltic Sea (Figure 6). In this seascape, the macrobenthic community shows spatial heterogeneity in size across the seascape (Figure 6A), as well as in longevity although slightly more evenly distributed in space with longer lived species in both the inner and the outer parts (Figure 6B). Mobility, on the other hand, shows higher values along a north-south gradient and particularly in the outer archipelago (Figure 6C). This approach can further be evaluated for more in-depth trait-environment relationships, but already now allows for an improved understanding of large-scale biodiversity patterns, that can ultimately aid in biodiversity conservation, restoration planning, and management decisions.

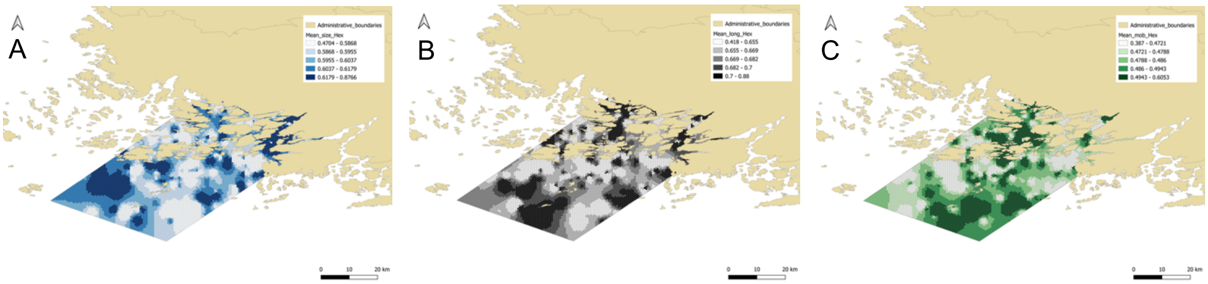

Figure 6 *Expression of individual traits A) size, B) longevity, and C) mobility in an area in the Archipelago Sea, Northern Baltic Sea for benthic macrofauna. Trait values represent combined community-weighted mean (CWM) values of five trait categories for each trait, after which inverse distance weighting (IDW) a simple interpolation has been applied. The darker the colour, the higher average CWM trait values. Not all categories are expressed in all communities.*

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Assessing Morphological Traits of Marine Invertebrates Related to Ocean Acidification and Climate Change Using Micro-Computed Tomography (Micro-CT) ***

Tool focus:	Ecosystem components, Ecosystem function
Tool type:	Equipment, 3D analysis from micro-CT data
Tool category:	Image-based approaches
Main users:	Scientists

Tool description: Micro-CT is an X-ray imaging technique that enables the visualization of both internal and external structures of a sample in three dimensions. The technique is based on generating a series of projection images by rotating a specimen positioned between an X-ray source and a detector. Owing to its non-destructive nature, micro-CT has become an important tool in taxonomic research, as it reveals taxonomically important internal structures in their original spatial context. Beyond taxonomy, micro-CT datasets allow for advanced 3D morphological analyses, including measurements of density, porosity, and structural thickness. Such quantitative traits can be proposed as novel numerical trait data for marine invertebrates, helping to address the gaps in the existing morphological data repositories and relevant literature. Moreover, these traits may also serve as "response traits," as they can reflect organismal response to environmental shifts induced by anthropogenic stressors such as climate change and ocean acidification. For instance, density and structural thickness of calcified structures can provide valuable insights into calcification processes and reef-building functions, while 3D porosity analysis can inform estimates of the filtration capacity in marine invertebrates such as sponges.

Data needs: The micro-CT tool generates data and so has no data pre-requisites. The use of micro-CT requires advanced expertise for both instrument operation and 3D data analysis. Furthermore, specimen preparation procedures also need a high level of technical skill, as the use of contrast agents is often necessary to enhance tissue contrast due to the inherent low X-ray absorption of many biological materials. Moreover, since the micro-CT protocol produces large image-based datasets, substantial storage capacity and powerful computational resources are essential for efficient processing and analysis.

Key references:

Byrne & Fitzer (2019). The impact of environmental acidification on the microstructure and mechanical integrity of marine invertebrate skeletons. *Conservation Physiology* 7: coz062

Vernadou *et al.* (2025). Micro-CT morphological traits for two invertebrate species in 2023. Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Center for Marine Research.
https://marineinfo.org/doc/dataset/8885
https://www.vliz.be/en/imis?module=dataset&dasid=8885
https://marinedataarchive.org/archive.php (MARBEFES/WP3/HCMR/)

MicroCT vlab (https://microct.portal.lifewatchgreece.eu/) A virtual laboratory which was created in the framework of the LifeWatchGreece project (https://www.lifewatchgreece.eu/) and offers virtual galleries and online tools for the 3D manipulation of the micro-CT datasets.

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Assessing Morphological Traits of Marine Invertebrates Related to Ocean Acidification and Climate Change Using Micro-Computed Tomography (Micro-CT)

Example of implementation: Trait-based approaches provide a valuable framework for the characterization of biological communities in response to anthropogenic stressors, such as climate change and ocean acidification. Within the MARBEFES project, selected morphological traits were investigated to assess their potential as key indicators for these impacts. Previous studies have shown that several morphological traits, including the density and thickness of calcified structures and the porosity of marine invertebrates, may be strongly influenced by increased temperature or low pH conditions (for a comprehensive review, see Byrne & Fitzer, 2019).

To assess the effects of climate change on marine invertebrates, individuals of the marine sponge *Chondrilla nucula* Schmidt, 1862, and the gastropod *Hexaplex trunculus* (Linnaeus, 1758) were collected from the Gulf of Heraklion and subjected into climate change experiments. Micro-CT scans were performed with a SkyScan 1172 micro-tomograph (Bruker, Kontich, Belgium) at the Hellenic Centre for Marine Research (HCMR). Sponges were dried with Hexamethyldisilazane (HMDS) and then scanned without any scanning medium at a voltage of 60 kV, a current of 167 µA and at a pixel size of 2.97 µm without filter. Gastropods were not stained with any contrast agent, and scans were performed at a voltage of 100 kV, a current of 100 µA and at a pixel size of 13.79 µm with a combined aluminium and copper filter. Projection images were reconstructed into cross-sections using SkyScan's NRecon software (Bruker, Kontich, Belgium). 3D analysis was performed on the reconstructed images using the CT Analyser software (CTAn, Bruker, Kontich, Belgium). From the reconstructed datasets, the following morphological traits were quantified: relative **density** (calculated as the mean grey scale values), 3D **structure thickness**, **separation** (thickness of pores), and **total porosity** (percentage of open and closed pore volume relative to the total volume of interest). These numerical values were compiled into a dataset designed to support future monitoring efforts. Examples of this analysis are illustrated in Figure 7. These results demonstrate that the micro-CT can effectively measure morphological traits sensitive to climate change and ocean acidification.

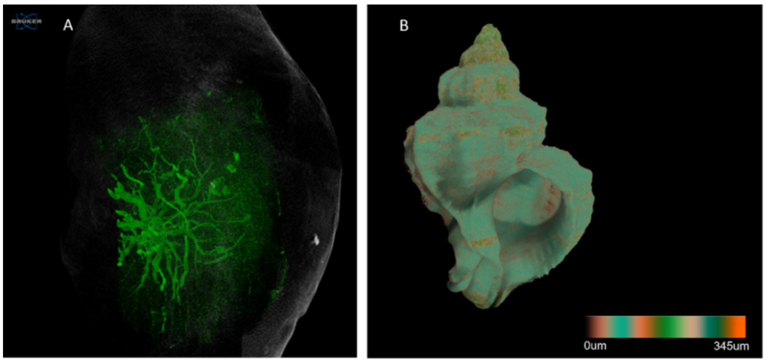

Figure 7 *Volume renderings of (A) the pore canal system of the sponge* Chondrilla nucula *and (B) structural shell thickness of the gastropod* Hexaplex trunculus.

Micro-CT has proven to be a powerful non-destructive tool for quantifying morphological and functional traits in marine invertebrates, offering new opportunities for taxonomic, ecological, and trait-based research. By providing accurate 3D measurements of density, porosity, and structural thickness, this technique can generate numerical trait datasets that complement traditional morphological information and improve our ability to detect organismal responses to environmental stressors, including climate change and ocean acidification. Looking forward, integrating micro-CT–derived traits into long-term monitoring and ecosystem assessment frameworks may strengthen our capacity to identify early warning signals of environmental stress and to better understand the functional roles of marine invertebrates in changing oceans.

Metric of Habitat Function *

Tool focus:	Biodiversity, Ecosystem functioning, Ecosystem services
Tool type:	Conceptual model, GIS, map of functions
Tool category:	Trait-based approaches
Main users:	Scientists

Tool description: The Metric of Habitat Function (MHF) is designed as a spatially explicit tool to assess and visualize the capacity of benthic habitats to sustain key ecosystem functions across the seascape. By providing a standardized scale to assess the potential contribution of different habitats to ecological processes—such as nutrient cycling, primary production, bioturbation, or carbon storage—the MHF establishes a common framework to evaluate ecosystem functioning at the seascape. Its purpose is to generate a comparable and scalable measure that informs conservation prioritization based on the potential of each habitat to support ecological functions. The MHF adopts an adaptive approach that initially relies on expert knowledge while progressively incorporating empirical and trait-based data to strengthen mechanistic links between habitats, functions, and the services they provide.

Data needs:

- Habitat data: EUNIS 2022 maps (≥Level 2).
- Environmental data: Marine Strategy Framework Directive (MSFD) indicators or other spatial assessments of ecological status.
- Functional data: Expert-derived functional scores, literature-based function–habitat linkages, and empirical measures (*e.g.*, bioturbation, carbon fluxes, habitat complexity indicators).
- Spatial framework: High-resolution (250 m) hexagonal grid for GIS integration.

Additional biological and environmental datasets enhance accuracy and enable scaling from expert-based to data-driven assessments.

Key references:

de Juan *et al.* (2015). Standardising the assessment of Functional Integrity in benthic ecosystems. *Journal of Sea Research*, *98*, 33-41.
de Juan *et al.* (2022). Biological traits approaches in benthic marine ecology: Dead ends and new paths. *Ecology and Evolution*, *12*(6), e9001.
Lavorel *et al.* (2017). Pathways to bridge the biophysical realism gap in ecosystem services mapping approaches. *Ecological Indicators*, *74*, 241-260.
Townsend & Lohrer (2019). Empirical validation of an ecosystem service map developed from ecological principles and biophysical parameters. *Frontiers in Marine Science*, *6*, 21.

Metric of Habitat Function

Example of implementation: The Menorca Channel serves as a case study for applying the MHF, setting an example for the transition from conceptual development to practical implementation. The workflow follows a standardized, stepwise procedure (Figure 8):

1. **Level 0 – Mapping:** Benthic habitats are mapped onto a 250 m hexagonal grid. For the study area, habitat data from previous projects provide high-resolution maps that underpin this activity.
2. **Level 1 – Functional Scoring:** Expert-based scores are assigned to each habitat-function pair.
3. **Level 2 – Weighting:** Not applied in this case, as the area is in uniformly good condition.
4. **Level 3 – Integration:** Scores are normalized and integrated into a composite MHF layer.

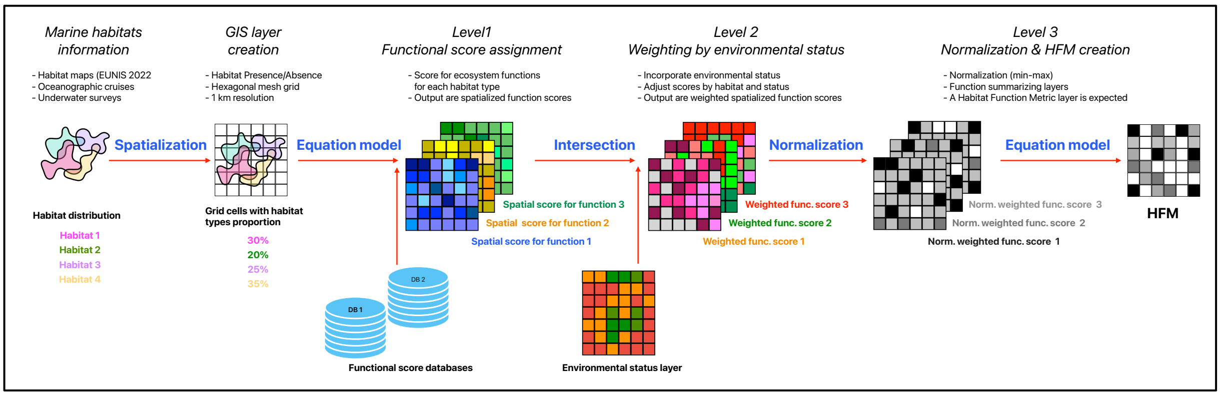

Figure 8 *Workflow chart for applying the Metric of Habitat Function in seascapes*

At the initial stage, the MHF relied entirely on expert knowledge in the assignment of functional scores to the habitats within the area. Each habitat was evaluated against a standardized list of ecological functions (extracted from Hinz *et al. unpublished*) that included primary production, nutrient cycling, carbon storage, and structural habitat provision. Functional importance scores were assigned based on each habitat's perceived capacity to support these processes, using the highest-scoring habitat as reference. The ecological condition across the Menorca Channel was assumed to be good, supported by its designation as a *Lugar de Importancia Comunitaria (LIC)*, which ensures protection from physical disturbance—the main pressure affecting seafloor integrity in this area. No additional anthropogenic impacts were identified, so no environmental weighting was applied to the MHF results. Experts identified seven key functions, for which scores were aggregated and normalized to generate the composite MHF (Figure 9). The metric can also be disaggregated to show the contribution of individual ecological functions across the seascape.

MARBEFES Project has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement no 101060937 and UKRI under Grant Agreements 10040216, 10048815 and 10041354'

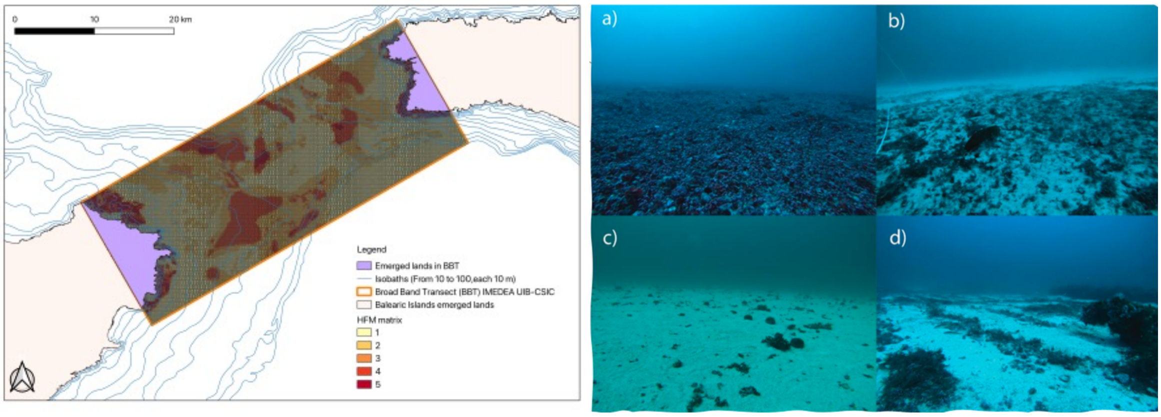

Figure 9 *Metric of Habitat Function in the Menorca Channel. The potential functions provided by habitats (right) are scaled and aggregated to produce a normalized measure of functional potential across spatial units in the area.*

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Biogeochemical Modelling: Linking Marine Physics, Chemistry, and Biology

**

Tool focus:	Ecosystem components, Ecosystem function, Ecosystem services
Tool type:	Numerical model
Tool category:	Biogeochemical approaches
Main users:	Scientist/modelling specialists

Tool description: Biogeochemical modelling is an approach for understanding how physical, chemical, and biological processes interact in oceans, lakes, and coastal areas, focusing on the **lower trophic food web**, including phytoplankton, zooplankton, and benthic fauna. The models are based on empirical mathematical relationships between nutrient concentrations, light levels, phytoplankton growth and faunal consumption and usually rely on computer solution of the resulting differential equations. These models help scientists to provide policymakers with predictions of changes in water quality, carbon cycling, and nutrient dynamics, that can apply to fisheries management, assessing climate impacts and the effect of changes in nutrient inputs.

What Can Biogeochemical Models Do?

- **Simulate Complex Interactions:** Models can represent how nutrients (like nitrogen and phosphorus), carbon, and oxygen move through water and sediments, and how these interact with lower trophic level organisms (*e.g.*, plankton, bacteria) and physical forces (*e.g.*, currents, temperature).
- **Support Decision-Making:** They are used to forecast the effects of climate change, pollution, or management actions (like reducing nutrient inputs) on marine and freshwater systems.
- **Upscale Observations:** By integrating field data, models can fill gaps in monitoring, helping to interpret trends and predict future conditions.

What Do They Not Generally Do?

- **Human activities.** Generally, these models do not include a representation of direct human pressures such as trawling, or the construction and operation of offshore and coastal infrastructure. The models can sometimes be combined with other types of models to include a representation of these processes.
- **Biodiversity.** These models generally do not include species level information that would allow diversity to be assessed (the models are generally built with lower trophic level functional groups rather than individual species).
- **Fish stocks and higher trophic levels.** Biogeochemical models generally simulate the lower trophic levels although some studies have coupled them to fisheries models.
- **Effect of pollutants on biota.** Although concentrations of pollutants can be predicted, biogeochemical models do not generally include their interaction with biota. Again, this could be added if studies of the effect of the pollutant on the model ecosystem components were available.

- **1D Models** focus on changes over time at a single location, typically representing vertical processes in the water column (from surface to seabed). They are computationally efficient and useful for studying detailed processes at specific sites, such as how oxygen or nutrients change with depth.
- **3D Models** simulate processes across large areas and at multiple depths, capturing spatial variability and complex interactions between regions. 3D models allow an understanding how currents, tides, and geography affect the distribution of nutrients, plankton, and pollutants across entire seas or coastal zones.

Data needs:

- **Setting up and running biogeochemical models requires a wide range of data including physical data** (temperature, salinity, currents, and mixing rates, often from oceanographic surveys or monitoring buoys), **chemical data** (concentrations of nutrients, oxygen, carbon, and other key elements in water and sediments), **biological data** (information on plankton, bacteria, and other organisms that drive biogeochemical cycles).

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For 3D models, high-resolution spatial data and boundary conditions (e.g., river inputs, atmospheric deposition) are needed, as well as large datasets for calibration and validation. Biogeochemical modelling is multidisciplinary and requires:

- **Understanding of Biogeochemical Processes:** Knowledge of how nutrients and carbon cycle through ecosystems, and how these are affected by physical and biological factors and how to represent these in mathematical form.
- **Numerical and Computational Skills:** Ability to work with complex software, set up simulations, and interpret model outputs,
- **Data Handling:** Skills in managing, processing, and quality-controlling large datasets.
- **Model Calibration and Validation:** Experience in comparing model results to observations and adjusting parameters to improve accuracy.
- **Collaboration:** Often, modelling teams include specialists in oceanography, ecology, chemistry, and computer science.

Key references:

Butenschön *et al.* (2016). ERSEM 15.06: A generic model for marine biogeochemistry and the ecosystem dynamics of the lower trophic levels. *Geoscientific Model Development*, 9: 1293–1339.

Fennel *et al.* (2006). Nitrogen cycling in the Middle Atlantic Bight: Results from a three-dimensional model and implications for the North Atlantic nitrogen budget. *Global Biogeochemical Cycles*, 20: 1–14.

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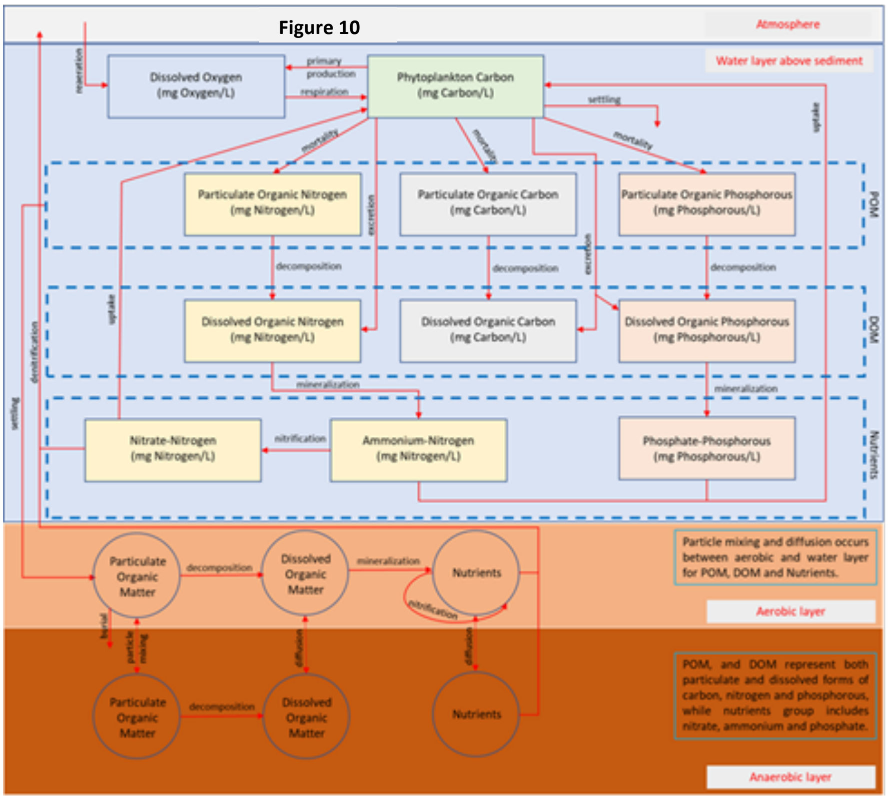
Biogeochemical Modelling: Examples of implementation

The Curonian Lagoon, Lithuania: This study used a 2-D configuration of a Python-based, mechanistic, and computationally efficient modelling tool for simulating primary production and analyzing eutrophication dynamics, EUTROPY (Kaynaroglu *et al.*, 2025). The pelagic compartment consists of 11 state variables (one biotic and 10 abiotic), while the sediment compartment is divided into two layers, as an upper aerobic layer and a bottom anaerobic layer and consists of 18 abiotic state variables (Figure 10). EUTROPY is openly accessible via GitHub (https://github.com/kaynarob/EUTROPY) and is developed for its potential integration into a decision support system for the end users.

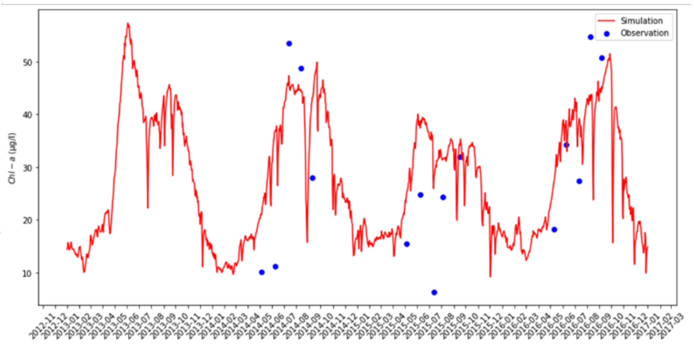
Figure 10



The Curonian Lagoon simulation with the EUTROPY model was performed for the period 2011–2017, leaving the first two years as the model spin-up period. Chlorophyll-*a* concentrations, a key water quality indicator for assessing eutrophication dynamics, were derived from phytoplankton carbon. The chlorophyll-*a* simulations over five years corresponded well with the three years of measured data from box 19 (Figure 11).

EUTROPY boxes

Figure 11



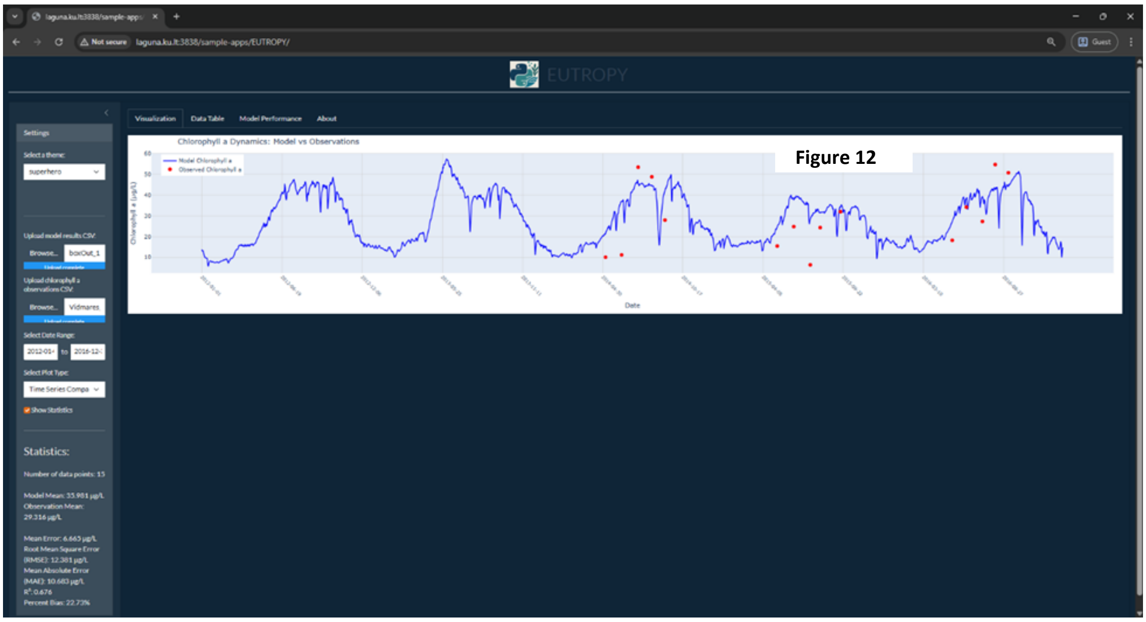


Figure 12

Settings

Select a theme: superhero

Upload model results CSV: boxOut_1

Upload chlorophyll a observations CSV: Vidmares

Select Date Range: 2012-01- to 2016-12-

Select Plot Type: Time Series Compa

☑ Show Statistics

Statistics:

Number of data points: 15

Model Mean: 35.981 µg/L
Observation Mean: 29.316 µg/L

Mean Error: 6.665 µg/L
Root Mean Square Error (RMSE): 12.381 µg/L
Mean Absolute Error (MAE): 10.683 µg/L
R^2: 0.676
Percent Bias: 22.73%

Additionally, simulation results can be explored through a web-based graphical user interface, developed in Shiny for Python, available at http://laguna.ku.lt:3838/sample-apps/EUTROPY/ (Figure 12). This interface facilitates post-processing and is particularly useful for users with limited modeling or programming experience. Combining simulation and analysis within a single programming environment makes EUTROPY a practical tool for effectively assessing eutrophication dynamics across various aquatic systems.

Reference:
Kaynaroglu, B., Razinkovas-Baziukas, A., Idzelytė, R., Tiškus, E., Zilius, M., Mėžinė, J., Umgiesser, G., (2025) EUTROPY: A Python-based software optimized with Just-In-Time compilation for simulating eutrophication dynamics in aquatic systems. SoftwareX 32, 102430. https://doi.org/https://doi.org/10.1016/j.softx.2025.102430

Mediterranean Sea: This study is based on the POSEIDON operational model, built upon the 3-D coupled hydrodynamic–biogeochemical POM–ERSEM model, implemented at ~10 km resolution (1/10°) across the Mediterranean Sea (Tsiaras et al., 2024 and references therein). Apart from model predictions for the entire Mediterranean basin, POSEIDON provides in situ observations of the Greek Seas from fixed platforms, gliders, Argo floats, regular R/V visits and a Ferrybox system. The operational model results (meteorological, waves, sea level, hydrodynamics and ecosystems variables) are available at https://poseidon.hcmr.gr (Figure 13). The operational biogeochemical-ecosystem model variables, forecasted daily, include water column chlorophyll, inorganic nutrients, bacteria, mesozooplankton, phytoplankton, primary production and bacterial

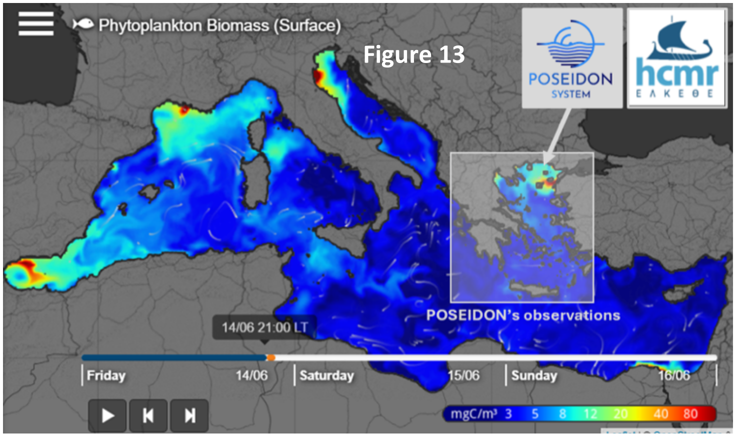


Figure 13

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production. The POSEIDON model has been further developed for several applications. Some examples from recent biogeochemical-ecosystem applications are presented below.

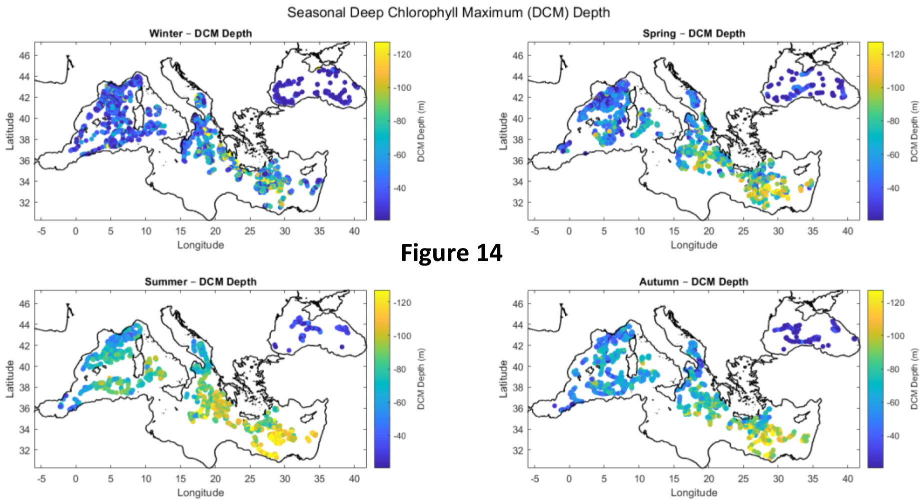

Figure 14

First, the ERSEM part of the POSEIDON model has been recently coupled with a carbonate system model (HALTAFALL) that enables the simulation of the spatiotemporal variability of seawater CO_2 partial pressure (pCO_2), oceanic CO_2 uptake, pH, and other carbonate system components (Tsiaras et al., 2024). The simulated spatial variability of carbonate system variables was validated with in situ carbonated system data (DIC, TA, pCO2), showing good agreement (except a slight overestimation of total alkalinity in the Eastern Levantine) that could allow thus to forecast carbonate system variables evolution.

The outputs ERSEM model have also been recently further calibrated and validated using data from Biogeochemical Argo (BGC-Argo) floats profiles collected in the Mediterranean since 2010, allowing improved prediction of the depth and intensity of the Deep Chlorophyll Maximum (DCM). The Argo BGC floats are equipped with sensors for chlorophyll, dissolved oxygen, nitrate, and pH. Figure 14 presents model outputs for the depth of the DCM. Comparison of model output with observations showed broadly consistent seasonal patterns between model and float data. Improvement in model parameterizations of nutrients and light, are currently being investigated to reduce model overestimates of surface chlorophyll and smoothing of vertical structure (Stamataki et al. 2025).

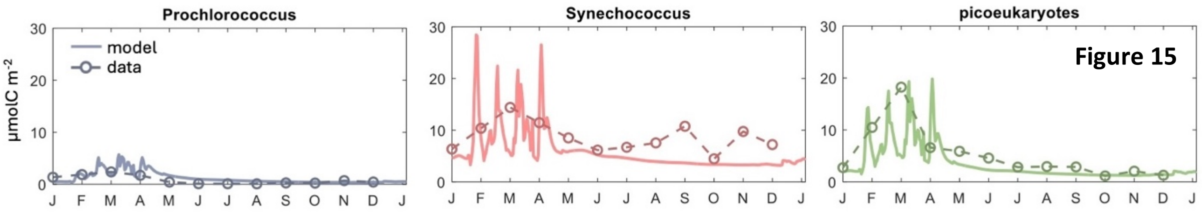

Figure 15

The ERSEM model has also been further developed to incorporate the three most abundant pico-phytoplankton functional groups in the Mediterranean Sea — *Prochlorococcus*, *Synechococcus*, and pico-eukaryotes in addition to the existing larger phytoplankton groups. A simulation of an annual cycle, compared to observations, is shown in Figure 15. The calibration process was guided by ecological theory, including the gleaner–opportunist trade-off and differences in photoprotection capacity among the groups (Tsakalakis *et al.* 2025). The model can be used to elucidate the mechanisms governing pico-phytoplankton dynamics and their resource competition. This model is currently being validated using data collected in the Aegean Sea.

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References:

Stamataki N., Tsakalakis I., Tsiaras, K. Frangoulis C., Petihakis G. (2025). Using Argo floats as validation tools of in-situ, satellite and model data. 8th Euro-Argo Science Meeting (23/09/2025)

Tsakalakis I., Stamataki N. Tsiaras K. Frangoulis C., Petihakis G. (2025). Pico-phytoplankton diversity, marine ecosystem function and services. ICES Annual Science Conference (15/09/2025), Klaipeda, Lithuania

Tsiaras, K., Frangoulis, C., Stamataki, N. (2024). Carbonate system variability in the Mediterranean Sea: a modelling study. *Frontiers in Marine Science*, 11: 1347990.

Examples of implementation

Irish Sea: This study used a configuration of the NEMO 3-D hydrodynamic model coupled to the ERSEM (European Regional Seas Ecosystem Model) biogeochemical model running at 7 km resolution on the European continental shelf and including the Irish Sea. ERSEM is very much at the higher end of complexity for this type of model with multiple phytoplankton, zooplankton, bacterial and benthic faunal groups and considering the full range of macro nutrients together with carbon in both organic and inorganic forms (Figure 16). The model requires a high-performance parallel processor computer system and simulation over multiple years will generally lead to very large (100 Gb/y) outputs containing, for example, 3D fields at daily intervals. The variables output and the time resolution can be adjusted (for example to minimise storage requirements). The number of potential output variables can be large.

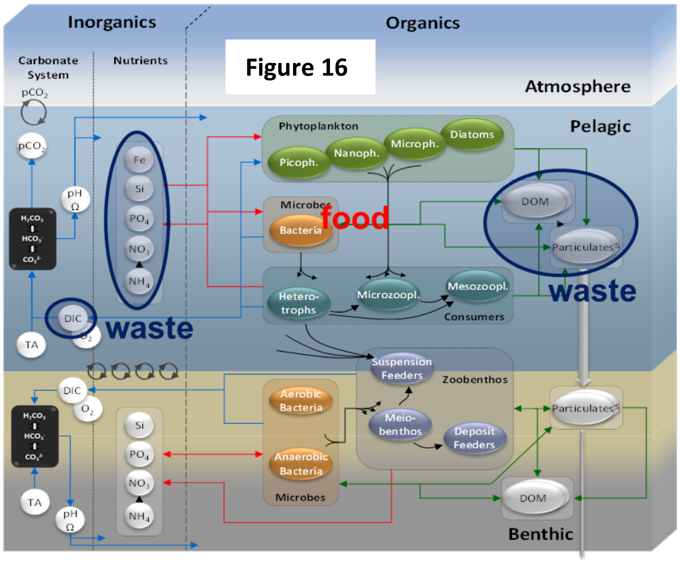

Figure 16

Figure 17 shows time series of daily chlorophyll concentration (summed over the 4 phytoplankton groups) over 2 years compared with observations. Clearly observed is the spring peak in concentrations typical of the region. Bespoke runs of the model could investigate the effect on chlorophyll concentrations of changes to anthropogenic nutrient inputs, for example.

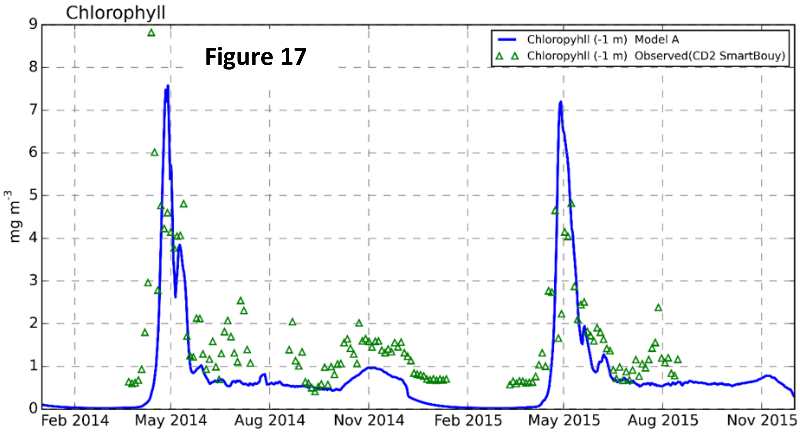

Figure 17

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Organic carbon accumulation in the seabed can also be simulated (Figure 3A). In the model this represents a temporary storage of carbon but also indicates locations that may sequester carbon over longer time scales. Comparison with observed sediment types (Figure 18) shows a correlation between model accumulation and muddier sediment types observed to contain higher organic carbon content than sand or gravel substrates.

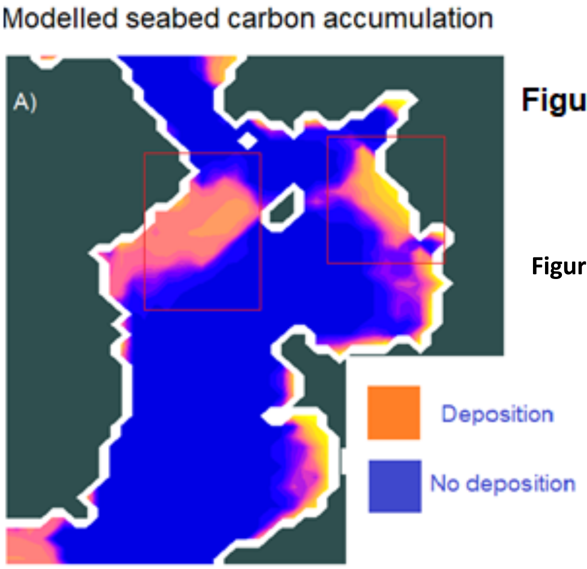
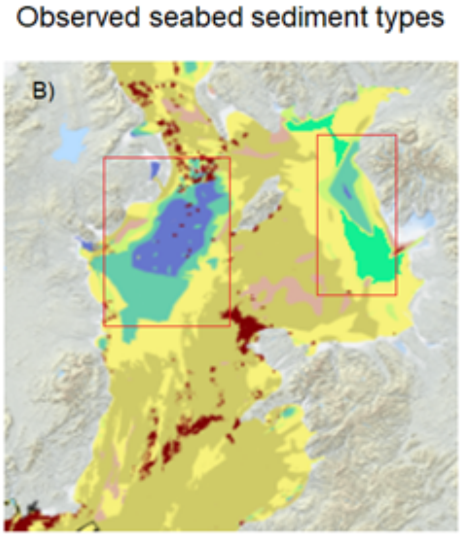


Figure 3:

Figure 18



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Modelling Ecosystem Service Dynamics Using Structural Equation Modelling **

Tool focus:	Ecosystem components, Ecosystem function, Ecosystem services
Tool type:	Statistical analysis process, Correlative (multivariate) model, R Environment
Tool category:	Network approaches
Main users:	Scientists

Tool description: This tool is based on two main principles: a causal path diagram and a Structural Equation Model (SEM) framework. The development of causal path analysis allows for the visual representation of the different cause-and-effect relationships among the environmental components from which ecosystem function and services emerge. They simplify the system by mapping how variables influence one another (*e.g.*, seabed type -> oxygen regime -> carbon recycling & storage -> climate regulation) and help identify direct, indirect and feedback effects that are difficult to detect. These diagrams rely on available information and first principles and represent testable hypotheses that can then be verified with available data using the SEM framework. SEM is a quantitative framework able to quantify and partition the relative direct and indirect pathways between multiple processes within a system. Some forms of the SEM approach consist of multiple sub-models within a global model, which allows for testing of direct and indirect effects within a causal path diagram to tease out the mechanisms driving ecosystem functioning and service provisioning. Once the causal path diagram is defined and the appropriate data formatted, the SEM models are fitted after checking for collinearity. The are refined and simplified iteratively, where necessary, until the model is determined to be an adequate representation of the data (determined by a statistical test). Pathways can be modified (added or removed) to fit the data better whilst retaining ecological realism. Equally statistically valid alternative models are compared, and the recurring parameters and consistent pathways are highlighted. Typical output represents a network of interactions going from the left (descriptors = environmental features) to the right (ecosystem function and services), positive (black) and negative (red) relationships are represented alongside the magnitude of effect (arrow thickness) and significance (stars).

Data needs: The design of the Causal Path Diagram requires no specific data as it is entirely conceptual. However, knowledge of or close consultation with experts is essential to consider all possible interactions of descriptors for a given ecosystem service. Parameterisation and running of SEM requires expertise in the approach and knowledge in a coding environment (*e.g.*, R). The tool is data greedy, and all components of the diagram must be supported by data to be tested appropriately, such that often a compromise between complexity and data availability is necessary. SEM works as a system of multivariate correlations so continuous data for each descriptor and the responses is essential. Although categorical data can technically be accommodated, they are generally dealt with through individual SEM versions for each data category separately.

Key references: Fan *et al.* (2016). Applications of structural equation modelling (SEM) in ecological studies: an updated review. *Ecological Processes*, 5. Grace *et al.* (2010). On the specification of structural equation models for ecological systems. *Ecological Monographs* 80:67–87. Lefcheck (2016). PiecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. *Methods in Ecology and Evolution* 7:573–579. Valdés *et al.* (2020) High ecosystem service delivery potential of small woodlands in agricultural landscapes. *Journal of Applied Ecology* 57:4–16.

Modelling Ecosystem Service Dynamics Using Structural Equation Modelling

Example of implementation: The tool was used here to describe carbon content within offshore sediment. Knowledge of the dynamics of organic carbon (OC) stock and sequestration in the offshore shelf seabed was used to draw the Causal Path Diagram. OC is controlled by several interacting variables: input from terrestrial sources (distance to coast), generation by carbon fixation (primary production, temperature) and seabed deposition. At or near the seabed, OC stock and sequestration rate are linked to local environment conditions. Sediment type (%fines) controls oxygenation (OPD) in the upper seabed layers, temperature controls carbon degradation rates and the faunal community (bioturbation) can mix organic matter to depths below the seafloor and control carbon degradation (remineralisation) rate.

Figure 19 visualises the service mechanisms. Carbon is directly influenced positively by an interplay between sediment type (mud) and temperature, whilst indirectly influenced positively by depth and temperature and negatively by hydrodynamics through their effects on mud. The model can also present some surprises like oxygen (OPD) and redox (aRPD) regime not influencing carbon - which suggests more that the effect of mud predominates rather than oxygen and redox not influencing carbon. This serves as a reminder that even though the tool has a causative baseline (causal path), it remains a correlative framework and therefore its outcomes need to be scrupulously appraised. The tool can be adapted to any service and integrated into a multi-service framework.

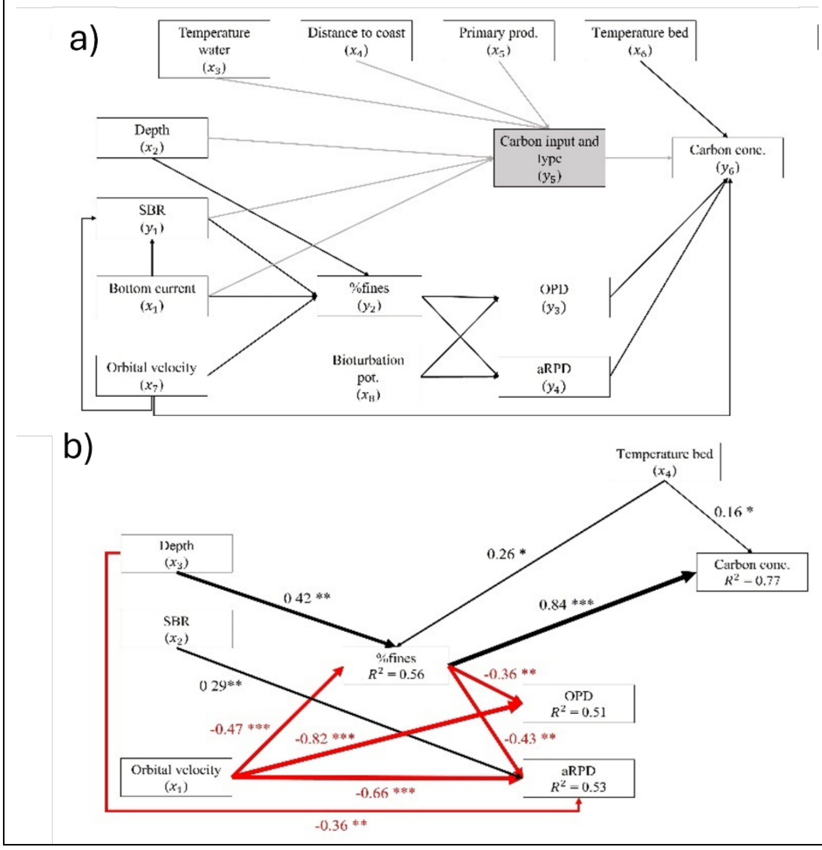

Figure 19 *The initial conceptual Causal Path Diagram a) and best fitting model for sedimentation rate (b) for carbon content; a) hypothesized pathways between variable (grey boxes/arrows indicating where a variable/pathways were removed due to data availability), b) coefficient estimates with arrow thickness representing effect size, positive (black) and negative (red) associations, and non-significant (dashed) and significant (solid) pathways. Significance is defined as* *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, *and* $\cdot\ p < 0.1$*).*

Assessing Food Web Structure and Function Using Ecological Interaction Network Approaches and Bioenergetic Modelling **

Tool focus:	Biodiversity, Ecosystem components, Ecosystem function, Ecosystem services
Tool type:	Application of graph theory, conceptual model
Tool category:	Network approaches
Main users:	Scientists

Tool description: Ecological interaction networks consist of nodes (or vertices) and links (or edges) representing species in a community and the interactions among them. The interactions can represent feeding links, or trophic interactions, describing "who eats whom" in the food web. Trophic interactions represent a dimension of biodiversity that is central to the functioning and stability of marine ecosystems. The organisation of the network – its topological structure – can be described and quantified using different metrics. These can either be network-level metrics, describing *e.g.* the number of links and the connectance of the network, or species-level metrics, such as generality and vulnerability, which are properties that show some of the functional roles of different taxa. The feeding links can be binary or weighted, *e.g.*, assigned an estimate of importance using bioenergetic modelling, taking advantage of allometric scaling laws to quantify metabolic rates. Interaction strength is here defined as energy flux, effectively a common currency of ecosystem functioning. Energy flux dynamics in networks elegantly link together biodiversity, structure, and ecosystem functioning, and many fluxes are further translatable to ecosystem services. In summary, flux-weighted interaction networks provide an approach with which one can examine shifts in biodiversity (species richness, composition, relative biomasses) and ecosystem functioning (flux of energy through the community) in changing marine ecosystems. The method has been upscaled for the spatial analysis of bioenergetic food webs and ecosystem functioning (see EcoFunMAP in D3.2, section *Food-web-informed cumulative effects assessment to evaluate ecosystem functioning across human-use scenarios*).

Data needs: For purely topological analyses (binary networks), the following data are needed: (i) taxa occurrences and (ii) a record of links among the taxa. The information on feeding links for defined communities is usually assembled in and available as so-called metawebs, *i.e.*, collections of interaction information derived from the literature, originally established through multiple approaches (feeding trials, observations, gut content analysis, DNA metabarcoding, stable isotopes and fatty acid analysis etc.). The bioenergetic modelling builds on these data and assigns weight to the interactions by quantifying the flux of energy between species. This is accomplished using information including biomasses or abundances of taxa, species metabolic rates, and the temperature in the environment. The approach and the analytical framework are presented in the *fluxweb* package available and well-supported in R, a free software environment for statistical computing and graphics. Hence, the approach is most accessible to those who are used to working with data in such environments. In addition, used for illustrative purposes, ecological interaction networks are valuable for a range of user groups.

Key references: Barnes *et al.* (2018) Energy flux: the link between multitrophic biodiversity and ecosystem functioning. *Trends in Ecology and Evolution* 33: 186-197; Delmas *et al.* (2019) Analysing ecological networks of species interactions. *Biological Reviews* 94: 16-36; Kortsch *et al.* (2021) Disentangling temporal food web dynamics facilitates understanding of ecosystem functioning. *Journal of Animal Ecology* 90: 1205-1216; Nordström & Bonsdorff (2017) Organic enrichment simplifies marine benthic food web structure. *Limnology and Oceanography* 62: 2179-2188.

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Assessing Food Web Structure and Function Using Ecological Interaction Network Approaches and Bioenergetic Modelling

Example of implementation (structure): Ecological interaction networks are here used to describe the spatial structuring of food webs in benthic communities encompassing basal resources and consumer communities consisting of zooplankton, benthic invertebrates, and fishes in the Finnish Archipelago Sea. The study area is shallow, characterized by a highly mosaic archipelago (>40 000 islands in the greater area) and brackish water conditions. It encompasses an environmental gradient with higher levels of nutrients, lower salinity, and lower visibility in the inner archipelago, shifting towards the middle archipelago zone and offshore. The Archipelago Sea food web metaweb used here contains 76 species and 595 links and was combined with species monitoring data to create 640 local networks.

The network size ranged between 21 and 50 species, whereas the number of links ranged between 81 and 333, connectance 0.12 and 0.20, and maximum trophic level between 3.41 and 3.87. Figure 20 shows networks for three soft/mixed-sediment sites from the inner, middle and outer zones of the Archipelago Sea. In the figure, the size of a node is relative to the number of incoming and outgoing links, displaying species differently connected to other taxa in the network. For these three local food webs, the highest complexity in terms of the number of species and the number of interactions was at the outer archipelago site.

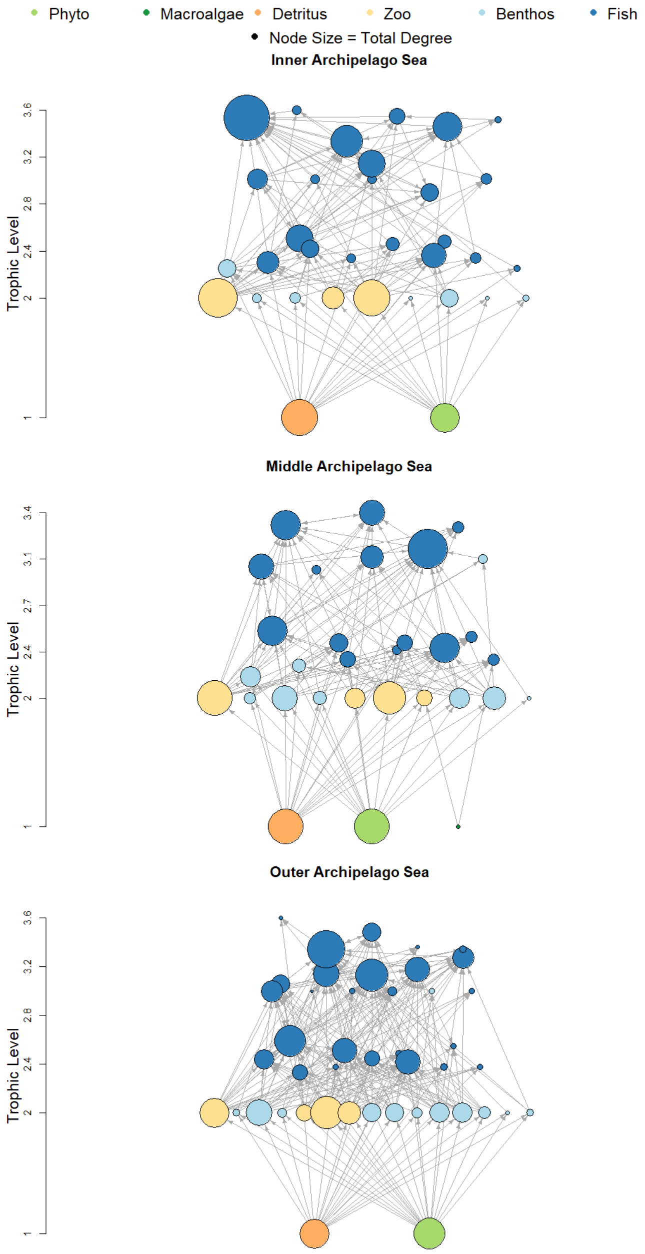

Figure 20 *Food web networks from the inner, middle and outer areas of the Finnish Archipelago Sea*

Assessing Food Web Structure and Function Using Ecological Interaction Network Approaches and Bioenergetic Modelling

Example of implementation (function): Ecological interaction networks are here used to describe the functioning of food webs in benthic communities encompassing basal resources and consumer communities consisting of zooplankton, benthic invertebrates, and fishes in the Finnish Archipelago Sea. The Archipelago Sea food web metaweb, as used here, contains 76 species and 595 links and was combined with species monitoring data to create 640 local networks. Using bioenergetic modelling, the interactions in the local networks were quantified to represent energy fluxes (kJ m^{-2} d^{-1}) among taxa. These energy fluxes represent analogous ecosystem functions, which in turn may correspond to ecosystem services, such as production (fish, plants), decomposition, or carbon storage or sequestration.

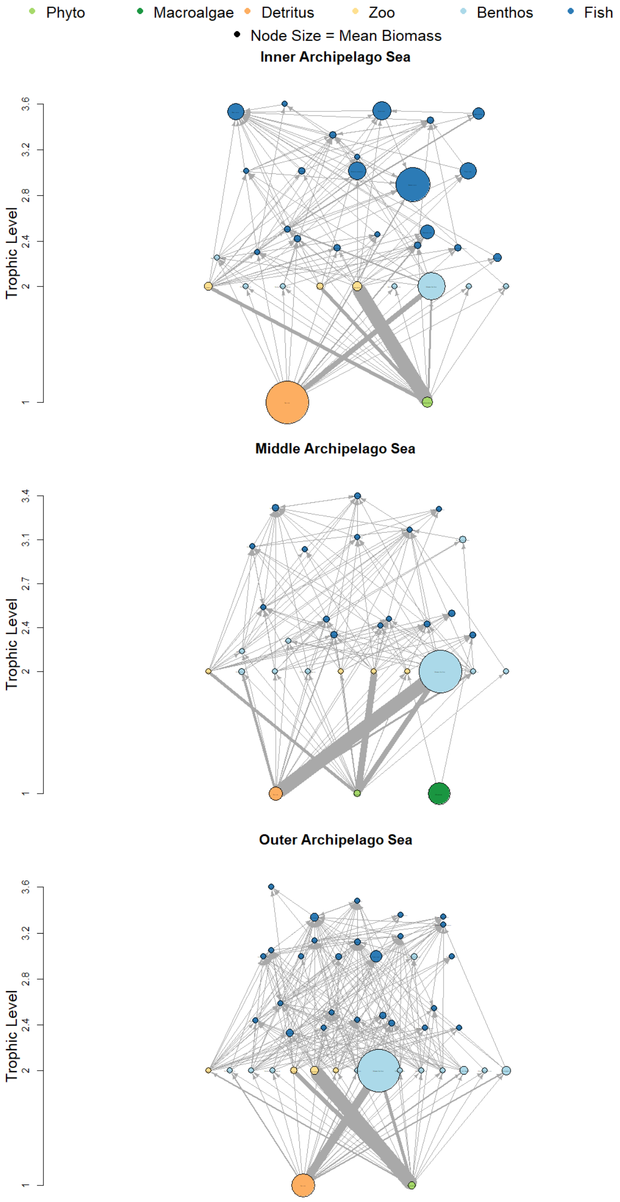

Figure 21 *Flux-weighted networks from the Archipelago Sea*

Using local-scale food web energy flux approaches, ecosystem functioning was quantified in benthic communities along the archipelago gradient. Figure 21 shows the weighted networks for three soft/mixed-sediment sites at 13-16m depth in the inner, middle and outer zones of the Archipelago Sea, respectively. In the figure, the size of a node is relative to species biomass (g wet weight m^{-2}). Using the summed energy fluxes for each trophic pathway, we can approximate how ecosystem functioning changes along the archipelago gradient. Total energy flux is similar in the inner and middle sites but increases at the outer site, suggesting higher overall system throughput offshore. Across sites, the relative contribution of trophic pathways shifts markedly:
the inner site is strongly dominated by planktivory (84.7%), whereas the middle area relies heavily on detritivory (68.3%) with a clear reduction in planktivory (30.1%). At the outer site, planktivory again becomes the primary pathway (73.7%) whereas detritivory decreases (25.6%), and benthivory and piscivory remain consistently minor throughout the gradient.

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5 Tool Integration

The tools in WP3 represent a diversity of approaches, not only in their focus on biodiversity, ecosystem structure, ecosystem functioning or ecosystem services, but also in how widely they can be used to understand the connections and linkages among these. This section of the deliverable reflects on the complementarity of the tools, to highlight ways in which these specialised approaches together offer comprehensive evidence of ecosystem properties. Figure 22 illustrates the span of the approaches across the focal areas. The illustration only relates to aspects of biodiversity, ecosystem functioning and ecosystem services, and should be considered together with its sister illustration from D3.2 on human multi-impacts.

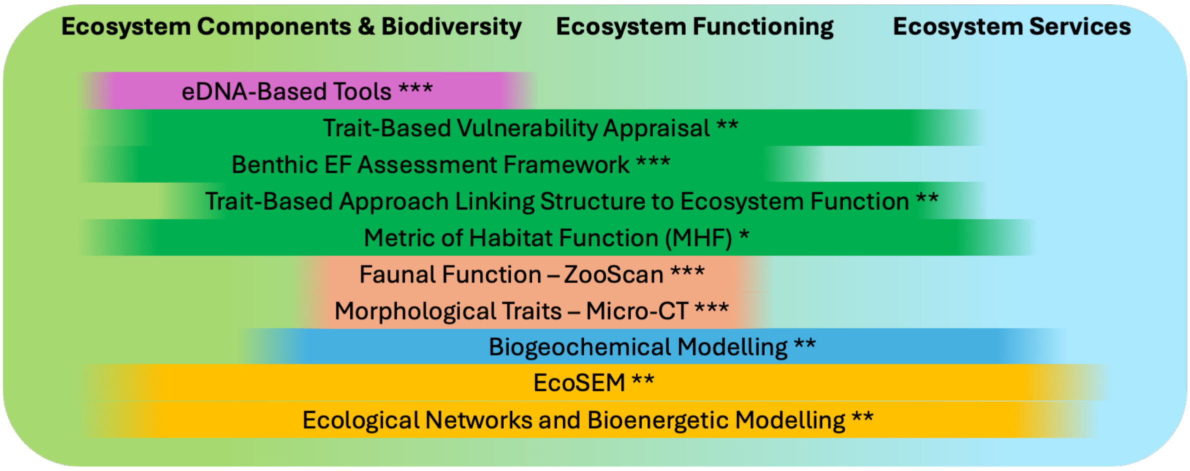

Figure 22 *Tools and their assigned category (trait-based approaches [green], network approaches [orange], physical-biogeochemical approaches[blue], molecular approaches [fuchsia], and imaging approaches [coral]). Note that tools are not necessarily restricted to a single category but, here, the most representative for the implementation was chosen. Stars indicate the level of possible stakeholder engagement and usability; * = direct, ** = semi-direct, and *** = indirect.*

Meeting the foundational need for broad knowledge acquisition identified by MARBEFES, the tools are proposed together to provide knowledge across different ecosystem components, from the physical and biogeochemical setting to marine life across multiple levels of biological organisation. Focusing on **assessments of biodiversity**, eDNA-based tools are highly complementary to many monitoring programs, offering information regarding the biodiversity across the seascape. Other approaches, such as ecological interaction networks, can incorporate this information. For example, food web assessments could combine monitoring data with complementary information from molecular approaches to determine the structure of the species community (*which species are present*), as well as to identify links (*who eats whom*) and, in some cases, quantify the interactions (*how much*). Apart from the eDNA-based approach, the selected tools meet around the purpose of **understanding ecosystem function** and aspects around quantifying functioning. At the centre of this focus, lies the trait-based approaches and the associated tools explored in WP3. These trait-based approaches include efforts to assess trait expression in ecological communities and individual trait biogeographies, trait-based vulnerability and relationships to the environment, as well as spatial representations of functioning at the habitat level. Contributing to these, the two imaging-based approaches exemplify ways through which intra-specific trait variability can be determined and quantified to subsequently feed into the other trait-based approaches, *i.a.*, ones assessing community trait diversity, focusing on vulnerability appraisal, or mapping habitat function. Together, the approaches thereby cover *acquiring trait information*, *using trait information* on individual, population, and in descriptions of functional diversity at community and habitat levels, as well as *understanding trait information* in relation to their use as proxies for ecosystem function and specific functional measurements obtained. In the same manner, although recognising that biodiversity and ecosystem functioning underpin and maintain **ecosystem services**, not all tools are intended for, or able to provide, assessments of ecosystem services provision. The metric of habitat function can use available information on ecosystem services in mapping the capacity of benthic habitats that participate in service provisioning. However, given the set of tools in this handbook, contributions to understanding and quantifying ecosystem services are mainly through network-based approaches, such as the ecological interaction networks, structural equation modelling and the physical-biogeochemical models. In general, the network-based approaches hold the greatest capacity to assess linkages across domains of biodiversity, functioning, and ecosystem service provisioning.

6 Conclusion

This Deliverable reports on the MARBEFES WP3 activities on biodiversity and ecosystem tools, specifically designed to assess biodiversity, ecosystem functioning and ecosystem services across the seascape. It forms the final deliverables from WP3 together with its sister document, the Handbook on Assessing Human Multi-impacts on Seascape Ecosystems. It was designed around themes spanning biodiversity and ecosystem structure, ecosystem function and ecosystem services, including ten (10) tools addressing all or a subset of these focal areas. The tools were further categorised by approach (trait-based approaches, network approaches, physical-biogeochemical approaches, molecular approaches, and imaging approaches), and the anticipated level of direct stakeholder engagement (from direct to indirect). In the tool section, each tool has been presented succinctly, and a worked example is provided to illustrate the type of outputs expected. The final section has provided an overview of how to use tools in an integrated manner complementing strengths and enabling tackling complex marine ecological issues across the seascape, with deeper insights into ecological processes.

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