Review of existing and emerging technologies for pelagic biodiversity observations

Palazo, Maialen; Artigas, Luis Felipe; Créa, Véronique; Boicenco, Laura; Børsen, Tom; Cabal, Ainhoa; Canals, Oriol; Casotti, Raffaella; Cenci, Alessandra; D'Alelio, Domenico; Innocentis, Sabrina De; Concetta Eliso, Maria; Faulkner, Rebecca; Fernandez-Salvador, Jose A.; Fonseca, Vera; Frangoulis, Constantin; Goñi, Nicolas; Grégori, Gérald; Haraguchi, Lumi; Harvey, Therese; Jakobsen, Hans H.; Karvine, Ville; Lavigne, Héloïse; Lilja, Juha; Metfies, Katja; Monchy, Sébastien; Pitois, Sophie; Righton, David; Sagarmínaga, Yolanda; Seddon, Rosalyn; Seppälä, Jukka; Thyssen, Melilotus; Stæhr, Sanjina Upadhyay; Van Der Kooij, Jeroen; Van der Zande, Dimitry; Vlas, Oana; Zambon, Tommaso

DOI (link to publication from Publisher):
10.5281/zenodo.14098959

Publication date:
2024

Document Version
Publisher's PDF, also known as Version of record

Review of existing and emerging technologies for pelagic biodiversity observations

OBAMA-NEXT

Co-funded by the European Union

UK Research and Innovation

Grant Agreement no. 101081642

Work Package	**2**
WP Co-Leader(s)	Véronique Créach (CEFAS) and Raffaele Siano (IFREMER)
Deliverable Title	Review of existing and emerging technologies for pelagic observation
Deliverable Number	2.1
Deliverable Beneficiary	
Authors	Maialen Palazot (CNRS), Luis Felipe Artigas (CNRS), Véronique Créach (CEFAS), Laura Boicenco (NIMRD), Tom Børsen (AAU), Ainhoa Caballero (AZTI), Oriol Canals (AZTI), Raffaella Casotti (SZN), Alessandra Cenci (AAU), Domenico D'Alelio (SZN), Sabina De Innocentiis (ISPRA), Maria Concetta Eliso (SZN), Rebecca Faulkner (CEFAS), Jose A. Fernandes-Salvador (AZTI), Vera Fonseca (CEFAS), Constantin Frangoulis (HCMR), Nicolas Goñi (LUKE), Gérald Grégori (CNRS), Lumi Haraguchi (SYKE), Therese Harvey (NIVA), Hans H. Jakobsen (AU), Ville Karvinen (SYKE), Héloïse Lavigne (RBINS), Juha Lilja (LUKE), Katja Metfies (AWI), Sébastien Monchy (CNRS), Sophie Pitois (CEFAS), David Righton (CEFAS), Yolanda Sagarmínaga (AZTI), Rosalyn Seddon (CEFAS), Jukka Seppälä (SYKE), Melilotus Thyssen (CNRS), Sanjina Upadhyay Stæhr (AU), Jeroen Van Der Kooij (CEFAS), Dimitry Van der Zande (RBINS), Oana Vlas (NIMRD), Tommaso Zambon (AAU)
Internal reviewer(s)	Philip Erm (UNEP-WCMC), Louise Forsblom (SYKE)
Dissemination level	
Submission date	22 May 2024
How to cite	Palazot, M., Artigas, L. F., Créach, V., Boicenco, L., Børsen, T., Caballero, A., ... & Zambon, T. (2024). OBAMA-NEXT Deliverable 2.1. Review of existing and emerging technologies for pelagic observation.

Title of project	Observing and mapping marine ecosystems – next generation tools
Instrument	HORIZON-CL6-2022-BIODIV-01-01
Contract number	101081642
Start date of project	1 December 2022
Duration	48 months
Project Coordinator	Jacob Carstensen

OBAMA-NEXT, OBSERVING AND MAPPING MARINE ECOSYSTEMS – NEXT GENERATION TOOLS (Grant Agreement: 101081642) project is funded by the European Union under the Horizon Europe programme (grant agreement no. 101081642). Views and opinions expressed are however those of the authors only and do not necessarily reflect those of the European Union or UK Research and Innovation. Neither the European Union nor the granting authority can be held responsible for them.

Table of Contents

List of figures

List of tables

Glossary

Platforms	Platforms on which the technologies presented in this deliverable can be implemented. These include: Satellites, Drones, Aerial platforms, ROV, AUV, Moorings, Floats, Fixed autonomous stations, Ships of Opportunity.
Ships of Opportunity	Volunteer commercial and research vessels.
Technology Readiness Level (TRL)	Estimated maturity of technologies. The TRL levels applied in this deliverable are: • 0 Idea • 1 Basic research • 2 Technology formulation • 3 Needs validation • 4 Small scale prototype • 5 Large scale prototype • 6 Prototype system • 7 Demonstration system • 8 First of a kind commercial system • 9 Full commercial application

Executive Summary

OBAMA-NEXT aims to develop a comprehensive toolbox that uses established and novel approaches to acquire accurate, cost-effective and precise data on marine ecosystems and biodiversity to support the effective management of marine ecosystems and the services they provide. Deliverable 2.1 aims to critically review and assess existing and emerging pelagic biodiversity monitoring methods. These methods include optical (bulk) measurements, flow cytometry (FCM), in vivo imaging (in flow or in situ), eDNA, acoustics, tagging/biologging, remote sensing and citizen science. The use of each method was specifically evaluated for monitoring the following pelagic organisms: bacteria, phytoplankton, microzooplankton, mesozooplankton, macrozooplankton/jellyfish/micronekton, pelagic fish and megafauna/marine mammals. The catalogue includes a general description of each method, followed by examples of its application to pelagic biodiversity monitoring through its deployment in different monitoring platforms. It also lists the Essential Biodiversity Variables (EBVs) and Essential Ocean Variables (EOVs) that can be addressed, as well as the advantages and limitations of each method. However, the conclusion highlighted the need to combine different technologies and traditional approaches to adequately address the different levels of variability in pelagic biodiversity in a changing ocean.

1. Introduction

1.1. Aim

The objective of task 2.1 is to review existing and emerging technologies for pelagic biodiversity observations. Deliverable 2.1 reviews the advantages, limitations, and the readiness of the technologies and the FAIRness (findability, accessibility, interoperability, and reusability) of the data for the observation of Essential Biodiversity Variables (EBVs), including their adaptability to platforms and their combination with Essential Ocean Variables (EOVs).

1.2. Approach

Eleven categories of sensors/methods/platforms/approaches were announced to be explored in the OBAMA-NEXT project for pelagic environment. It was proposed to subdivide them into six methods targeting seven categories of organisms that could be deployed on six different platforms according to three approaches, as shown in Figure 1. This deliverable provides detailed descriptions of all six methods and two approaches for monitoring pelagic target organisms, together with guidance on their effective implementation in monitoring platforms.

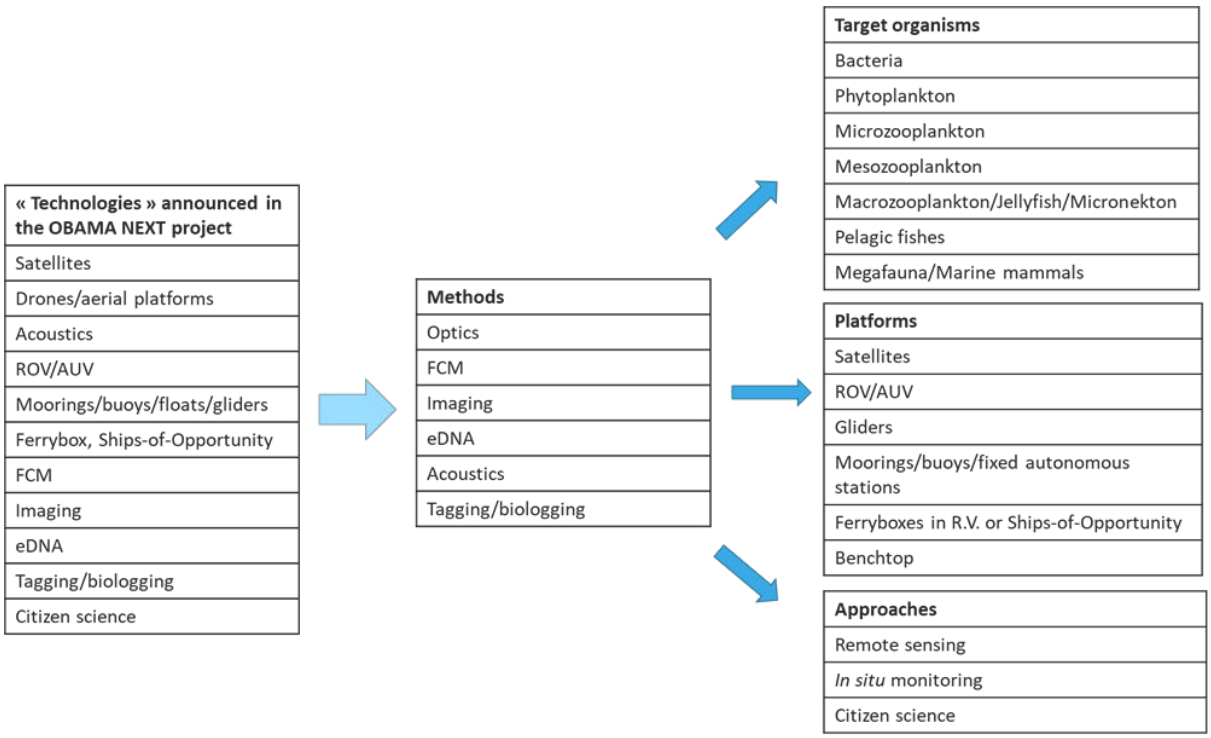

Figure 1: Proposed reorganisation of categories for T2.1.

Most of the OBAMA-NEXT scientists involved in pelagic biodiversity observations were involved in writing and commenting this deliverable. We have also drawn on work, results and findings from previous or ongoing sister

projects such as JERICO NEXT/JERICO S3 (www.jerico-ri.eu), TechOceans (https://techoceans.eu/), MARCO BOLO (https://marcobolo-project.eu/).

1.2.1. Technologies

Deliverable 2.1 focuses on the most commonly used and innovative methods for observing pelagic biodiversity, such as: optical measurements, flow cytometry (FCM), imaging, eDNA, acoustics, tagging/biologging and the remote sensing approach. Citizen science is separated from the other methods and approaches and has its own section. To avoid an excessively long list of sensors, it was decided to focus on emerging techniques and sensors for field measurements and to detail the platforms on which they can be deployed (e.g., underwater, on moorings, fixed autonomous stations, on vessels). Already well-established and automated analytical laboratory approaches as well as long-established techniques for pelagic observations are only mentioned and not considered in detail.

1.2.2. Target organisms

Deliverable 2.1 will focus on the following target organisms: bacteria, phytoplankton, microzooplankton, mesozooplankton, macrozooplankton/jellyfish/micronekton, pelagic fish and megafauna/marine mammals.

1.2.3. Essential Biodiversity Variables (EBVs) and Essential Ocean Variables (EOVs)

Essential Biodiversity Variables provide a common framework for monitoring efforts, like Essential Climate Variables (ECVs) for Climate Change (GCOS), but based on Essential Ocean Variables, which includes only phytoplankton and zooplankton observations. However, current efforts to monitor EBVs across the broad range of size and diversity of marine organisms using *in situ* observations are hampered by a paucity of data in the coastal and open ocean areas and the latency of information. For instance, the Ocean Biogeographic Information System (OBIS, 2024) has an uneven coverage of the open ocean, with better coverage in the North Atlantic than in the South Atlantic Ocean. It also has less biodiversity data in the open ocean and a 5-to-10-year lag between data production and delivery (Muller-Karger et al., 2018). Paucity of data, both in spatial coverage and in temporal frequency, is ultimately associated with the cost of data acquisition, remoteness of locations and difficulty of access (Muller-Karger et al., 2018). High-frequency monitoring of multidimensional data – from species' morphological and behavioural traits to species' abundances, distributions and interactions – will allow the implementation of a diverse set of indicators to assess changes at multiple levels of ecological organisation, fostering a hierarchical view of the mechanisms driving ecological change, as envisioned by the EBV framework (Kissling et al., 2018; Schmeller et al., 2017). Central to this framework is the notion that EBVs are a small set of variables which collectively capture biodiversity change at multiple levels of biological organization and spatial scales that are of scientific and management interest (Jetz et al., 2019; Valdez et al., 2023). Automated and semi-automated systems can provide both biological EOVs and EBVs such as those required by the MSFD regulation (Uusitalo et al., 2016). Therefore, we will evaluate how the different techniques presented in this deliverable

could provide data that meet both EBVs and EOVs requirements and contribute to improving our assessment of changes in marine pelagic biodiversity.

1.2.4. SWOT analysis

The information for each technology will be assessed using a strengths, weaknesses, opportunities, and threats (SWOT) approach. Strengths and weaknesses refer to internal factors or characteristics of the techniques described. The aim of their analysis is to evaluate how the use of these techniques achieves its goals for efficiency, research/data provision and development. Opportunities and threats, on the other hand, refer to external factors, the analysis of which aims to assess whether the use of these techniques can meet challenges (opportunities) in biodiversity assessment and prevent problems (threats) when faced with an uncontrollable (and unpredictable) external environment (including human decisions, economic issues and also technological issues) (Kaymaz Muhling, 2022).

Below are examples of the questions addressed by experts for each technology and the types of information summarised in the SWOT tables (Table 1).

Strengths questions:

- What do the scientists like about the technology?
- What does this technology do better than others?
- What are the most positive aspects of the technology?
- What is unique about this technology?

Weakness questions:

- What do the scientists dislike about the technology?
- What problems or complaints are often mentioned?
- Why is the technology not used?
- What are the most negative aspects?
- What are the biggest obstacles/challenges?
- What resources do other technologies have that the technology of interest does not have?

Opportunities questions:

- How can the technology be improved?
- How can the technology be promoted?
- How can new applications be identified?

Threat questions:

- What are the impacts of technological changes?
- Lack of suppliers?
- Restricted applications?
- Are there mandatory requirements?
- What is the confidence in the data?

Table 1: Examples of information summarised in the technology SWOT tables.

Internal factors	Strengths	Performance Unique and specific
Internal factors	Weaknesses	Inefficiency Competitivity Resources limitations (people's knowledge and cost)
External factors	Opportunities	Improvements Emergent needs
External factors	Threats	Suppliers Small market Lack of best practices for collecting and processing the data No change in ecological assessment

1.2.5. References

Jetz, W., McGeoch, M. A., Guralnick, R., Ferrier, S., Beck, J., Costello, M. J., ... & Turak, E. (2019). Essential biodiversity variables for mapping and monitoring species populations. *Nature ecology & evolution*, *3(4)*, 539-551.

Kaymaz Muhling S.M. (2022). Understanding the Strengths, Weaknesses, Opportunities and Threats (SWOT) of Marine Protected Area (MPA): A Brief Review. *Green Rep.*, *3(8)*, 31-34.

Kissling, W. D., Ahumada, J. A., Bowser, A., Fernandez, M., Fernández, N., García, E. A., ... & Hardisty, A. R. (2018). Building essential biodiversity variables (EBVs) of species distribution and abundance at a global scale. *Biological reviews*, *93(1)*, 600-625.

Muller-Karger, F. E., Miloslavich, P., Bax, N. J., Simmons, S., Costello, M. J., Sousa Pinto, I., ... & Geller, G. (2018). Advancing marine biological observations and data requirements of the complementary essential ocean variables (EOVs) and essential biodiversity variables (EBVs) frameworks. *Frontiers in Marine Science*, *5*, 373058.

OBIS. (2024). Ocean biogeographic information system. Intergovernmental Oceanographic Commission of UNESCO. www.iobis.org.

Schmeller, D. S., Mihoub, J. B., Bowser, A., Arvanitidis, C., Costello, M. J., Fernandez, M., ... & Isaac, N. J. (2017). An operational definition of essential biodiversity variables. *Biodiversity and Conservation*, *26*, 2967-2972.

Uusitalo, L., Fernandes, J. A., Bachiller, E., Tasala, S., & Lehtiniemi, M. (2016). Semi-automated classification method addressing marine strategy framework directive (MSFD) zooplankton indicators. *Ecological Indicators*, *71*, 398-405.

Valdez, J. W., Callaghan, C. T., Junker, J., Purvis, A., Hill, S. L., & Pereira, H. M. (2023). The undetectability of global biodiversity trends using local species richness. *Ecography*, *2023*(3), e06604.

2. Catalogue of existing and emerging methods for pelagic observation

2.1. Optical measurements

2.1.1. General overview

Optical *in situ* and *in vivo* techniques have been deployed for over 50 years for continuous recording in different marine observation platforms. They include several methods for monitoring phytoplankton distribution in aquatic systems using optical analysis, such as:

- Single waveband LED fluorometers (generally targeting either eukaryotes and/or cyanobacteria),
- Spectral fluorometers (aimed at discriminating main pigmentary/spectral phytoplankton groups),
- Spectral absorption meters,
- Variable fluorescence instruments (dedicated to estimating photo-physiology and primary productivity).

Single waveband (monospectral) LED fluorometers are widely available in various models from several manufacturers and are currently integrated into most types of observing platforms. They typically target the fluorescence signal of the pigment chlorophyll *a* (Fchla), the most widely used measure of phytoplankton biomass, as well as phycocyanin (Fpc) or phycoerythrin (Fpe) using excitation light at their maximum absorption wavelength. While practically all LED fluorometers are designed for underwater measurements, they can also be used in flow-through measurements, as in FerryBox systems, and some manufacturers supply flow cells for their fluorometers. Technically, the instrumentation is well developed and relatively simple, but do not of themselves provide information on biodiversity. The main methodological concerns with them involve issues of instrument calibration and validation, which need to be done on a regular basis and for which there does not exist standardised and metrologically traceable practices (Ove Möller et al, 2019). These sensors measure fluorescence in relative units and empirical approaches are used to convert *in vivo* fluorescence to chlorophyll *a* concentrations.

The spectral chlorophyll *a* fluorescence of phytoplankton cells can be determined by the absorption properties of photosynthetic pigments of cells' Photosystem II (MacIntyre et al., 2010). As higher taxonomic groups differ in their photosynthetic pigments, their fluorescence spectra are also distinguishable. Spectral fluorometers measure the *in vivo* fluorescence emission of chlorophyll *a* after excitation through accessory pigments using LEDs of different wavelengths. Spectral LED fluorometers are available from two manufacturers, each with different optical properties, complicating comparisons between analyses.

As with spectral fluorescence, phytoplankton pigment groups may be detected from *in vivo* spectral absorption data, but with two complications: i) absorption includes signal from organic matter, which may overlap strongly

with pigment absorption, and ii) absorption by photoprotective pigments can be very strong in some cases, and these photoprotective pigments are often not group-specific. A couple of different commercial sensors for measuring *in situ* absorption spectra exist, using either integrating cavity (OSCAR, Trios) or subsequent measuring of absorption spectra and beam attenuation (ac-s, Sea-Bird Scientific). Detailed procedures for both chlorophyll *a* fluorescence and ac-meters in flow-through systems can be found in the IOCCG Protocol Series (Boss et al., 2019).

Variable fluorescence retrieves information on the photo-physiological state of phytoplankton communities and can be used to estimate primary production. Such devices are based on slightly different methods of measuring the variable fluorescence levels of cells (corresponding to different ways of closing photosystem reaction centres). Recent instruments utilise several wavelengths, potentially allowing the assessment of the physiological status of different pigment groups.

Table 2: Techniques and sensors for monitoring phytoplankton by means of optical methods. The wavelength column refers to excitation/emission wavelengths for fluorometers and to wavelength ranges for spectrophotometers. Chl a: Chlorophyll a. TRL: Technology Readiness Level. λ: Wavelength.

Technique	Sensor name	Wavelength (nm)	Variables measured	Detection limit/range	TRL	Level of automation	Platform
Mono-spectral fluorometry	Seapoint Chlorophyll Fluorometer	470/695	Fchl *a*	5-150 µg Chl *a* eq.L^{-1}	9	Fully automated	Moorings, floats, fixed autonomous stations, ROV, AUV
	ECO FL (SeaBird)	470/695 370/460 518/595 630/680	Fchl *a* fDOM FPhycoerythrin FPhycocyanin	0-125 µg eq.L^{-1} 0-500 ppb 0–230 ppb 0–230 ppb	9	Fully automated	Moorings, floats, fixed autonomous stations, ROV, AUV
	UniLux (Chelsea Technologies)	- 530/590 -	Fchl *a* FPhycoerythrin FPhycocyanin	0-400 µg eq.L^{-1} 0-400 µg eq.L^{-1} 0-400 µg eq.L^{-1}	9	Fully automated	Moorings, floats, fixed autonomous stations, ROV, AUV
	MicroFlu V2 (TriOS)	470/685 620/655 375/460	Fchl *a* FPhycocyanin CDOM	Depending on the sensor version: 0-200 or 500 µg eq.L^{-1} 0-200 or 500 µg eq.L^{-1} 0-500 µg.L^{-1}	9	Fully automated	Moorings, floats, fixed autonomous stations, ROV, AUV
	ECO-Triplet (SeaBird)	470/695 370/460 530/595 630/680	Fchl *a* fDOM FPhycoerythrin FPhycocyanin	0-50 µg eq.L^{-1} 0-375 ppb 0-175 ppb	9	Fully automated	Moorings, floats, fixed autonomous stations, ROV, AUV
Multi-spectral fluorometry	Cyclops C3 (3 λ) / C6P (Turner Designs)	460/696 635/695 365/470 525/>590 590/>645	Fchl *a* (blue) Fchl *a* (red) FCDOM/fDOM FPhycoerythrin FPhycocyanin	0-500 µg eq.L^{-1} 0-500 µg eq.L^{-1} 0-1,500 ppb 0-750 ppb 0-4,500 ppb	9	Fully automated	Moorings, floats, fixed autonomous stations, ROV, AUV
Multi-spectral fluorometry	Fluoroprobe (5 λ) and	370, 470, 525, 570,	Total Chl *a* [µg Chl *a* eq.L^{-1}] Brown algae	0.5-200 µg Chl *a* eq. L^{-1} (estimated)	9	Fully automated	Moorings, floats, fixed autonomous

	AlgaeOnlineAnalyser (AOA) (bbe Moldaenke)	590, 610 /690, 710	Cyanobacteria Green algae Cryptophytes Haptophyes fDOM [µg Chl *a* eq. L^{-1}]				stations, Ships of Opportunity
	Multiexciter (9 λ) (JFE Advantech)	375, 400, 420, 435, 470, 505, 525, 570, 590 />640	Fchla Fpe Fpc FDOM (Possible to calculate Total Chl *a* Brown algae Cyanobacteria Green algae Cryptophytes CDOM)	0-400 ppb	9	Fully automated	Moorings, floats, fixed autonomous stations, ROV, AUV
	MatrixFlu VIS (TriOS)	470/682 375/460, 655, 682 590/655, 682	Fchl *a* FDOM FPhycocyanin	0-200 µg.L^{-1} 0-500 µg.L^{-1} 0-200 µg.L^{-1}	9	Fully automated	Moorings, floats, fixed autonomous stations, ROV, AUV
	TriLux (Chelsea Technologies)		FChl *a* FPhycoerythrin FPhycocyanin	0-100 µg Chl *a* eq.L^{-1} (in acetone)	9	Fully automated	Moorings, floats, fixed autonomous stations, ROV, AUV
Variable / induced fluorometry	Phyto PAM II (Walz)	440, 480, 540, 590, 625 / >650	Variable fluorescence parameters	Detection limit 0.1 µg Chl *a* eq.L^{-1}	9	Semi-automated	Ships of Opportunity
	Algae Online Monitor (AOM) (Photon Systems Instruments)	455, 590 or 630/ 660-750	Variable fluorescence parameters	Detection limit 0.03 µg Chl *a* eq.L^{-1}	9	Semi-automated	Ships of Opportunity
	LabSTAF (Chelsea Technologies)	452, 472, 505, 417, 534, 594, 622/685, 730	Variable fluorescence parameters	0.001 µg eq.L^{-1}	9	Fully automated	Ships of Opportunity
Spectro-photometry	OSCAR (TriOS)	Detector 360-750 nm, 3.3 nm pixel	Raw optical density (absorption coefficient)	NA	9	Fully automated	Moorings, floats, fixed autonomous stations, ROV, AUV
	AC-S (SeaBird)	Detector 400-700 nm, 4 nm pixel	Optical density (absorption coefficient)	NA	9	Fully automated	Moorings, floats, fixed autonomous stations, ROV, AUV

2.1.2. Applications

The measure Fchl*a* is used as a proxy for chlorophyll *a* concentration (which is itself a proxy for total phytoplankton biomass), while phycocyanin and phycoerythrin are often used as proxies for differently pigmented cyanobacteria (Bertone et al 2018), although some other phytoplankton groups, like cryptophytes

can also contribute to the signal. Whether it is beneficial to measure phycocyanin or phycoerythrin or both as a proxy for cyanobacteria abundance depends on the pigmentation of local cyanobacteria populations. Typically, oceanic cyanobacteria are rich of phycoerythrin, with different forms (reflecting whether blue or green light penetrates deepest in the area) while in more coastal areas (with high loads of organic carbon, and thus contributions of red light in the underwater light field) phycocyanin is often the dominant phycobilin pigment among cyanobacteria.

In practice, phytoplankton can be differentiated into 4-9 pigment groups using spectral fluorescence and the spectral fluorescence method can be used to derive the chemotaxonomic information of a community, or to monitor certain pigmentary groups (E.g., Houliez et al., 2012; Lefebvre and Poisson-Caillault, 2019; Kodama et al., 2022). The biodiversity information obtained from spectral fluorescence is not comparable with microscopy, pigment based (HPLC) or imaging methods. However, fluorometric sensors are less expensive, relatively easy to deploy, and results can be obtained in real time, and therefore can be used as tools for rapid diagnostics of changes in phytoplankton community structure. This type of portable technology on different platforms can monitor the environment from both the surface and the water column. There is a need to better understand the relationship between *in vivo* fluorescence (raw values) and measured chlorophyll *a* concentration, as the empirical conversion factors show variability according to the main phytoplankton groups present and their physiological status.

From spectral absorption data, bulk chlorophyll *a* concentration, concentrations of other pigments or contribution of different phytoplankton spectral groups may be retrieved (E.g. Liu et al 2019). Long-term deployment of these sensors may be compromised by strong effects of biofouling, and improvements in their calibration methods are needed (e.g., Wollschläger et al 2019). Measuring pigment-group specific differences in the variable fluorescence, and thus in photosynthetic capacity and physiological status, is not straightforward with newly developed sensors with two or more wavebands (Houliez et al. 2017, Schuback et al 2021). So far, only the separation of cyanobacteria and other algae has been shown to work (Courtecuisse et al, 2023). However, their measurements can provide important information on the photosynthetic parameters and proxies of primary productivity of the whole community.

2.1.3. Level of biodiversity information (EBV/EOV)

EBVs

Table 3: EBVs that can be addressed by bulk optical methods, as defined by the GEOBON (https://geobon.org/ebvs/what-are-ebvs/).

EBV class	EBV name
Community composition	Trait diversity
Ecosystem functioning	Primary productivity Ecosystem phenology
Ecosystem structure	Ecosystem Vertical Profile

EOVs

Table 4: EOVs that can be addressed by optical methods. Sub-variables: Components of the EOV that may be measured, derived or inferred from other elements of the observing system and used to estimate the desired EOV. Derived products: Outputs calculated from the EOV and other relevant information, in response to user needs. For each EOV, a specification sheet can be downloaded from https://goosocean.org/what-we-do/framework/essential-ocean-variables/.

EOV name	Sub-variables	Derived products
Phytoplankton biomass and diversity	- Presence/Absence - Diversity/Taxonomy-*In vivo* pigment fluorescence - Primary productivity (different methods)	- Phytoplankton Functional Types - Harmful or beneficial algal bloom indices, including Harmful Algal Events - Global biogeography / spatial distribution - Primary production and carbon and nutrient cycling, storage, and export

2.1.4. SWOT analysis

Table 5: SWOT analysis of optical methods used for phytoplankton monitoring.

Strengths	Weaknesses
- Optical bulk sensors are available at different price and performance levels (Carstea et al., 2020), achieving high cost efficiency for phytoplankton monitoring. - Most of the instruments offer the advantage of being portable and easy to deploy on any oceanographic platform as well as in the lab. - They can be sent for calibration regularly. - They provide fast, high frequency and often real-time *in vivo/in situ* data (Courtecuisse et al. 2022). - Most of them have an excellent level of automation. - Data can either be stored or sent in various ways.	- Fluorescence does not provide the actual measurement of total biomass or biomass of target organisms, but a proxy. - Many types (e.g. excitation/emission wavelengths for fluorometers) and formats of outputs, providing different types of either raw and/or processed data. - For single waveband and spectral fluorometers, calibration methods have not been agreed (either with pure pigment extracts, monocultures of reference pigmentary groups or local phytoplankton) and solutions for traceable calibration are still under scrutiny. - Although there are measures to mitigate this, optical sensors can be affected by biofouling, requiring high degrees of maintenance and regular calibration.
Opportunities	**Threats**
- They can be installed on mobile platforms to cover different geographical areas from the sea surface (FerryBoxes) to deep waters (CTD, profilers, gliders, R.O.V.s, tagging of animals). - They can be deployed on fixed platforms (moorings, fixed stations). - High spatial and temporal resolution, providing high data throughput. -Can be used to validate Earth Observation outputs	- Lack of best practices for data processing : difficulty in defining time series unless calibration is carried out with a known fluorescence source. - Technical upgrades (at least for variable fluorometers) may prevent intercomparisons with older instruments. - The outputs are not accepted yet in any ecological assessment - Limited network of experts in spite of a world wide use of some of these sensors

2.1.5. References

Bertone, E., Burford, M. A., & Hamilton, D. P. (2018). Fluorescence probes for real-time remote cyanobacteria monitoring: A review of challenges and opportunities. *Water research*, *141*, 152-162.

Boss, E., Haëntjens, N., Ackleson, S., Balch, B., Chase, A., Dall'Olmo, G., ... & Westberry, T. (2019). Inherent Optical Property Measurements and Protocols: Best practices for the collection and processing of ship-based underway flow-through optical data. *Ioccg Ocean Optics and Biogeochemistry Protocols for Satellite Ocean Colour Sensor Validation; IOCCG: Dartmouth, NS, Canada*. http://dx.doi.org/10.25607/OBP-664

Carstea, E. M., Popa, C. L., Baker, A., & Bridgeman, J. (2020). In situ fluorescence measurements of dissolved organic matter: a review. *Science of the Total Environment*, *699*, 134361.

Courtecuisse, E., Oxborough, K., Tilstone, G. H., Spyrakos, E., Hunter, P. D., & Simis, S. G. (2022). Determination of optical markers of cyanobacterial physiology from fluorescence kinetics. *Journal of Plankton Research*, *44(3)*, 365-385.

Courtecuisse, E., Marchetti, E., Oxborough, K., Hunter, P. D., Spyrakos, E., Tilstone, G. H., & Simis, S. G. (2023). Optimising Multispectral Active Fluorescence to Distinguish the Photosynthetic Variability of Cyanobacteria and Algae. *Sensors*, *23*(1), 461.

Houliez E., Lizon F., Thyssen M., Artigas L.F., Schmitt F.G. (2012) Spectral fluorometric characterization of Haptophyte dynamics using the FluoroProbe : an application in the Eastern English Channel for monitoring Phaeocystis globosa. *Journal of Plankton Research*, 34(2), 136–151

Houliez, E., Simis, S., Nenonen, S., Ylöstalo, P., & Seppälä, J. (2017). Basin-scale spatio-temporal variability and control of phytoplankton photosynthesis in the Baltic Sea: The first multiwavelength fast repetition rate fluorescence study operated on a ship-of-opportunity. *Journal of Marine Systems*, *169*, 40-51.

Kodama, T., Taniuchi, Y., Kasai, H., Yamaguchi, T., Nakae, M., & Okumura, Y. (2022). Empirical estimation of marine phytoplankton assemblages in coastal and offshore areas using an in situ multi-wavelength excitation fluorometer. *Plos one*, *17*(2), e0257258. https://doi.org/10.1371/journal.pone.0257258

Lefebvre, A., & Poisson-Caillault, E. (2019). High resolution overview of phytoplankton spectral groups and hydrological conditions in the eastern English Channel using unsupervised clustering. *Marine Ecology Progress Series*, *608*, 73-92. https://doi.org/10.3354/meps12781

Liu, Y., Boss, E., Chase, A., Xi, H., Zhang, X., Röttgers, R., ... & Bracher, A. (2019). Retrieval of phytoplankton pigments from underway spectrophotometry in the Fram Strait. *Remote Sensing*, *11*(3), 318.

MacIntyre, H. L., Lawrenz, E., & Richardson, T. L. (2010). Taxonomic discrimination of phytoplankton by spectral fluorescence. *Chlorophyll a fluorescence in aquatic sciences: methods and applications*, 129-169.

Möller, K.O., Petersen, W., Nair, R. (2019) D2.5 Report on Best Practice in the utilization of sensors used for measuring nutrients, biology related optical properties, variables of the marine carbonate system, and for coastal profiling. JERICO-NEXT-WP2-D2.5-100919-V1.0 https://www.jerico-ri.eu/download/jerico-next-deliverables/JERICO_NEXT%20_Deliverable%20_2.5_Version%201.0.pdf

Schuback, N., Tortell, P. D., Berman-Frank, I., Campbell, D. A., Ciotti, A., Courtecuisse, E., ... & Varkey, D. R. (2021). Single-turnover variable chlorophyll fluorescence as a tool for assessing phytoplankton photosynthesis and primary productivity: opportunities, caveats and recommendations. *Frontiers in Marine Science*, *8*, 690607.

Wollschläger, J., Röttgers, R., Petersen, W., & Zielinski, O. (2019). Stick or dye: evaluating a solid standard calibration approach for point-source integrating cavity absorption meters (PSICAM). *Frontiers in Marine Science*, *5*, 534. doi: 10.3389/fmars.2018.00534

2.2. Flow cytometry (FCM) and single-particle analysis

2.2.1. General overview

Single cell observation using flow cytometry in the marine environment was developed more than 40 years ago. It has provided marine science with key discoveries such as the discovery of a widely distributed pico-cyanobacterium, *Prochlorococcus*, and its first ecological description (Chisholm et al., 1988). Flow cytometry enables the optical description of single particles at very high resolutions through the precise combination of fluidics, electronics and optics. Particles suspended in a liquid are aligned one by one to be hydrodynamically focused in a laser beam, generating a set of light scatter and fluorescence intensities per particle. These properties are perfectly adapted to the marine microbial environment, composed of suspended particles in a fluidic environment. The instrument, a flow cytometer, records the optical properties generated and provides high-resolution information in a numerical format. Particles can then be grouped into populations that share optical similarities, allowing the identification of coherent ecological groups. Despite the limited taxonomic identification power of optical values, important phytoplankton groups can be easily identified from a combination of size and autofluorescence, such as cell content of chlorophyll *a*, phycoerythrin and phycocyanin. Synthetic stains of DNA which can be used in flow cytometry also allows the distinction of living, non-autofluorescent cells, like heterotrophic prokaryotes and small flagellates, from non-living particles. The main strength of this technology is single particle counting, which provides precise counts of diverse ecologically relevant groups, and particularly small organisms that can be easily overlooked by other methods. While many instruments have a limited size range for measuring organisms, some devices are designed to cover a wide range of individual sizes, spanning several orders of magnitude and providing a snapshot of the entire community in one record. The high throughput capacity of such technology provides a solution to resolving the large spatial and temporal coverages required to understand the link with oceanic processes. A breakthrough came in the early 2000s, when commercial or homemade instruments became more autonomous with an online functionality. Since then, large datasets of optically identified and described plankton groups have been generated, and promising steps towards automated data processing are under development or already operational (Fuchs et al., 2022). These statistical and mathematical processes, which rapidly generate data associated with the recent common vocabulary for phytoplankton and microbial groups (Thyssen et al., 2022),

represent a step forward in the interoperability and reusability of biological information generated by flow cytometry.

The main groups identified by flow cytometry belong to the size classes of pico- (< 2-3 µm), nano- (2-3/20 µm) and microphytoplankton (> 20 µm), as well as to functional groups based on their pigments (chlorophyll *a*, phycoerythrin and phycocyanin). This depends on the instrument's laser and detector configuration and provides information on the optical characteristics of each particle, individually. Some instruments, such as the CytoSense/Sub, which has a wide flow cell and records the entire pulse shape, can collect information from can collect information from a single cell to an entire chain of microalgae.

More recently, FCM has been combined with high-resolution imaging (e.g. Imaging Flow Cytobot, CytoSense, and the new Attune "Cytpix" model), allowing visualisation and identification at a higher taxonomic level (see section 2.3). The combination of nano-microphytoplankton cell pulse shapes, which could picture the morphological traits of some characteristic groups (such as *Pseudo-nitzschia* and some dinoflagellates), can strengthen the classification of the counted cells.

Heterotrophic prokaryotes and viruses which do not exhibit any autofluorescence require a prior staining of their nucleic acid with a fluorescent dye to be observed using a flow cytometer. Automated staining modules for heterotrophic prokaryotes have recently been developed and can be adapted to various benchtop flow cytometers to run high frequency sampling and resolve small scale distributions (Pernice and Gasol, 2023).

Table 6: Techniques and sensors used by FCM. TRL: Technology Readiness Level.

Technique	Sensor name	Target organisms	Variables measured (units)	Detection limit/range	TRL	Level of automation	Platform
Automated FCM	Cytosense	Phytoplankton	Abundance (cells.ml^{-1}), Single cell scatter and autofluorescence (pulse shapes, a.u.) Proxy of Chl *a* (µg) per cell and biomass (pgC). Phycoerhytrin detection	1-800 µm	9	Fully automated	Ships of Opportunity
	CytoSub CytoBuoy	Phytoplankton	Abundance (cells.ml^{-1}), Single cell scatter and autofluorescence (pulse shapes) Proxy of Chl *a* per cell and biomass Phycoerythrin detection	1-800 µm	9	Fully automated	Moorings, Floats
	FlowCytoBot	Phytoplankton	Abundance (cells.ml^{-1}), Single cell scatter and autofluorescence (max and area) Proxy of Chl *a* per cell and biomass	0.5-20 µm	9	Fully automated	Moorings, Ships of Opportunity

			Phycoerythrin detection				
	SeaFlow	Phytoplankton	Abundance (cells.ml^{-1}), Single cell scatter and autofluorescence (max and area) Proxy of Chl *a* per cell and biomass Phycoerythrin detection	0.5-20 µm	7	Fully automated	Ships of Opportunity
	CYTOCHIP (See Mowlem et al., 2023)	Phytoplankton	Abundance (cells.ml^{-1}), Single cell scatter and autofluorescence (max and area) Proxy of Chl *a* per cell and biomass. Can add Phycoerythrin detector.	2-25um	3	Fully automated	Moorings, Ships of Opportunity
FCM with automated staining module	OnCyt + Accuri or CYTOFLEX FCM	Heterotrophic prokaryotes	Abundance (cells.ml^{-1}), Single cell scatter Induced - fluorescence (max and area)	0.9 and < 0.1-15 µm	9	Fully automated	Ships of Opportunity
	CYTOPRO	Heterotrophic prokaryotes	Abundance (cells.ml^{-1}), Single cell scatter Induced-fluorescence (pulse shapes)	0.4-15 µm	4	Fully automated	Ships of Opportunity
Bench top FCM	ACCURI	Pico Nano phytoplankton Heterotrophic prokaryotes	Abundance (cells.ml^{-1}), Single cell scatter and induced and auto-fluorescence	0.9-15 µm	9	Fully assisted	Ships of Opportunity
	Attune	Pico Nano phytoplanktonHeterotrophic prokaryotes	Abundance (cells.ml^{-1}), Single cell scatter and induced and auto-fluorescence	0.6-15 µm	9	Fully assisted	Ships of Opportunity
	Cytoflex	Pico Nano phytoplanktonHeterotrophic prokaryotes and nanoflagellates and viruses	Abundance (cells.ml^{-1}), Single cell scatter and induced and auto-fluorescence	<0.1-15 µm	9	Fully assisted (plate loader in option)	Ships of Opportunity

2.2.2. Applications

Phytoplankton can be analysed automatically with an online flow cytometer, mainly targeting surface waters. From the online flow of pumped seawater, the automated flow cytometer can sample every hour or even less, providing high-resolution mapping of phytoplankton groups (Louchart et al., 2020). Although it targets surface observations only, it is an unprecedented way to map fine-scale physical oceanographic structures and their associated communities (Marrec et al., 2018). Furthermore, mapping at this resolution level can provide a powerful validation of remote sensing algorithms for plankton functional groups. Submersible flow cytometers are fixed under a buoy or dike or on a submerged rosette to provide a multi-dimensional description of the

microbial distribution. When a FCM is onboard, discrete samples collected from Niskin bottles are analysed immediately after collection and storage in the dark. High-frequency sampling in time provides precise information on the diel cycle, which is mainly influenced by the cell cycle (Dugenne et al., 2014).

2.2.3. Level of biodiversity information (EBV/EOV)

EBVs

Table 7: EBVs that can be addressed by FCM, as defined by the GEOBON (https://geobon.org/ebvs/what-are-ebvs/).

EBV class	EBV name
Species traits	Morphology Phenology Physiology
Community composition	Community abundance Trait diversity Interaction diversity
Ecosystem functioning	Ecosystem phenology
Ecosystem structure	Ecosystem Vertical Profile

EOVs

Table 8: EOVs that can be addressed by FCM. Sub-variables: Components of the EOV that may be measured, derived or inferred from other elements of the observing system and used to estimate the desired EOV. Derived products: Outputs calculated from the EOV and other relevant information, in response to user needs. For each EOV, a specification sheet can be downloaded from https://goosocean.org/what-we-do/framework/essential-ocean-variables/.

EOV name	Sub-variables	Derived products
Phytoplankton biomass and diversity	- Presence/Absence/Relative Abundance - Diversity - In vivo pigment fluorescence	- Phytoplankton Functional Types - Diversity indices: other
Microbe biomass and diversity (*pilot)		- Heterotrophic prokaryotes, viruses, abundances

2.2.4. SWOT analysis

Table 9: SWOT analysis of FCM technologies for pelagic biodiversity monitoring.

Strengths	Weaknesses
- Single cell *in vivo/in situ* analysis and counts of nano- and picoplankton cells below 5 µm is only possible with flow cytometry. - Including large cells such as microphytoplankton is an advance in automated flow cytometers. - They provide high-throuput optical data that could be related to phytoplankton functional diversity (Fragoso et al., 2019).	- Flow cytometry provides taxonomic optical data and counts that need to rely on a common vocabulary to be intercompared and entered into databases. - Installing on a rosette can delay the sampling of the water column, as steps of several minutes would be required at each depth to allow proper FCM measurements. - Submersible flow cytometers require more technical expertise and care than on-board devices and are subject to bio-fouling.

	- Instruments are expensive (especially automated submersible devices). - Power consumption can represent an issue in autonomous deployments - Raw data is heavy and requires appropriate communication pipelines when transmitted from an autonomous device in the field to the central processing station. - A lack of automation in data analysis limits the processing of large raw data sets.
Opportunities	**Threats**
- The autonomous functionality of some flow cytometers can provide a high spatial and temporal resolution of the phytoplankton community. - Automated staining modules (although not yet submersible) open the way to automated assessment of the heterotrophic part of the microbial community and cell physiology (e.g., viable/dead cells via fluorescent dyes). - Automated FCM allows studies with high spatio-temporal resolution, at the scale of organism life cycles and physical/chemical events, allowing growth rates to be taken into account by using appropriate models. - The robustness of the sensors could allow long-term observations without significant changes in the optical characteristics of the machine. - Automated sensors can sample subsurface waters when installed on Ships of Opportunity/Researh Vessels connected to a FerryBox or flow-through system. - Submersible sensors can sample at different depths, provided the intake systems are adapted. - A common vocabulary has recently been published (Thyssen et al., 2022). An active network of expert users have been and are developing automated analytical tools (as Fuchs et al., 2020), data pipelines and best pratices.	- Limitations in standardisation (although work is in progress). - Difficulties remain in storing data in biodiversity databases, as most groups are mostly functional groups. - Lack of data visibility in European plaftforms for biodiversity study and monitoring. - Under constant development, technical upgrades and changes that may prevent intercomparisons with older machines. - Lack of choice for instruments and high dependence on suppliers. - High cost to purchase and maintain - Limited network of experts. - The ouputs have not yet been used in any ecological assessment.

2.2.5. References

Chisholm, S. W., Olson, R. J., Zettler, E. R., Goericke, R., Waterbury, J. B., Welschmeyer, N. A. (1988). A novel free-living prochlorophyte abundant in the oceanic euphotic zone. *Nature*, *334(6180)*, 340–343. doi: 10.1038/334340a0

Dugenne, M., Thyssen, M., Nerini, D., Mante, C., Poggiale, J. C., Garcia, N., ... & Grégori, G. J. (2014). Consequence of a sudden wind event on the dynamics of a coastal phytoplankton community: an insight into specific

population growth rates using a single cell high frequency approach. *Frontiers in microbiology*, *5*, 485. https://doi.org/10.3389/fmicb.2014.00485

Fuchs, R., Thyssen, M., Creach, V., Dugenne, M., Izard, L., Latimier, M., ... & Pommeret, D. (2022). Automatic recognition of flow cytometric phytoplankton functional groups using convolutional neural networks. *Limnology and Oceanography: Methods*, *20(7)*, 387-399. https://doi.org/10.1002/lom3.10493

Louchart, A., Lizon, F., Lefebvre, A., Didry, M., Schmitt, F. G., & Artigas, L. F. (2020). Phytoplankton distribution from Western to Central English Channel, revealed by automated flow cytometry during the summer-fall transition. *Continental Shelf Research*, *195*, 104056. doi: 10.1016/j.csr.2020.104056

Marrec, P., Grégori, G., Doglioli, A. M., Dugenne, M., Della Penna, A., Bhairy, N., ... & Thyssen, M. (2018). Coupling physics and biogeochemistry thanks to high-resolution observations of the phytoplankton community structure in the northwestern Mediterranean Sea. *Biogeosciences*, *15*(5), 1579-1606. doi: 10.5194/bg-15-1579-2018

Mowlem, M., Carvalho, F., Hanz, R., Abdi, E., Catalano, C., Mougiou, K. H., ... & Oxborough, K. (2023, June). Technologies for Ocean Sensing project developments in imaging and sensing. In *OCEANS 2023-Limerick* (pp. 1-13). IEEE.

Pernice, M. C., & Gasol, J. M. (2023). Automated flow cytometry as a tool to obtain a fine-grain picture of marine prokaryote community structure along an entire oceanographic cruise. *Frontiers in Microbiology*, *13*, 1064112. doi: 10.3389/fmicb.2022.1064112

Thyssen M., Grégori G., Créach V., Lahbib S., Dugenne M., Aardema H. M., Artigas L.F., Huang B., Barani A., Beaugeard L., Bellaaj-Zouari A., Beran A., Casotti R., Del Amo Y., Denis M., Dubelaar G.B.J., Endres S., Haraguchi L., Karlson B., Lambert C., Louchart A., Marie D., Moncoiffe G., Pecqueur D., Ribalet F., Rijkeboer M., Silovic T., Silva R., Marro S., Sosik H., Sourisseau M., Tarran G., Van Oostende N., Zhao L., Zhen S. (2022) Interoperable vocabulary for marine microbial flow cytometry. *Frontiers in Marine Science*, 975877. doi : 10.3389/fmars.2022.975877

2.3. Imaging *in situ/ex situ*

2.3.1. General overview

Imaging marine life has the advantage of providing images of either fixed (preserved) or living organisms. In classical observational biology, microscopes and stereomicroscopes are the mainstay. The traditional technique for analysing plankton (nano-, microplankton and mesozooplankton) taxonomic composition and cell counts relies on an experienced biologist who processes the samples by light microscopy (Moncheva et al., 2010, Hillebrand et al., 1999, Alexandrov et al., 2014) These methods are challenged by the use of fixed material, which is often associated with alterations in morphology, colouration and numbers (Jakobsen & Carstensen, 2011). Furthermore, the material is often destroyed during analysis, making further post-processing and data verification impossible. Sharp and detailed images of marine life at scales from microns (phytoplankton) to

centimetres (zooplankton, fish) are the norm, and images can be further processed to provide key parameters such as taxonomy and biomass information. In addition, some groups of organisms, such as gelatinous plankton, are very fragile and do not maintain morphological integrity during fixation. Therefore, *in situ/in vivo* imaging using cameras would be the most appropriate solution, although preserved samples and in lab processing may still be necessary to maintain long-term time series, or if there are limitations on the equipment to be allocated on research vessels (Fernandes et al., 2009). The collected images can be processed into species in real time using automated processing by Artificial Intelligence (AI) (Luo et al., 2018; Kraft et al., 2022). *In situ/in vivo* imaging systems target organisms as small as 5 µm, where the laws of optics define the lower size limit. For plankton measurements, the largest particles that can be processed are limited by the capacity to view a large enough volume to obtain significant counting statistics. However, there is no imaging instrument that is suitable for all applications and size classes, a limitation that is also shared by more classical sampling techniques like net sampling. The choice of instrumentation for a particular analysis is determined by the size and taxonomy of the organisms being studied, the ecosystem being studied and the logistics of the application, i.e., is *in vivo* analysis possible, or is it necessary to use an imaging instrument that can work with fixed material back in the lab? Lombard et al. (2019) wrote a review on the challenges of plankton observations, describing the need to combine different imaging and optical systems to deal with the full size range of plankton. Fishing vessels can also be used as Ships of Opportunity to obtain both fish species data (Lekunberri et al., 2022) and environmental variables (Uriondo et al., 2024).

Table 10: Techniques and sensors used by imaging methods. Please consider that the "Ships of opportunity" category includes various mode of operation, i.e., the systems can be used by casting or towed or on board (flow-through). TRL: Technology Readiness Level.

Technique	Sensor name	Target organisms	Variables measured (units)	Detection limit/range	TRL	Level of automation	Platform
In flow imaging	FlowCam	Phytoplank-ton, Micro-zooplankton, Zooplankton	Particle counts and individual sizes (cm)	10-1000 µm 300-5000 µm (macro version)	9	Semi-automated	Ships of Opportunity
	Imaging FlowCytobot	Phytoplankton	Particle counts, size	<10-150 µm	9	Fully automated	Moorings, Ships of Opportunity
	Plankton Imager	Zooplankton	Particle counts and individual sizes (cm)	10- 2000 µm	9	Fully automated	Moorings, Ships of Opportunity
	ZooCAM	Zooplankton, fish eggs	Particle counts, size	300-2000 µm	9	Semi-automated	Ships of Opportunity
	PlanktoScope	Phytoplank-ton, Micro-zooplankton	Particle counts, size	20-200 µm	7	Semi-automated	Ships of Opportunity
	CytoSense	Phytoplank-ton, Micro-zooplankton	Particle size	<5-800 µm	9	Fully automated	Ships of opportunity, Moorings
In situ Imaging	UVP (5 & 6)	Zooplankton and colonial phytoplankton	Particle counts, size	500-10000 µm	9	Fully automated	Moorings, AUV, Ships of Opportunity

	CPICS	Zooplankton and big phytoplankton	Particle counts, size	60-20000 µm	9	Fully automated	Moorings, Ships of Opportunity
	VPR-based (VPR, BIOMAPER II)	Zooplankton and big phytoplankton	Particle counts, size	50-10000 µm	9	Fully automated	ROV, AUV, Moorings, Ships of Opportunity
	LOKI	Zooplankton and big phytoplankton	Particle counts, size	50-2000 µm	9	Fully automated	Ships of Opportunity
	SIPPER	Zooplankton	Particle counts, size	65-na	9	Fully automated	AUV, Ships of Opportunity
	Silcam	Zooplankton And big phytoplankton	Particle counts, size	50-11000 µm	9	Fully automated	AUV, Ships of Opportunity
Laser field	LOPC	Zooplankton and colonial phytoplankton	Particle counts, size	100-35000 µm	9	Fully automated	AUV, Ships of Opportunity
Electronic eye + deep learning	Digital camera	Pelagic fish	Counts by species and individual sizes (cm)	5 cm	5	Fully automated	Ships of Opportunity
Holographic camera	HOLOCAM	Phytoplankton and Zooplankton	Particle counts, size	1-1000 µm	9	Fully automated	Ships of opportunity
	LISST-HOLO	Zooplankton and big phytoplankton	Particle counts, size	50-2500 µm	9	Fully automated	Ships of Opportunity
	4-Deep HoloSea	Zooplankton	Particle counts, size	10-2000 µm	9	Fully automated	Ships of Opportunity
Focused shadowgraph	ISIIS	Zooplankton and colonial phytoplankton	Particle counts, size	600-1300 µm	9	Fully automated	Ships of Opportunity
	ZOOVIS	Zooplankton and big phytoplankton	Particle counts, size	40-5000 µm	9	Semi-automated	Ships of Opportunity
Submersible video cameras	PelagiCAM, BRUV	Pelagic fish, marine fauna	Organism counts	-	9	Fully automated	Moorings and fixed platforms

In addition to the information given in the table, depending on the application, additional information like the volume (flow) imaged, the instrument dimensions/weight, the possibility of combining with other instruments (e.g. CTD), etc. needs to be considered. For example, the BIOMAPPER II uses a combination of imaging, acoustics and a CTD, which can be towed at high speed (up to 6 knots), processing large volumes, being thus more adapted to study macrozooplankton, but its dimensions/weight limit its applicability to only a subset of research vessels. In addition, imaging approaches are also used to study pelagic fauna and can be installed on fixed platforms or moorings (PelagiCAM, Sheehan et al., 2020; Bait-based remote underwater video (BRUV) systems; Prat-Varela et al., 2023).

2.3.2. Applications

Imaging systems enable functional groups detection, fast species identification, biomass estimation and conversion to carbon, spatial and temporal dynamics, biodiversity assessment and provide support for modelling. Imaging data are important for assessing, often in real-time, changes in biodiversity and biomass and

their coupling to environmental pressures. Two recent studies have been carried out, for example, on marine plankton traits obtained from automated image analysis (Orenstein et al., 2022) and on data management procedures (Martin-Cabrera et al., 2022). Imaging data can be used for policy, such as MSFD indicators (Uusitalo et al., 2016). They are also useful for climate observations (Chust et al., 2024).

2.3.3. Level of biodiversity information (EBV/EOV)

EBVs

Table 11: EBVs that can be addressed by imaging methods, as defined by the GEOBON (https://geobon.org/ebvs/what-are-ebvs/).

EBV class	EBV name
Species populations	Species distributions Species abundances
Species traits	Morphology Phenology
Community composition	Community abundance Trait diversity
Ecosystem functioning	Ecosystem phenology
Ecosystem structure	Ecosystem Vertical Profile

EOVs

Table 12: EOVs that can be addressed by imaging methods. Sub-variables: Components of the EOV that may be measured, derived or inferred from other elements of the observing system and used to estimate the desired EOV. Derived products: Outputs calculated from the EOV and other relevant information, in response to user needs. For each EOV, a specification sheet can be downloaded from https://goosocean.org/what-we-do/framework/essential-ocean-variables/.

EOV name	Sub-variables	Derived products
Phytoplankton biomass and diversity	- Presence/Absence/Relative Abundance - Diversity	- Phytoplankton Functional Types - Diversity indices: other - Harmful or beneficial algal bloom indices, including Harmful Algal Events
Zooplankton biomass and diversity	- Biomass overall. - Biomass or abundance (or presence/absence) by taxon, - Functional group - Size class	- Geographical distributions by taxon or functional group - Life history timing - Community size structure

2.3.4. SWOT analysis

Table 13: SWOT analysis of imaging technologies for pelagic biodiversity monitoring.

Strengths	Weaknesses
- A variety of imaging sensors allow for adaptation to different size classes of organisms (Lombard et al., 2019). They range from benchtop scanning and in-flow systems (associated with FerryBoxes or deployed *in situ*) to *in situ* fixed or profiling devices.	- Each instrument is designed for a specific size range or organisms to be detected and recorded for further analysis. This means that many devices, or sometimes many optics within the same device, are required to cover the whole plankton realm, and even more for larger fauna.

- Images can be stored and analysed later using advanced image recognition methods, or to screen previously unknown or unlabelled classes. - Automated image data pipelines are already available (Martin-Cabrera et al., 2022) - Best practices are available for many sensors - Imaging can provide information on traits such as nutrition (chloroplasts retained in ciliates) and preferred prey and feeding modes, as well as tracking parasite infections. - Imaging provides high data throughput. - It provides a cost-effective approach to process hundreds or thousands of samples that would otherwise probably not be processed. - The error associated with automated classification tools can be considered as stable over time, as it does not depend on the changing expertise/experience of a human taxonomist or their level of fatigue. -	- There is usually a trade-off between the number of groups or taxa and the performance of the automated classification. - Visual differences between certain classes or taxa are small and depend on the resolution of the imaging system. - The variation in image size and aspect ratio is very large and can only be detected by using the most appropriate optical or device combination. - Depending on the device, image quality may be poor or vary widely. - If not properly calibrated or out of focus, images can be very blurry. - The amount of image data is massive (Eerola et al 2024) and requires significant storage capacity. - Most instruments are expensive.
Opportunities	**Threats**
- Automated imaging solutions enable studies with high spatio-temporal resolution, at the scale of organism life cycles and physical/chemical events. - Combined with near real-time image recognition, results are available for applications such as harmful algal bloom (HAB) early warning, satellite validation or model assimilation. - Permanent improvements in image analysis are being tested and implemented using Artificial Intelligence. - Rapid technological development allows for continuous improvement of available instruments and/or the release of new devices. - Permanent image databases allow the data to be re-analysed by various automated analytical tools.	- The amount of labelled data for training is limited. - Decrease in taxonomy experts to build the training sets. - The accuracy of defining taxa to a species or genus level is somewhat dependent on the resolution and quality of the images provided by the imaging system. - Continuous effort to build the training sets as the community can change in time and space. - High cost to purchase and maintain. - Species recognition by images is still not accepted nor applied in ecological assessment.

2.3.5. References

Alexandrov, B., Arashkevich, E., Gubanova, A., & Korshenko, A. (2014). Manual for Mesozooplankton Sampling and Analysis in the BlackSea Monitoring; Black Sea Commission: Istanbul, Turkey.

Chust, G., Villarino, E., McLean, M., Mieszkowska, N., Benedetti-Cecchi, L., Bulleri, F., ... & Lindegren, M. (2024). Cross-basin and cross-taxa patterns of marine community tropicalization and deborealization in warming European seas. *Nature Communications*, *15(1)*, 2126.

Eerola, T., Batrakhanov, D., Barazandeh, N. V., Kraft, K., Haraguchi, L., Lensu, L., ... & Kälviäinen, H. (2024). Survey of automatic plankton image recognition: Challenges, existing solutions and future perspectives. *Artificial Intelligence Review*, *57(5)*, 114.

Fernandes, J. A., Irigoien, X., Boyra, G., Lozano, J. A., & Inza, I. (2009). Optimizing the number of classes in automated zooplankton classification. *Journal of Plankton Research*, *31*(1), 19-29.

Hillebrand, H., Dürselen, C. D., Kirschtel, D., Pollingher, U., & Zohary, T. (1999). Biovolume calculation for pelagic and benthic microalgae. *Journal of phycology*, *35*(2), 403-424.

Jakobsen, H. H., & Carstensen, J. (2011). FlowCAM: sizing cells and understanding the impact of size distributions on biovolume of planktonic community structure. *Aquatic Microbial Ecology*, *65*(1), 75-87.

Kraft, K., Velhonoja, O., Eerola, T., Suikkanen, S., Tamminen, T., Haraguchi, L., Ylöstalo, P., Kielosto, S., Johansson, M., Lensu, L., Kälviäinen, H., Haario, H. and Seppälä, J. (2022) Towards operational phytoplankton recognition with automated high throughput imaging, near-real-time data processing, and convolutional neural networks. *Frontiers in Marine Science*, *9*, 867695.

Lekunberri, X., Ruiz, J., Quincoces, I., Dornaika, F., Arganda-Carreras, I., & Fernandes, J. A. (2022). Identification and measurement of tropical tuna species in purse seiner catches using computer vision and deep learning. *Ecological Informatics*, *67*, 101495.

Lombard, F., Boss, E., Waite, A. M., Vogt, M., Uitz, J., Stemmann, L., ... & Appeltans, W. (2019). Globally consistent quantitative observations of planktonic ecosystems. *Frontiers in Marine Science*, *6*, 196.

Luo, J.Y., Irisson, J.-O., Graham, B., Guigand, C., Sarafraz, A., Mader, C. and Cowen, R.K. (2018), Automated plankton image analysis using convolutional neural networks. *Limnology and Oceanography: methods*, *16*(12), 814-827. https://doi.org/10.1002/lom3.10285

Martin-Cabrera, P., Perez Perez, R., Irisson, J. O., Lombard, F., Möller, K. O., Rühl, S., ... & Schepers, L. (2022). Best practices and recommendations for plankton imaging data management: Ensuring effective data flow towards European data infrastructures. Version 1.

Moncheva, S., Parr, B., Sarayi, D., & Hareket, I. I. (2010). Manual for phytoplankton sampling and analysis in the black sea. *Phytoplankton Manual, UP-GRADE Black Sea Scene Project, FP7*, *226592*.

Orenstein, E. C., Ayata, S. D., Maps, F., Becker, É. C., Benedetti, F., Biard, T., ... & Irisson, J. O. (2022). Machine learning techniques to characterize functional traits of plankton from image data. *Limnology and oceanography*, *67*(8), 1647-1669, doi : 10.1002/lno.12101.

Prat-Varela, A., Torres, A., Cervantes, D., Aquino-Baleytó, M., Abril, A. M., & Clua, E. E. (2023). Improved baited remote underwater video (BRUV) for 24 h real-time monitoring of pelagic and demersal marine species from the epipelagic zone. *Journal of Marine Science and Engineering*, *11*(6), 1182.

Sheehan, E. V., Bridger, D., Nancollas, S. J., & Pittman, S. J. (2020). PelagiCam: A novel underwater imaging system with computer vision for semi-automated monitoring of mobile marine fauna at offshore structures. *Environmental monitoring and assessment*, *192*(1), 11.

Uriondo, Z., Fernandes-Salvador, J. A., Reite, K. J., Quincoces, I., & Pazouki, K. (2024). Toward Digitalization of Fishing Vessels to Achieve Higher Environmental and Economic Sustainability. *ACS Environmental Au*.

Uusitalo, L., Fernandes, J. A., Bachiller, E., Tasala, S., & Lehtiniemi, M. (2016). Semi-automated classification method addressing marine strategy framework directive (MSFD) zooplankton indicators. *Ecological Indicators*, *71*, 398-405.

2.4. eDNA

2.4.1. General overview

Environmental DNA (eDNA) analysis encompasses a diverse array of methodologies aimed at characterizing the biodiversity within an ecosystem without direct observation of organisms. This approach relies on amplifying and/or sequencing nucleic acids (DNA) shed by organisms into their environment or contained within microbial cells. For more than a decade, numerous studies have demonstrated the power of this approach for biodiversity monitoring, species detection, invasive species surveillance, and ecological research. More recently, this non-invasive technique has become also a valuable tool in conservation biology and environmental management. Here, quantitative and digital PCR (qPCR and dPCR, respectively) (Andruszkiewicz, Yamahara et al., 2020; Scriver et al., 2024) are efficient techniques for providing quantitative information on single targeted species, while DNA metabarcoding and metagenome studies allow for profiling communities across a wide range of taxonomic groups and provide information about relative taxon abundances (Adams, Jeunen et al., 2023). When reviewing existing and emerging methods for eDNA analysis, it is crucial to account for the different steps and methods encompassed by this term. This involves determining strategies and methodologies for sampling, followed by the chosen approach for biodiversity analysis, and finally, the specific taxonomic groups or target organisms to be covered (Fonseca et al., 2023, 2018). In marine biodiversity studies focused on pelagic species, eDNA is typically obtained by filtering water samples collected using a variety of containers (from buckets to self-closing Niskin bottles mounted on rosettes), with filtration normally done on board using peristaltic pumps. More recent developments involve automation of eDNA sampling to enable sampling in remote areas, or with high spatial and temporal coverage onboard ships or at fixed stations. Automated eDNA samplers offer the possibility to filter the water *in situ* as it is being collected (instead of after a period of storage) coupled with DNA preservation strategies such as the use of RNAlater (a concentrated solution of ammonium and csium sulfates) or liquid nitrogen (Truelove et al. 2022). Passive eDNA samplers, such as metaprobe (Maiello et al. 2020), allow capturing DNA in the water without filtration; these can be added to a trawling net or other devices to capture eDNA from larger areas. For community studies, third-generation sequencing technologies offer the possibility of sequencing long genomic regions in a compact and portable device (e.g. Minion-Oxford Nanopore technologies), thus allowing DNA sequencing on board, provided that a suitable laboratory is available.

Table 14: Techniques and sensors used by eDNA methods. TRL: Technology Readiness Level.

Technique	Sensor name	Target organisms	Sampling Volume	TRL	Level of automation	Platform
In situ autonomous oceanographic sampler	eDNA sampler (Dartmouth Ocean Technologies)	Any, depending on primers/probe used.	Variable	9	Fully automated	- Ships of Opportunity - ROV/AUV - Floats, fixed autonomous stations
	AUTOFIM	Any, depending on primers/probe used.	0.5 - 5 liter	6	Fully automated	- Ships of Opportunity, fixed stations

	Remote Access Sampler (McLane)	Any, depending on primers/probe used.	48 x 500 ml	9	Fully automated	- Moorings, floats, fixed autonomous stations
	Robotic Cartridge Sampling Instrument (RoCSI) (McLane)	Any, depending on primers/probe used.	Flexible, depending on filtration time, as it is a flow through filtration	9	Fully automated	- Ships of Opportunity - ROV/AUV - Floats, fixed autonomous stations
PCR	MIC (qPCR) (Bio Molecular systems)	Any, depending on primer/probes used	Flexible depending on the sampling approach used to collect eDNA samples for subsequent analyses	9	Not yet fully automated. Requires automated or manual solutions for sampling of eDNA, extraction of DNA and set up of analyses procedures	Ships of Opportunity
Portable sequencer	Minion (Oxford Nanopore Technologies)	Any, depending on primers used.	Depends on the target species but for macrofauna and in this case fish the minimum to allow for any degree of reliable detection is >5L for micro and meiofauna between 500ml up to 2L should suffice	9 (Hatfield et al., 2020)	Requires assistance for sampling, DNA extraction and library preparation	Ships of Opportunity
	Environmental Sample Processor (MBARI)	Any, depending on primers/probe used.		9		Fixed autonomous stations

2.4.2. Applications

Molecular methods can be used to characterize and monitor biodiversity, including temporal and spatial variations (community composition through DNA metabarcoding, e.g., Canals et al., 2021).

It is possible to address species distribution and mapping, including NIS detection and tracking (species-specific through q/dPCR, e.g. Jerde et al. 2011). The presence of endangered/elusive species as well as species abundance/fish biomass estimates can be obtained from eDNA analysis (in relative terms through DNA metabarcoding, e.g., Fraija-Fernandez et al., 2020; in absolute terms through q/dPCR e.g. Urban et al., 2023).

2.4.3. Level of biodiversity information (EBV/EOV)

EBVs

Table 15: EBVs that can be addressed by eDNA methods, as defined by the GEOBON (https://geobon.org/ebvs/what-are-ebvs/).

EBV class	EBV name
Genetic composition	Genetic diversity Genetic differentiation (number of genetic units and genetic distance)

	Inbreeding
Species populations	Species distributions Species abundances
Species traits	Physiology
Community composition	Community abundance Taxonomic/phylogenetic diversity Interaction diversity
Ecosystem functioning	Ecosystem disturbances
Ecosystem structure	Live cover fraction Ecosystem distribution

EOVs

Table 16: EOVs that can be addressed by eDNA methods. Sub-variables: Components of the EOV that may be measured, derived or inferred from other elements of the observing system and used to estimate the desired EOV. Derived products: Outputs calculated from the EOV and other relevant information, in response to user needs. For each EOV, a specification sheet can be downloaded from https://goosocean.org/what-we-do/framework/essential-ocean-variables/.

EOV name	Sub-variables	Derived products
Phytoplankton biomass and diversity	- Phylogenetic diversity - Genomic diversity	- Phytoplankton composition - Harmful taxa or beneficial blooms - Spatial and temporal distribution - Information on the physiological potential
Zooplankton biomass and diversity	- Phylogenetic diversity - Genomic diversity - Gut content composition	- Zooplankton composition - Spatial and temporal distribution - Trophic interactions - Information on the physiological potential
Fish distribution	- Phylogenetic diversity - Genomic diversity - Gut content composition	- Fish community composition - Spatial and temporal distribution - Information on the physiological potential - Trophic interactions
Marine turtles, mammals distribution	- Phylogenetic diversity - Genomic diversity - Gut content composition	- Spatial and temporal distribution - Trophic interactions - Species composition - Information on the physiological potential
Microbial biomass and diversity	- Phylogenetic diversity - Genomic diversity	- Spatial and temporal distribution - Species composition - Information on the physiological potential

2.4.4. SWOT analysis

Table 17: SWOT analysis of eDNA methods for pelagic biodiversity monitoring.

Strengths	Weaknesses
- Non-invasive sampling: eDNA methods are less disruptive to ecosystems, as they do not necessarily require the capture of individuals. This minimizes the impact on species and habitats, making it more	- Inability to distinguish DNA sources: eDNA cannot differentiate between DNA from live and dead individuals, leading to potential misinterpretation of presence or activity levels of species.

environmentally friendly and far less invasive than traditional sampling methods. - Higher detection rates: eDNA provides a greater potential for detecting rare, cryptic, and less abundant species compared to traditional methods, leading to more comprehensive biodiversity assessments. - Remote sampling: Allows for easier sampling and exploration of remote environments such as deep-sea habitats. - Access to functional insights: Enhances the possibility to access functional capability, adaptability, and physiology of organisms through gene expression analysis on the entire community, specific species, or individual cells. - Cost-effective sampling: For large-scale studies involving numerous samples, eDNA methods can be more cost-effective than traditional approaches. - Technology is increasingly affordable, and an extensive range of suppliers exist. - eDNA approaches provides high data throughput.	- Primer bias in DNA metabarcoding: The use of specific primers in DNA metabarcoding can result in the selective amplification of certain species, causing underrepresentation or even omission of other species. This bias can skew the assessment of relative abundances. - Complexities in quantitative measurements (qPCR/dPCR): The relationship between DNA abundance and organisms' biomass is not always straightforward. It depends on several factors such as number of gene copies per individual and DNA shedding rates in the environment, which can differ among species, due to activity (feeding, mating, etc.) or developmental stage (adult, larvae, etc.) as well as degradation rate and transport with currents. - Dependency on well-curated reference databases: Accurate taxonomic assignment in DNA metabarcoding and the development of species-specific probes for qPCR/dPCR require well-maintained reference databases. Similarly, poorly-curated references can lead to misidentification and inaccurate data interpretation.
Opportunities	**Threats**
- eDNA provides a novel source material to monitor biodiversity at a taxonomical and functional level. - eDNA can provide information about the spatial distribution and temporal variability of the species, particularly in remote locations. - Makes possible to conduct comprehensive ecosystem studies. - Automation of both sample collection and DNA extraction (i.e. ESP or RocSi) allows these new generation devices to be deployed in moorings, fixed stations and/or connected to inflow chambers connected to FerryBoxes. - Can be appropriate for citizen science (e.g. Ocean Sampling Day). - Permanently under development, providing new applications	- Lack of standardised methodologies despite current interoperable exercices and discussions. - If reference databasesare not regularly updated, samples may only be assigned to higher taxonomic levels. - Under constant development, both technically and analytically, making it difficult to compare results from long-time series. - Automated samplers are expensive. - Some analyses require more careful sample preservation (RNA-based metatranscriptomics)

2.4.5. References

Adams, C. I., Jeunen, G. J., Cross, H., Taylor, H. R., Bagnaro, A., Currie, K., ... & Knapp, M. (2023). Environmental DNA metabarcoding describes biodiversity across marine gradients. *ICES Journal of Marine Science*, *80*(4), 953-971.

Andruszkiewicz, E. A., Yamahara, K. M., Closek, C. J., & Boehm, A. B. (2020). Quantitative PCR assays to detect whales, rockfish, and common murre environmental DNA in marine water samples of the Northeastern Pacific. *Plos one*, *15*(12), e0242689. https://doi.org/10.1371/journal.pone.0242689

Canals, O., Mendibil, I., Santos, M., Irigoien, X., & Rodríguez-Ezpeleta, N. (2021). Vertical stratification of environmental DNA in the open ocean captures ecological patterns and behavior of deep-sea fishes. *Limnology and Oceanography Letters*, *6*(6), 339-347. https://doi.org/10.1002/lol2.10213

Fonseca, V. G. (2018). Pitfalls in relative abundance estimation using eDNA metabarcoding. *Molecular Ecology Resources*, *18(5)*, 923-926. doi: 10.1111/1755-0998.12902

Fonseca, V. G., Davison, P. I., Creach, V., Stone, D., Bass, D., & Tidbury, H. J. (2023). The Application of eDNA for Monitoring Aquatic Non-Indigenous Species: Practical and Policy Considerations. *Diversity*, *15(5)*, 631.

Fraija-Fernández, N., Bouquieaux, M. C., Rey, A., Mendibil, I., Cotano, U., Irigoien, X., ... & Rodríguez-Ezpeleta, N. (2020). Marine water environmental DNA metabarcoding provides a comprehensive fish diversity assessment and reveals spatial patterns in a large oceanic area. *Ecology and Evolution*, *10*(14), 7560-7584. https://doi.org/10.1002/ece3.6482

Hatfield, R. G., Batista, F. M., Bean, T. P., Fonseca, V. G., Santos, A., Turner, A. D., ... & Martinez-Urtaza, J. (2020). The application of nanopore sequencing technology to the study of dinoflagellates: a proof of concept study for rapid sequence-based discrimination of potentially harmful algae. *Frontiers in Microbiology*, *11*, 844. doi:10.3389/fmicb.2020.00844

Jerde, C. L., Mahon, A. R., Chadderton, W. L., & Lodge, D. M. (2011). "Sight-unseen" detection of rare aquatic species using environmental DNA. *Conservation letters*, *4*(2), 150-157. https://doi.org/10.1111/j.1755-263X.2010.00158.x

Maiello, G., Talarico, L., Carpentieri, P., De Angelis, F., Franceschini, S., Harper, L. R., ... & Russo, T. (2022). Little samplers, big fleet: eDNA metabarcoding from commercial trawlers enhances ocean monitoring. *Fisheries Research*, *249*, 106259. https://doi.org/10.1016/j.fishres.2022.106259.

Scriver, M., von Ammon, U., Youngbull, C., Pochon, X., Stanton, J. A. L., Gemmell, N. J., & Zaiko, A. (2024). Drop it all: extraction-free detection of targeted marine species through optimized direct droplet digital PCR. *PeerJ*, *12*, e16969. https://doi.org/10.7717/peerj.16969.

Thomas, A. C., Howard, J., Nguyen, P. L., Seimon, T. A., & Goldberg, C. S. (2018). eDNA Sampler: A fully integrated environmental DNA sampling system. *Methods in ecology and evolution*, *9(6)*, 1379-1385.

Truelove, N. K., Patin, N. V., Min, M., Pitz, K. J., Preston, C. M., Yamahara, K. M., ... & Chavez, F. P. (2022). Expanding the temporal and spatial scales of environmental DNA research with autonomous sampling. *Environmental DNA*, *4*(4), 972-984. https://doi.org/10.1002/edn3.299

Urban, P., Bekkevold, D., Degel, H., Hansen, B. K., Jacobsen, M. W., Nielsen, A., & Nielsen, E. E. (2023). Scaling from eDNA to biomass: Controlling allometric relationships improves precision in bycatch estimation. *ICES Journal of Marine Science*, *80*(4), 1066-1078. https://doi.org/10.1093/icesjms/fsad027

2.5. Acoustics

2.5.1. General overview

Acoustic methods used in fisheries include a spectrum of instruments which are based on the concept that a pulse of sound is emitted from a transducer and the returning echo contains information on objects in the water-column. Most used in marine research are echosounders, which collect high resolution backscatter information. It is therefore unrivalled in its ability to monitor organisms in the (whole) water-column at high vertical (cm) and horizontal (m) resolution over 100s of kilometres. When calibrated, echosounders can provide quantitative information on the organisms in the water column, which is why fisheries acoustic methods are the primary tool used for fisheries independent monitoring of small pelagic fish species worldwide: The aggregative (schooling) behaviour and patchy distribution of small pelagic fish requires the high sampling resolution achieved by fisheries acoustics to representatively capture their distribution and abundance. Information on other organisms, such as plankton, can also be obtained although the application of this in routine monitoring exercises, although such attempts are still relatively limited. The variable measured is the nautical area scattering coefficient (NASC), which is proportional to the abundance of each species. The data comprise raw files generated by the transceivers, intermediate files produced by processing the raw files through hydroacoustic data processing software, and tables containing the numeric outputs of the echo integration, a fundamental principle of (fish) abundance estimation (Simmonds & MacLennan, 2005). Echosounders can operate at different frequencies, depending on the target organism. Their range is inversely related to frequency, with lower frequencies propagating further than high frequencies. The main disadvantage is its limited ability to distinguish between different species; while the use of multiple frequencies (and particularly recent commercially available broadband systems) can help in distinguishing between “echotypes” based on the frequency response, the vertical distribution and the shape of the echoes, alternative data sources (e.g., direct observation after fishing the detected schools with pelagic trawl or purse seine) are often required to validate the marks seen on the echosounder. Likewise, the use of broadband systems can provide estimates of individuals' sizes in the detected fish schools, but it requires calibration by catch and manual measurement.

Acoustic methods are also particularly relevant to marine mammals. Marine mammals produce sound to communicate, to maintain group structure, and to sense their surroundings. Vocalisations include characteristic whistles, with some species also using echolocation to navigate, and to detect objects and prey by emitting short pulses of sound (clicks) and listening for the echoes. Passive Acoustic Monitoring (PAM) systems use hydrophones (underwater microphones) to detect marine mammal vocalisations. PAM systems consist of a variety of platforms including bottom mounted acoustic recorders, moored recording buoys, towed hydrophone arrays, autonomous underwater or surface vehicles, or profile drifters (Van Parijs et al., 2021). There are two broad types of static (or fixed) PAM that are widely used to detect/monitor cetaceans: (i) sound recorders (**e**.**g**., LS1/LS1X, Loggerhead Instruments) and (ii) click detectors (**e**.**g**., C-PODS, F-PODS) (Embling et al., 2014). Sound recorders are designed to record a wide frequency range to capture sounds produced by a wide variety of sources (geological, biological and human produced). Recording either on a duty cycle (a % of the time) or

continuously, these acoustic recorders are limited by their data storage and battery life capacity. **C**lick detectors, on the other hand, are specifically designed to use real-time processing to select clicks that may have come from cetaceans (dolphins, porpoises and other toothed whales) and many other sources such as acoustic fish tags. Click detectors record compact summaries of each click; therefore, the data typically occupies much less storage than a PAM system that is recording continuously over a wide frequency range. However, click detectors are only appropriate for detecting and monitoring species that vocalise frequently, such as dolphins (i.e., bottlenose dolphins) and porpoises (i.e., harbour porpoises)**,** and are less effective for those with low vocalisation rates, such as baleen whales and seals (Embling et al., 2014). Importantly, PAM efforts complement visual surveys (aerial, boat based and land surveys) in order to provide a more accurate record of species presence and abundance, as they can record at night and during inclement weather conditions when visual surveys are not possible. Furthermore, PAM can provide valuable information on species behaviour in the absence of human presence from a survey vessel (Michel et al., 2024).

Beyond mammals, progress in ocean acoustics during the early 1980s allowed for the estimation of relative distribution patterns and biomass of zooplankton and micronekton using echosounders and acoustic Doppler current profilers (ADCPs). The sound transmitted is scattered by particles in the water column, and the change in frequency between the transmitted and reflected sound — the Doppler shift — is dependent upon the motion of the reflecting particles. In many instances (e.g., in oligotrophic waters) the predominant particles reflecting sound and causing the Doppler shift are planktonic. In addition to measuring Doppler shift, ADCPs are also able to determine the intensity of received echoes. If these particles are zooplankton, then the magnitude of the returned echo intensity is proportional to zooplankton biomass. The instruments can be vessel-mounted, bottom-mounted, or fixed on moorings or towed vehicles (Aparna et al., 2022 and references therein). Interestingly, in some cases, there is a relationship between the backscatter signal and the biomass of organisms of smaller size than expected at the frequency used, which has been attributed to predator-prey relationships (Aparna et al., 2022 and references therein). Using a combination of different frequencies and examining the difference of mean volume backscattering strength between frequencies, it is possible to distinguish certain groups of zooplankton (Sakinan et al., 2019 and references therein).

*Table 18: Techniques and sensors used by acoustic methods. TRL: Technology Readiness Level. ** Many options are available for acoustic recorders and PAM systems (too many to list) so selected examples are provided. See also Seiche, RTsys (i.e., RESEA, SYLENCE-LP, TRIPOD)*

Technique	Sensor name	Target organisms	Variables measured (units)	Detection limit/range	TRL	Level of automation	Platform
Fisheries acoustics	Echosounder (EK80)	Pelagic fish	NASC (s_A) in m^2nmi^{-2}	Dependent on frequency, Power and pulse duration Limit: millimeters to meters and range: centimeters to 100s meters	9	Semi-automated	ROV, AUV, Moorings etc, Ships of Opportunity
Zooplankton acoustics	Echosounder (EK80) or	Meso & Macro	NASC (s_A) in m^2nmi^{-2}	Dependent on frequency, Power and pulse duration	9	Semi-automated	ROV, AUV, Moorings

	ADCPs (e.g., Teledyne RDI)	zooplankton		Limit: millimeters to meters and range: centimeters to 100s meters			etc, Ships of Opportunity
Mammal acoustics **	The Chelonia C-POD (click detector)	Marine mammals	Click snippets recorded (audio), Statistics including positive detection hours (PDH)	Maximum detection range for porpoises is approximately 400 metres. Dolphins may be detected at >1 km	9	Fully automated (just needs to be programmed initially)	Moorings, floats, Ships of Opportunity
	The Chelonia F-POD (click detector)	Marine mammals	Click snippets recorded (audio), Statistics including positive detection hours (PDH)	Maximum detection range for porpoises is approximately 400 metres. Dolphins may be detected at >1 km.	9	Fully automated (just needs to be programmed initially)	Moorings, floats, Ships of Opportunity
	LS1 / LS1X Loggerhead Instruments (sound recorder)	Marine mammals, Pelagic fish	Records audio (.wav files) that can be processed to understand received dB levels	Detection range depends on the acoustic propagation and ambient noise at the time of recording.	9	Fully automated (just needs to be programmed initially)	Moorings, floats, Ships of Opportunity
	SoundTraps Ocean Instruments (sound recorder and click detection) [Latest model – ST600 for long-term deployments]	Marine mammals, Pelagic fish	Records audio (.wav files) that can be processed to understand received dB levels	Detection range depends on the acoustic propagation and ambient noise at the time of recording	9	Fully automated (just needs to be programmed initially)	Moorings, floats, Ships of Opportunity

2.5.2. Applications

For fisheries acoustics, the main application is monitoring small pelagic fish during annual fisheries independent surveys. These surveys provide information on the distribution of target species, and trends in acoustic-derived annual biomass estimates can be used as indices in stock assessments. The use of increasing number of frequencies and the commercialization of broadband systems (e.g., Simrad EK80) have led to an increased ability to extract quantitative information on other organisms such as plankton and gelatinous organisms. An example of consistent use of acoustics for monitoring of plankton abundance and distribution is work on Antarctic krill (Valdez et al., 2022).

For mammals, PAM can serve as an alternative to, or complement of, real-time visual observations and surveys of marine mammals. It can support the estimation of various individual-level ecological metrics (i.e., species presence, abundance and density, behaviour, and population viability and structure) as well as community-level metrics including species composition and richness (Fleishman et al., 2023). PAM is also often required as a mitigation tool to reduce the risk of potential impact of anthropogenic noise generating activities on marine mammals, notably pile driving, seismic and geophysical surveys. PAM may be required to supplement the visual surveys undertaken by marine mammal observers, and it has been increasingly used for monitoring marine

mammals in poor visibility conditions and during night, especially during winter months when daylight hours are reduced (JNCC, 2017). The role of the PAM operative is to advise a delay in the commencement of the activity if an animal is detected within the mitigation zone.

The main application of zooplankton acoustics is to monitor the relative spatiotemporal changes in zooplankton biomass including aggregations and vertical migration (Aparna et al., 2022 and references therein). In some cases, the intensity of the backscattered sound is used in conjunction with net samples to estimate zooplankton biomass (Yang et al., 2019 and references there in). When using multiple frequencies, it is also possible to discriminate certain groups of zooplankton (e.g., copepods and krill) from fish and other zooplankton (Sakinan et al., 2019 and references therein).

2.5.3. Level of biodiversity information (EBV/EOV)

EBVs

Table 19: EBVs that can be addressed by acoustic methods, as defined by the GEOBON (https://geobon.org/ebvs/what-are-ebvs/).

EBV class	EBV name
Species populations	Species distribution Species Abundance
Species traits	Movement

EOVs

Table 20: EOVs that can be addressed by acoustic methods. Sub-variables: Components of the EOV that may be measured, derived or inferred from other elements of the observing system and used to estimate the desired EOV. Derived products: Outputs calculated from the EOV and other relevant information, in response to user needs. For each EOV, a specification sheet can be downloaded from https://goosocean.org/what-we-do/framework/essential-ocean-variables/.

EOV name	Sub-variables	Derived products
Fish abundance and distribution	- Number, biomass or abundance index of fish of different taxa per unit volume or area of water in a specific region, stock or population, and measured by a standard or known protocol - Numbers or biomass of fish by size/age/stage	- Fish abundance indices
Marine turtle, bird, mammals abundance and distribution	- Species presence/absence, - Age, - Sex, - Count data, - Repeated individual presence (tracking/resights)	- Density - Hotspots - Home range - Utilization distribution (relative occupation of home range) - Movement patterns - Migration pathways - Habitat maps - Population status (increasing, decreasing, or stable)

Zooplankton Biomass and Diversity	Overall biomass; biomass or abundance (or presence/absence) by taxon, functional group or size class	- Geographical distributions by taxon or functional group - Life history timing - Community size structure

2.5.4. SWOT analysis

Table 21: SWOT analysis of acoustic technologies for pelagic biodiversity monitoring (fisheries, mammals and zooplankton).

Strengths	Weaknesses
- Acoustic methods are passive and, therefore, non invasive. They can be deployed from a range of different platforms and operate relatively autonomously. - Advantages of fisheries acoustics include large-scale, high resolution data collection. Fisheries acoustic methods can operate relatively autonomously and cheaply. - PAM systems can be used to monitor marine mammal over large marine areas, as well as over long time periods (Michel et al., 2024). When collecting duty-cycled data (Van Parijs et al., 2021), they can record for over a year before download is required, providing long time series of data (Embling et al., 2014). - Zooplankton acoustics allow monitoring of changes in zooplankton biomass at small spatial (patches) and temporal scales (hourly, thus permitting the monitoring of vertical migration). This is particularly important for studying populations of large individuals (macrozooplankton), which are underestimated with the usual net sampling (200 µm mesh size and speed <1 m s−1) due to their patchy distribution and net avoidance behavior (Sakinan et al., 2019 and references therein).	- Fisheries acoustics suffers from several limitations: • Taxonomic identification of species is limited. • Purchase costs can be significant. • Power supplies can be significant depending on system used. • The number of frequencies required can be high. • The raw data recorded requires specialist processing software which can be expensive and/or requires a high threshold of scientific understanding. • The signal/noise ratio of higher frequencies is highly dependent on the physical properties of the water column on-site. - Sound recorders are limited by the size of the memory of the equipment in terms of the amount of data that can be recorded as well as battery capacity, however, in recent years this has exponentially increased with smaller batteries and larger integrated hard drives and SD cards becoming available. - For zooplankton acoustics, in addition to the limitations mentioned for fisheries acoustics, the relationship between recorded signals and zooplankton biomass may be site, period or instrument specific. Discrimination between zooplankton groups is also limited.
Opportunities	**Threats**
- The possibility of transmitting the acoustic data provides an opportunity to obtain zooplankton stock data in real time. - For mammal acoustics one of the greatest benefits of PAM is the capability to record information about animals during times that human presence is impossible, dangerous, or difficult (e.g., at night, during inclement weather and in remote areas such as further offshore or polar regions). - Algorithms to process large data sets and to automatedly classify habitats and species are under study.	- Acoustic instruments can be expensive - The number of experts is limited - One obvious limitations with PAM is that animals may go undetected: The systems only work when marine mammals are vocalising. - Effective use of PAM requires an understanding of the acoustic behaviour of the animals (frequency range and calling rate) to train automated detectors. - There can also be challenges associated with the deployment and retrieval of PAM systems, particularly in offshore environments where good weather windows are required.

	- There is always the risk that devices will get lost or trawled, and data can also be lost through battery or equipment failure.

2.5.5. References

Aparna, S. G., Desai, D. V., Shankar, D., Anil, A. C., Dora, S., & Khedekar, R. (2022). Seasonal cycle of zooplankton standing stock inferred from ADCP backscatter measurements in the eastern Arabian Sea. *Progress in Oceanography*, *203*, 102766.

Embling, C.B., Wilson, B., Benjamins, S., Pikesley, S., Thompson, P., Graham, I., Cheney, B., Brookes, K.L., Godley, B. J. and Witt, M. (2014). Use of Static Passive Acoustic Monitoring (PAM) for monitoring cetaceans at Marine Renewable Energy Installations (MREIs) for Marine Scotland. Guidance document. 26 pp.

Fleishman, E., Cholewiak, D., Gillespie, D., Helble, T., Klinck, H., Nosal, E. M., & Roch, M. A. (2023). Ecological inferences about marine mammals from passive acoustic data. *Biological Reviews*, *98*(5), 1633-1647. doi: 10.1111/brv.12969.

JNCC. (2017). JNCC guidelines for minimising the risk of injury to marine mammals from geophysical surveys. *Joint Nature Conservation Committee*.

Michel, M., Guichard, B., Béesau, J., & Samaran, F. (2024). Passive acoustic monitoring for assessing marine mammals population in European waters: Workshop conclusions and perspectives. *Marine Policy*, *160*, 105983.

Sakinan, S., Lawson, G. L., Wiebe, P. H., Chu, D., & Copley, N. J. (2019). Accounting for seasonal and composition-related variability in acoustic material properties in estimating copepod and krill target strength. *Limnology and Oceanography: Methods, 17*(11), 607-625. doi: 10.1002/lom3.10336.

Simmonds, J., & MacLennan, D. (2005). *Fisheries Acoustics Theory and Practice*. Second edition. Blackwell Publishing.

Valdez, C., Grados, G., La Cruz, L., Cuadros, G., Cornejo, R., & Castillo, R. (2022). Acoustic characterization of automatically detected krill (Euphausia superba) aggregations in the Bransfield Strait and Elephant Island. *Marine & Fishery Sciences (MAFIS)*, *35*(3), 315-331. doi: 10.47193/mafis.3532022010903.

Van Parijs, S. M., Baker, K., Carduner, J., Daly, J., Davis, G. E., Esch, C., ... & Staaterman, E. (2021). NOAA and BOEM minimum recommendations for use of passive acoustic listening systems in offshore wind energy development monitoring and mitigation programs. *Frontiers in Marine Science*, *8*, 760840. doi: 10.3389/fmars.2021.760840.

Yang, C., Xu, D., Chen, Z., Wang, J., Xu, M., Yuan, Y., & Zhou, M. (2019). Diel vertical migration of zooplankton and micronekton on the northern slope of the South China Sea observed by a moored ADCP. *Deep Sea Research Part II: Topical Studies in Oceanography, 167*, 93-104. doi: 10.1016/j.dsr2.2019.04.012.

2.6. Biologging

2.6.1. General overview

Biologging is a technique whereby an electronic instrument (hereafter 'tag' or 'electronic tag') is attached to a living organism to log the environmental and biological conditions that the organism experiences. In some cases, tags also measure the physiological status of the host organism. The basic concept of biologging was established in the 1960s by Kooyman (1966), who designed a mechanical logging device based on a timer, a pressure sensor and a recording mechanism, and attached it to Weddell seals in the Arctic to measure the depth and duration of their dives. Miniaturisation of biologgers for use in a wide range of circumstances did not occur until solid-state electronics became available in the 1980s, and since then there has been a considerable diversification in the sensors that can be used, and the ways in which data from the sensors are recorded and relayed back to the user. In many cases, loggers need to be physically retrieved to download the data that they have stored (see Metcalfe & Arnold, 1997 for the first example using fish species), but tags that transmit stored data to low earth orbiting satellite systems are increasingly popular, as are tags that transmit measurements via acoustic pulses to listening stations deployed on coasts, in the ocean, or on a vessel that is tracking the tagged organism (Hussey et al., 2015). These transmission methods overcome the limitations of physical data recovery, albeit at a cost: Satellite transmitting tags are very expensive compared to simple data loggers, while the limited listening range of acoustic arrays requires that very large numbers of tagged organisms and listening stations are required. For tags that log data, the length or resolution of the time-series depends on several factors, the most important being the memory available onboard the tag, but also the length of time that the tag is attached to the host organism. Short-study periods enable the logging of very high-resolution time-series (e.g., acceleration, which is often measured at 30-60Hz), but even when logging periods are intended to be lengthy, premature detachment of tags or capture of the host organism can cut them short. The trade-offs between cost, attachment method, impact on the host etc. all need to be considered when designing studies – some methods are more suitable than others depending on the purpose or question being addressed. However, the number and diversity of biologging studies attests to the flexibility and adaptability of the technique: The range of organisms and sizes to which tags have been attached to, and the range of scenarios in which they have been deployed, is large and is only limited by the organism: tag size ratio. As such, tags have been attached to a wide range of fish species, marine turtles, marine mammals, seabirds and even jellyfish.

Most tags deployed in marine studies measure pressure and temperature, with light intensity also commonly included. Sensors to measure other oceanographic variables such as fluorescence, salinity, oxygen, sound and magnetism are also available in various forms (Harcourt et al, 2019; Watanabe & Papastamatiou, 2019). However, it is important to remember that the purpose of biologging is typically to develop knowledge and understanding of the host organism and its ecology, meaning that sensors that measure compass direction, acceleration or movement, geographic position, heart rate, blood chemistry and, in some cases, field of view through still photos or video are also available and can be valuable (Hussey et al., 2015 ; Harcourt et al., 2019). Data from these sensors are integrated with the measurements of the physical environment so that they can be

used alone or integrated with oceanographic information. Furthermore, measurements from tags are often used to derive metrics that describe aspects of the host's activity, movement or behaviour. For example, acceleration data can be used to measure tailbeat frequency or energy expenditure, or as a measure of activity. Alternatively, the data can be used to infer posture and behaviour type.

A critical element that influences or limits how biologging data can be used or interpreted is the extent to which the geographic location of the tag can be deduced, and how often. This is because GPS cannot be used to measure position unless the target organism spends enough time at the surface and can be tagged in a way that a GPS antenna will be able to collect or send a signal (Harcourt et al., 2019). This solution applies to many marine mammal or turtle studies but is not generally applicable to non-air breathers. For these species, assuming that the study period is longer than a few hours or days, methods to determine or estimate geographic location from logged and transmitted data have been developed based on light (Harcourt et al., 2019; Watanabe & Papastamatiou, 2023). The uncertainty associated with light-based estimates can be large, generally on the magnitude of tens of km, and in some cases hundreds of km. State-space modelling approaches are often used to overcome these limitations (Harcourt et al., 2019). The problem of estimating position is negligible if acoustic tags are being used, since the detection of a signal from a tag at a listening station defines the approximate location (within a few hundred metres) of the organism. Sophisticated 3D acoustic positioning systems are available for studies where fine-scale resolution is required (Hussey et al., 2015).

Table 22: Techniques and sensors used by biologging methods. The potential for developing derived indices that measure aspects of behaviour or ecology is not included but is arguably more diverse than the parameters measured. TRL: Technology Readiness Level.

Technique	Sensor name	Target organisms	Variables measured (units)	Detection limit/range	TRL	Level of automation	Platform
Biologging	Pressure	Any	Decibar (dBar)	Immediate vicinity	9	Fully Automated	NA
	Temperature	Any	Centigrade (°C)	Immediate vicinity	9	Fully Automated	NA
	Light	Any	Engineering units, varies per device	Immediate vicinity	9	Fully Automated	NA
	Salinity	Any	Conductivity ($mS.cm^{-1}$)	Immediate vicinity	9	Fully Automated	NA
	Fluorescence	Any	F_{chla} (A.U.) (see section on optical methods)	Immediate vicinity	9	Fully Automated	NA
	Acceleration	Any	Gravity (g)	Immediate vicinity	9	Fully Automated	NA
	Magnetism	Any	Tesla (T)	Immediate vicinity	9	Fully Automated	NA
	Flow rate	Any	m s	Immediate vicinity	9	Fully Automated	NA
	Location	Any	Degrees latitude or longitude	Immediate vicinity	9	Fully Automated	NA
	Video	Any	Images (see section on imaging methods)	Immediate vicinity	9	Fully Automated	NA

2.6.2. Applications

The main application of biologging is to determine the position, migrations and behaviour of pelagic organisms in relation to their environment, and thereby define habitat occupation (Hussey et al., 2015; Harcourt et al., 2019; Watanabe and Papastamiatiou., 2019). By combining the results from multiple individuals, species, or studies, it is possible to assess preferred habitat, changes in the suitability of habitat or the drivers of habitat selection. Given the focus on the measurement of environmental variables at the level of the individual organism, it is also possible to understand aspects of movement and behaviour at a range of time-scales and, depending on the number and types of tag used, social or predator-prey interactions. Collecting data from many individuals using biologging techniques can identify important regions/habitats or 'hotspots'. Furthermore, by integrating biologging and animal spatial data with data from other sources, e.g., fishing effort, the extent and risks to populations or habitats can be identified. A good example of this type of work is the interaction between pelagic sharks and the global long-line fishing fleet (Quieroz et al., 2016).

A developing application of biologging is the use of animal-collected data for oceanographic measurements (March et al., 2019), in particular because animals often visit or exploit areas that are ecologically important (e.g., frontal systems) or because animals can sample the water column in places or in ways that oceanographic instruments cannot (e.g., under ice; Fedak, 2004) due to their ability to navigate difficult environments. However, the ethics of using animals as platforms of opportunity for oceanographic studies need to be considered, as attachment of biologging devices can have impacts on the host animal (Fedak, 2004).

2.6.3. Level of biodiversity information (EBV/EOV)

EBVs

Table 23: EBVs that can be addressed by biologging methods, as defined by the GEOBON (https://geobon.org/ebvs/what-are-ebvs/).

EBV class	EBV name
Species populations	Species distribution Species abundance
Species traits	Physiology Phenology Movement Reproduction
Ecosystem functioning	Ecosystem phenology Ecosystem disturbances

EOVs

Table 24: EOVs that can be addressed by biologging methods. Sub-variables: Components of the EOV that may be measured, derived or inferred from other elements of the observing system and used to estimate the desired EOV. Derived products: Outputs calculated from the EOV and other relevant information, in response to user needs. For each EOV, a specification sheet can be downloaded from https://goosocean.org/what-we-do/framework/essential-ocean-variables/.

EOV name	Sub-variables	Derived products

Fish abundance and distribution	- Abundance index of fish of different taxa per unit volume or area of water in a specific region, stock or population, and measured by a standard or known protocol - Numbers or biomass of fish by size/age/stage	- Fish abundance indices - Fish habitat
Marine turtle, bird, and mammal abundance and distribution	- Species presence/absence - Repeated individual presence (tracking/resights)	- Density - Hotspots - Home range - Utilisation distribution (relative occupation of home range) - Movement patterns - Migration pathways - Habitat maps

2.6.4. SWOT analysis

Table 25: SWOT analysis of biologging technologies for pelagic biodiversity monitoring.

Strengths	Weaknesses
- Different types of data can be collected from host animals that move at will in the marine environment, often providing data and information at times of the year or in geographic locations that would otherwise be impossible or expensive to collect. - The wide range of sensors and form factors available means that there are many applications of biologging, suited to different species, environments and timescales, both for studying animal behaviour and the distribution of their prey (when fluorometers and/or imaging sensors are included). - Advancements in tagging technology enhance data accuracy, battery life, and the range of measurable parameters.	- Tags can only be used on organisms that are relatively large (e.g., tens of cm in size). - Tagged devices might disturb swimming and potentially modify animal's behaviour. - Colourful loggers may alter interaction dynamics with conspecifics/heterospecifics. - Information can only be collected from a limited number of individuals at a time due to the logistical or ethical challenges of deploying large numbers of tags, but also because (a) physical recovery of tags is sometimes required to recover the information they collect, and (b) tags that communicate by satellite are expensive, thereby limiting tag numbers by cost. - The ability to estimate the locations of tagged animals can be limited by their behaviour and the accuracy of geolocation models, or by the scale or location of the listening arrays used in acoustic tag studies. The loss of tags can be non-negligible.
Opportunities	**Threats**
- Since it is generally possible to integrate biologging data with other forms of environmental data (e.g., from satellites or synoptic survey programmes), an integrated and holistic view of species ecology can be obtained. - Furthermore, it enables decisions on the deployment of large and expensive tracking or observational infrastructure to be targeted more efficiently.	- Animal welfare, legal and ethical concerns. - Limited available training for the tagging process - Limited experts.

- Outreach initiatives based on biologging can raise public awareness about marine ecology and conservation issues.	

2.6.5. References

Fedak, M. (2004). Marine animals as platforms for oceanographic sampling: a" win/win" situation for biology and operational oceanography. *Memoirs of National Institute of Polar Research. Special issue*, *58*, 133-147.

Harcourt, R., Sequeira, A. M., Zhang, X., Roquet, F., Komatsu, K., Heupel, M., ... & Fedak, M. A. (2019). Animal-borne telemetry: an integral component of the ocean observing toolkit. *Frontiers in Marine Science*, *6*, 326. https://doi.org/10.3389/fmars.2019.00326

Hussey, N. E., Kessel, S. T., Aarestrup, K., Cooke, S. J., Cowley, P. D., Fisk, A. T., ... & Whoriskey, F. G. (2015). Aquatic animal telemetry: a panoramic window into the underwater world. *Science*, *348*(6240), 1255642. doi: 10.1126/science.1255642

Kooyman, G. L. (1966). Maximum diving capacities of the Weddell seal, Leptonychotes weddelli. *Science*, *151*(3717), 1553-1554.

March, D., Boehme, L., Tintoré, J., Vélez-Belchi, P. J., & Godley, B. J. (2020). Towards the integration of animal-borne instruments into global ocean observing systems. *Global change biology*, *26*(2), 586-596. https://doi.org/10.1111/gcb.14902

Metcalfe, J. & Arnold, G. (1997) Tracking fish with electronic tags. *Nature*, *387*(6634), 665-666.

Queiroz, N., Humphries, N. E., Mucientes, G., Hammerschlag, N., Lima, F. P., Scales, K. L., ... & Sims, D. W. (2016). Ocean-wide tracking of pelagic sharks reveals extent of overlap with longline fishing hotspots. *Proceedings of the National Academy of Sciences*, *113*(6), 1582-1587. https://doi.org/10.1073/pnas.1510090113

Watanabe, Y. Y., & Papastamatiou, Y. P. (2023). Biologging and biotelemetry: tools for understanding the lives and environments of marine animals. *Annual Review of Animal Biosciences*, *11*, 247-267. https://doi.org/10.1146/annurev-animal-050322-073657

2.7. Remote sensing

2.7.1. General overview

The potential of remote sensing for marine pelagic biodiversity includes the direct detection of the presence of some biodiversity groups (e.g. phytoplankton groups, macroalgae or marine mammals and bird colonies) and their spatial and temporal distribution, and the remote estimation of biophysical properties (temperature, salinity, turbidity, etc.) and features (fronts, seascapes, etc.) that can determine the distribution and abundance of species in different habitats (Kavanaugh et al., 2021). Remote sensing (RS) systems can be divided into passive and active. Passive RS record reflected electromagnetic energy in the visible, Near-Infrared (NIR), and Shortwave

Infrared (SWIR) bands, as well as emitted electromagnetic energy in the Thermal Infrared (TIR) bands. They are used, for instance, for optical earth observation (ocean colour in aquatic environments) or surface temperature. Active RS systems (e.g., microwave systems) measure, after emission, the backscattering radiation from different objects on the Earth. Active remote sensing is typically used to measure the sea surface height. The spectral resolution of the sensors (i.e., the width, wavelength, and number of spectral bands) will determine the aquatic parameters that can be retrieved. Although turbidity and suspended particulate concentration can be retrieved with a single band in the red or NIR, chlorophyll *a* requires at least two bands in blue and green regions in oceanic waters and even more in optically complex waters. Finally, retrieval of phytoplankton groups is the most exigent in term of spectral resolution. In addition to the spectral characteristics, the spatial and temporal resolutions provided by the platforms are crucial to define the applicability of RS observations to different research objectives. For ocean colour applications, water bodies require short revisit period with high (coastal and inland waters) to moderate (ocean) spatial resolution.

Satellite ocean colour missions are the most used RS systems for marine pelagic research. Ocean colour instruments measure ultraviolet, visible, and near infrared light at the top of Earth's atmosphere, and atmospheric correction algorithms are applied to obtain remote-sensing reflectances (Rrs), a measure of the colour of Earth's surface. Rrs are then related to biogeochemical quantities (i.e. chlorophyll *a*, turbidity, particulates, etc.) of interest using statistics or descriptive algorithms. Although the technique of ocean colour satellite remote sensing was demonstrated in 1978 with the successful launch of NASA's Coastal Zone Color Scanner (CZCS), it was not until the launch of SeaWiFS in 1997 that the method started to be used regularily within research, with a continuously growing field supported by new research missions with a spatial resolution between 300m–1 km, such as MODIS-Aqua, MODIS-Terra, MERIS-ENVISAT and VIIRS-SNPP. Other commercial higher resolution (60–100 m) missions such as Landsat-TM have also been applied. With the long-term commitment from the European Space Agency and the Copernicus programme including the Sentinel satellites since 2015, the Sentinel-3 satellite constellation can now provide ocean colour data with 1–2 days revisiting time, making it possible to monitor fast changes in the marine environment. The OLCI sensors onboard Sentinel-3 are a typical ocean colour sensors based on the MERIS heritage, whereas the MSI sensor on Sentinel-2 is primarily dedicated to land monitoring, but with an additional band in the blue, making it also applicable to ocean colour applications. This is likewise the case for the OLI sensor onboard Landsat-8 and landat-9. Those sensors provide a lower spectral resolution but higher spatial resolution (10–60 m) for a 5-10 day revisit time. New hyperspectral satellites are currently being developed with applications for pelagic habitats and biodiversity mapping, the latest of which, PACE, was launched in early 2024. The use of drones equipped with multi-spectral or hyper spectral sensors are also becoming more common but uses are so far limited to restricted localities and largely for research applications.

Table 26: Description of the main aquatic optical sensors for potential use in OBAMA-NEXT.

Platform	Sensor name	Period covered	Coverage	Spatial resolution	Temporal resolution	Spectra ranges (nm)	Number of bands

Satellite	Sentinel 3 OLCI A/B	Since Sept. 2016	Global, polar orbiting	300 m	Every day	400-1020	21
Satellite	MODIS	Since Sept. 2002	Global, polar orbiting	1km, 500 m, 250 m	Every day	405-14.385	36
Satellite	VIIRS	Since 2011	Global, polar orbiting	750 m,375 m	Every day	415-1200	22
Satellite	Sentinel 2 MSI A/B	Since June 2015	Global, polar orbiting	10 to 60 m	Every 5 days	443-2202	12
Satellite	Landsat 8 - OLI	Since Feb. 2013	Global, polar orbiting	100 m	Every 16 days	440 -1370	9
Satellite	Landsat 9 – OLI2	Since Sept. 2021	Global, polar orbiting	30 m	Every 16 days	440 -1370	9
Satellite	PACE	From spring 2024	Global, polar orbiting	1.2 km	Every 2 days	315-895	119
Piers, ferries, boats	TRIOS / HYPSTAR	Since 2020	Fixed stations	NA	Every 20 minutes	400-1000	Every 1 nm to 2.5 nm
Drones	RGB, multispectral or hyperspectral cameras		Restricted areas where applied	Centimetres to meter scale	One measurement	variable	variable

Table 27: Water parameters retrieval feasibility as a function of sensor and optical water type.

Parameter	**MODIS, VIIRS & S3 OLCI**	**S2 MSI**	**Landsat 8/ Landsat 9**	**PACE**	**Drones**	**Piers, ferries boats**
Chlorophyll *a*	Potentially feasible for all water types but complex in very high CDOM waters	Feasible in case-1 waters[1] and eutrophic waters	Only feasible in case-1 waters[1]	Potentially feasible for all water types	Depends on the spectral resolution (see text for discussion and examples).	
Turbidity	Feasible	Feasible	Feasible	Feasible		
SPM	Feasible	Feasible	Feasible	Feasible		
Rrs	Feasible	Feasible	Feasible	Feasible		
Light attenuation, Kd	Potentially feasible for all water types	Feasible in case-1 waters and case-2 turbid and eutrophic waters[1]	Feasible in case-1 waters and turbid case-2 waters[1]	Potentially feasible for all water types		
Particulate backscatter	Potentially feasible for all water types	Feasible in case-1 waters and case-2 turbid and eutrophic waters[1]	Feasible in case-1 waters and turbid case-2 waters[1]	Potentially feasible for all water types		
CDOM absorption coefficient at 443nm	Potentially feasible for all water types	Very complex without a band at 412 nm	Very complex without a band at 412 nm	Potentially feasible for all water types		

[1] Case-1 waters are typically oceanic waters with Phytoplankton (Chl *a*) as the main contribution to the Rrs and Case-2 waters are typically coastal, lakes and waters affected by land, also called complex waters, containing Chl *a*, CDOM and SPM.

Cyanobacteria bloom	Potentially feasible for all water types	Very complex without a band at 620 nm	Not feasible	Potentially feasible for all water types	
Net Primary Production	Feasible for case-1 waters and more complex for case-2 waters[1]	Not feasible	Not feasible	Feasible for case-1 waters and more complex for case-2 waters[1]	
Phytoplankton groups	Feasible for case-1 waters and more complex for case-2 waters[1]	Very complex due to the limited spectral resolution	Not feasible	Feasible for case-1 waters and more complex for case-2 waters[1]	
Floating vegetation detection	Feasible	Feasible	Feasible	Feasible	

2.7.2. Applications

There has been considerable success in retrieving accurate chlorophyll *a* estimates from water reflectance with blue/green-ratio algorithms in oceanic Case-1 waters[1]. In contrast, the optical complexity of coastal waters makes it difficult to accurately retrieve biogeochemical parameters using satellite remote sensing (Lee and Hu, 2006; Dierssen, 2010). Chlorophyll *a* retrieval by blue/green band-ratio algorithms tends to fail when applied to coastal waters where optical properties may be strongly influenced by non-covarying concentrations of suspended particulate matter (SPM) and coloured dissolved organic matter (CDOM) or where the chlorophyll *a* can reach extremely high values (Gower et al., 2005; Smith et al., 2018). In Case-2 waters[1], a large variety of regional algorithms have been developed to improve chlorophyll *a* estimates from water reflectance in these types of waters (see Odermatt et al., 2012 for a review). However, in general, there is no clear definition of when an algorithm will give reliable output nor which algorithm will perform best for each image pixel. This lack of knowledge has been handled by, e.g., Lavigne et al. (2021), where the authors proposed a methodology based on water reflectance only to determine when three complementary algorithms can be used with limited error. This work allows for an automatic processing of different types without a priori knowledge on water properties. However, it does not allow for the processing of all kinds of waters.

Ocean colour remote sensing has become increasingly important in studying phytoplankton phenology. It allows for the collection of long-term, continuous data at large spatial scales, contributing to significant advances in phenology analysis (e.g., Friedland et al., 2018; Sagarminaga et al., 2024). Most studies cover regional scales and typically focus on the spring bloom, specifically the timing of its initiation, duration and peak, indicated by the maximum chlorophyll *a* concentration. There are fewer addressing indicators on the number, timing, and patterns of secondary and/or episodic blooms. Although daily resolution is optimal for bloom phenology applications, most studies use fortnightly or monthly composites to avoid gaps. However, using a temporal resolution coarser than one day may be insufficient to accurately assess changes in bloom phenology in areas where bloom-inducing conditions change rapidly. As climate change threatens to alter bloom phenology worldwide, it is crucial to fill these knowledge gaps, particularly as different regions may be affected differently.

New algorithms and processing methods are continuously being proposed to provide improved products for the identification of HAB risk areas. For instance, a web alert system to track the development, magnitude and spread of HABs (*Karenia mikimotoi, Phaeocystis globosa, Pseudo-nitzschia* spp.) in the French-English Channel with satellite data has been developed within the INTERREG-VA FCE project S-3 EUROHAB (https://www.s3eurohab.eu/) and in the in the HELCOM region of the Baltic Sea for the cyanobacterial bloom index (HELCOM, 2018). Similar studies using satellite data for HAB risk identifications have been proposed for many other species in many other regions, and the applications are many.

Cyanobacterial blooms are a familiar sight in freshwater and brackish water bodies near centres of human activity, posing health and economic threats. This can sometimes be problematic in the Baltic Sea, where cyanobacterial blooms occur every summer. Although these blooms are naturally occurring, they have been intensified due to human pressures. The methods for identification of cyanobacterial blooms rely on algorithms targeting the pigment phycocyanin, with a broad absorption spectrum at ~620 nm. The algorithms used can either target the phycocyanin spectral absorption (Kutser, 2009) or make use of the changes in the spectral shape between 665, 681, and 709 nm (e.g., Matthews et al. 2012). Hence, the RS sensors used to detect cyanobacteria blooms need to have a high and specific spectral resolution. Despite advances in development of both semi-analytical phycocyanin targeting methods and spectral shape methods, remote-sensing methods for detection of cyanobacterial HABs are still limited. For this objective, Sentinel 3 OLCI, and potentially the upcoming PACE hyperspectral sensor, represent the best adapted sensors.

To improve our understanding of the role of phytoplankton for marine ecosystems and global biogeochemical cycles, information on the global distribution of major phytoplankton groups is essential. Although algorithms have been developed to assess phytoplankton diversity from space for over two decades, so far, the application of these data sets has been limited. Ocean colour algorithms to assess phytoplankton diversity make use of information originating from phytoplankton abundance, cell size, bio-optical properties, and environmental features to differentiate phytoplankton groups (Phytoplankton Functional Types – PFTs). The abundance-based approaches of, e.g., Hirata et al. (2011) use satellite chlorophyll *a* as an input to derive phytoplankton size group or phytoplankton types based on empirical relationships linking *in situ* marker pigments to chlorophyll *a* which are determined using high precision liquid chromatography (HPLC). Abundance-based approaches use satellite chlorophyll *a* as an input and by that they exploit the largest signal in water, leaving radiance to extract variability due to phytoplankton groups (out of chlorophyll *a*). This is then a simple calculation and can be applied easily to chlorophyll *a* outputs from different sensors. However, they cannot predict atypical associations are not adapted to optically complex waters. Another class of algorithms relies on spectral features in reflectance, absorption, and/or backscattering spectra caused by the variation in phytoplankton structure and pigment composition. Spectral-based approaches exploit as much of the backscattered spectrum observed by satellite as necessary to extract the signatures of specific phytoplankton group to ocean colour to retrieve main phytoplankton groups (Alvain et al., 2008; Bracher et al., 2009; Sadeghi et al., 2012; Xi et al., 2020). For instance, PhytoDOAS (Bracher et al., 2009; Sadeghi et al., 2012) retrieves the imprints of absorption characteristics of specific phytoplankton

groups among all other atmospheric and oceanic absorbers from data from the top of the atmosphere from the hyperspectral satellite sensor SCIAMACHY (Scanning Imaging Absorption Spectrometers for Atmospheric Chartography). Another approach incorporates various environmental parameters to predict phytoplankton groups based on their ecological preferences (Raitsos et al., 2008). This method uses artificial neural networks to link different biological and physical data sets and allowed for the development of a regional algorithm in optically complex waters, where a main phytoplankton groups retrieval algorithm did not apply.

Applications involving direct identification of pelagic wildlife like large whales, resting seals, and bird colonies need very high resolution (VHR) satellites with submeter spatial resolution. A review of different projects applying VHR satellite missions for whale detection was provided by Cubaynes et al. (2019) and for monitoring cetaceans mass strandings by Clarke et al. (2021). These VHR systems are also being used to make censuses of southern elephant seals (Fudala and Bialik, 2022) Weddell seals in Antarctica (LaRue et al., 2021), emperor penguins (Fretwell and Trathan, 2021), and albatross colonies on inaccessible islands (Fretwell et al., 2017). Increasing interest, combined with the advancements in VHR satellite resolution and revisit rates with the launches of new optical satellites in the coming years, drone technology, machine learning, and cloud computing provides the momentum needed for the creation of a rapid management tool for the detection of marine mammals (Rodofili et al., 2022; Khan et al., 2023).

A more common approach in many of the studies using remote-sensing data to assess marine wildlife biodiversity is to correlate information collected in situ on species numbers and diversity with certain variables detectable in RS data (e.g. sea surface temperature, chlorophyll *a*, turbidity, surface roughness, etc). Good correlations between species representations and certain variables have been found by many authors (Kuenzer et al., 2014). They use this as an argument supporting the high value of remote-sensing data for biodiversity assessments and habitat suitability products that can act as a proxy for species occurrence or richness (Kuenzer et al., 2014).

A review of RS applications for fisheries oceanography can be found in Santos (2000). Although fish schools cannot (at least with the sensors presently available on non-commercial satellites) be seen directly from satellites, RS systems have been used to identify and/or predict potential favourable zones of fish aggregation (e.g., ocean fronts) or support studies on the development and survival of eggs and larvae, as well as the distribution, aggregation, migration and schooling behaviour of juvenile and adults of several fish stocks. However, some initiatives on direct identification of schools of juvenile anchovies, jack mackerel, skipjack tuna, southern bluefin tuna, dolphins and mixed schools of tuna and dolphins and have been successful using airborne active sensors like Light Detection And Ranging (LIDAR) (Churnside and Hunter, 1996) and Side-Looking Airborne Radar (SLAR) (Griffiths et al., 1989).

Remotely sensed parameters have been used for aquaculture site selection of fish, shellfish and macroalgae. RS images in Geographic Information Systems (GIS) are used for spatial multi-criteria evaluation methodologies to resolve complex environmental and socioeconomic constraints. Besides site-selection and planning, aquaculture could also benefit from EO for water quality monitoring, notably in the case of HAB detection, assessment of

fish farming environmental impact, and modelling of species invasion associated with aquaculture (Gernez et al., 2021).

2.7.3. Level of biodiversity information (EBV/EOV)

EBVs

Table 28: EBVs that can be addressed by remote sensing methods, as defined by the GEOBON (https://geobon.org/ebvs/what-are-ebvs/).

EBV class	EBV Names
Species populations	Species distributions Species abundances
Species traits	Phenology Movement
Community composition	Community abundance Trait diversity
Ecosystem function	Primary productivity Ecosystem phenology Ecosystem disturbances
Ecosystem structure	Ecosystem distribution

EOVs

Table 29: EOVs that can be addressed by remote sensing methods. Sub-variables: Components of the EOV that may be measured, derived or inferred from other elements of the observing system and used to estimate the desired EOV. Derived products: Outputs calculated from the EOV and other relevant information, in response to user needs. For each EOV, a specification sheet can be downloaded from https://goosocean.org/what-we-do/framework/essential-ocean-variables/.

EOV name	Sub-variables	Derived products
Biology and ecosystems	- Phytoplankton biomass and diversity	- Chlorophyll *a* concentration - Phytoplankton functional Types - Phytoplankton Size Class - Harmful algal bloom - Cyanobacteria bloom - Eutrophication
	- Fish abundance and distribution - Marine turtles, mammals abundance and distribution	- Species presence/absence - Repeated individual presence (tracking/resights)
Cross disciplinary	- Ocean colour	- All

2.7.4. SWOT analysis

Strengths	Weaknesses
- Ocean colour satellite observations offer many advantages for ecosystem and biodiversity mapping. The main one is global coverage and temporal revisits, which allows to capture all features of spatial and temporal variability as soon as these features are of sufficient scale. Satellite data also ensure a regular revisit time ranging from 1 day for	- Ocean colour observations are limited by cloud and ice cover, especially in high latitudes with frequent cloud cover and during the winter when the sun angle is too low for acquisitions. - As all satellite remote sensing products are based upon a signal reaching a sensor, i.e., the radiance at the top of atmosphere, in which the water leaving radiance (Rrs) represents only 1% to 10% of the

Sentinel-3A/B, to 3-5 days with Sentinel-2, and 8 days for LANDSAT8/9. - Satellite acquisitions are very reliable and cannot be cancelled by a 'bad' weather forecast, instrument disruption or human issues. - Satellites data represents a low-cost source of information as, e.g., NASA and ESA generally deliver data as well as different products for free. Only very high-resolution images (meter-scale) like the Pléiades or Planetscope constellations are ordered on request and so can be expensive. - The developments, maintenance and calibration of the sensors and data from the large space agencies ensure the consistency of a QA dataset over its full period of activity which can reach several decades and the inter-operability of different sensors like, e.g., Sentinel-3 A and B. This also permits climatic studies to be performed using remote sensing observations. - With appropriate atmospheric corrections and algorithms, and appropriate sensors (e.g. hyperspectral), it is possible to derive proxies for phytoplankton biomass from suspended detrital matter and phytoplankton functional types (Alvain et al., 2004).	signal, correct inversion algorithms and removing of the atmospheric signal is crucial. Although atmospheric corrections are less complicated over clear waters and works well, it is always based on assumptions and extrapolations which can propagate to errors, especially in the blue part of the spectrum. Therefore, the application of atmospheric corrections in complex waters usually requires specific selections and adaptations to minimize errors. Ensuring the validity of the atmospheric correction is therefore important to consider when using ocean colour RS data. - Many algorithms exist for each parameter, and the difficulty is to know which algorithm is valid for the study area, which requires expertise knowledge and understanding of the optical properties and their effects on the data. - Other efforts are made to provide algorithms adapted to different optical water types that can be used in different regions without a priori knowledge. These algorithms are either based on machine learning or use a switching approach which includes existing algorithms. - Inexperienced end-users of RS data usually need inputs and guidance from experts to understand the uncertainties and the validity of the data
Opportunities	**Threats**
- Global coverage largely helps to improve spatial understanding of ecosystems mapping and can be used in complement of specific *in situ* data which generally only provide information on scattered stations and can easily miss the position of oceanic fronts, for instance. - Repeatable large-scale global monitoring. - Different satellites and/or associated sensors provide upscaling/downscaling spatial resolution and different temporal resolutions. - Advances in sensor technology that could improve pelagic diversity mapping. - Cloud processing. - Combining satellites with other sensors such as drones and AUVs, or automated underway sub-surface recording and measurements with optical, automated flow cytometry and/or automated imaging devices to improve mapping. - New capabilities using AI to improve classification and sensor development.	- Mixed classification between spectrally similar species groups - this often requires expertise in marine ecology/taxonomy, which could then end up being costly. - Data load and computational requirements may create bottlenecks or require large resources. - The decrease of in situ data for validation - The availability of images from providers

2.7.5. References

Alvain, S., Moulin, C., Dandonneau, Y., & Loisel, H. (2008). Seasonal distribution and succession of dominant phytoplankton groups in the global ocean: A satellite view. *Global Biogeochemical Cycles*, *22(3)*.

Bracher, A., Vountas, M., Dinter, T., Burrows, J. P., Röttgers, R., & Peeken, I. (2009). Quantitative observation of cyanobacteria and diatoms from space using PhytoDOAS on SCIAMACHY data. *Biogeosciences*, *6*(5), 751-764.

Churnside, J. H., & Hunter, J. R. (1996). Laser remote sensing of epipelagic fishes. in , eds. V. I. Feigels and Y. I. Kopilevich (St.Petersburg, Russia), 38–53. doi: 10.1117/12.258352

Clarke, P. J., Cubaynes, H. C., Stockin, K. A., Olavarría, C., de Vos, A., Fretwell, P. T., & Jackson, J. A. (2021). Cetacean strandings from space: challenges and opportunities of very high resolution satellites for the remote monitoring of cetacean mass strandings. *Frontiers in Marine Science*, *8*, 650735. doi: 10.3389/fmars.2021.650735

Cubaynes, H. C., Fretwell, P. T., Bamford, C., Gerrish, L., & Jackson, J. A. (2019). Whales from space: four mysticete species described using new VHR satellite imagery. *Marine Mammal Science*, *35*(2), 466-491. doi: 10.1111/mms.12544

Dierssen, H. M. (2010). Perspectives on empirical approaches for ocean color remote sensing of chlorophyll in a changing climate. *Proceedings of the National Academy of Sciences*, *107*(40), 17073-17078.

Fretwell, P. T., Scofield, P., & Phillips, R. A. (2017). Using super-high resolution satellite imagery to census threatened albatrosses. *Ibis*, *159*(3), 481-490. doi: 10.1111/ibi.12482

Fretwell, P. T., & Trathan, P. N. (2021). Discovery of new colonies by Sentinel2 reveals good and bad news for emperor penguins. *Remote Sensing in Ecology and Conservation*, *7*(2), 139-153. doi: 10.1002/rse2.176

Friedland, K. D., Mouw, C. B., Asch, R. G., Ferreira, A. S. A., Henson, S., Hyde, K. J., ... & Brady, D. C. (2018). Phenology and time series trends of the dominant seasonal phytoplankton bloom across global scales. *Global Ecology and Biogeography*, *27*(5), 551-569. doi: 10.1111/geb.12717

Fudala, K., & Bialik, R. J. (2022). Seals from outer space-Population census of southern elephant seals using VHR satellite imagery. *Remote Sensing Applications: Society and Environment*, *28*, 100836. doi: 10.1016/j.rsase.2022.100836

Gernez, P., Palmer, S. C., Thomas, Y., & Forster, R. (2021). remote sensing for aquaculture. *Frontiers in Marine Science*, *7*, 638156. doi: 10.3389/fmars.2020.638156

Gower, J., King, S., Borstad, G., & Brown, L. (2005). Detection of intense plankton blooms using the 709 nm band of the MERIS imaging spectrometer. *International Journal of Remote Sensing*, *26*(9), 2005-2012. https://doi.org/10.1080/01431160500075857.

Griffiths, S. G., & Lynch, W. H. (1989). Characterization of Aeromonas salmonicida mutants with low-level resistance to multiple antibiotics. *Antimicrobial agents and chemotherapy*, *33*(1), 19-26. doi: 10.1128/AAC.33.1.19

HELCOM (2018). Cyanobacteria bloom index. HELCOM pre-core indicator report. Online. Available at: https://helcom.fi/wp-content/uploads/2019/08/Cyanobacterial-bloom-index-HELCOM-pre-core-indicator-2018.pdf (Accessed January 1, 2023).

Hirata, T., Hardman-Mountford, N. J., Brewin, R. J. W., Aiken, J., Barlow, R., Suzuki, K., ... & Yamanaka, Y. (2011). Synoptic relationships between surface Chlorophyll-a and diagnostic pigments specific to phytoplankton functional types. *Biogeosciences*, *8(2)*, 311-327.

Kavanaugh, M. T., Bell, T., Catlett, D., Cimino, M. A., Doney, S. C., Klajbor, W., ... & Siegel, D. A. (2021). Satellite remote sensing and the marine biodiversity observation network. *Oceanography*, *34*(2), 62-79.

Khan, C. B., Goetz, K. T., Cubaynes, H. C., Robinson, C., Murnane, E., Aldrich, T., ... & Lavista Ferres, J. M. (2023). A biologist's guide to the galaxy: Leveraging artificial intelligence and very high-resolution satellite imagery to monitor marine mammals from space. *Journal of Marine Science and Engineering*, *11*(3), 595. doi: 10.3390/jmse11030595

Kuenzer, C., Ottinger, M., Wegmann, M., Guo, H., Wang, C., Zhang, J., ... & Wikelski, M. (2014). Earth observation satellite sensors for biodiversity monitoring: potentials and bottlenecks. *International Journal of Remote Sensing*, *35*(18), 6599-6647. doi: 10.1080/01431161.2014.964349

Kutser, T. (2009). Passive optical remote sensing of cyanobacteria and other intense phytoplankton blooms in coastal and inland waters. *International Journal of Remote Sensing*, *30*(17), 4401-4425.

Lavigne, H., Van der Zande, D., Ruddick, K., Dos Santos, J. C., Gohin, F., Brotas, V., & Kratzer, S. (2021). Quality-control tests for OC4, OC5 and NIR-red satellite chlorophyll-a algorithms applied to coastal waters. *Remote Sensing of Environment*, *255*, 112237.

LaRue, M., Salas, L., Nur, N., Ainley, D., Stammerjohn, S., Pennycook, J., ... & Rotella, J. (2021). Insights from the first global population estimate of Weddell seals in Antarctica. *Science Advances*, *7*(39), eabh3674. doi: 10.1126/sciadv.abh3674

Lee, Z., & Hu, C. (2006). Global distribution of Case-1 waters: An analysis from SeaWiFS measurements. *Remote Sensing of Environment*, *101*(2), 270-276.

Odermatt, D., Gitelson, A., Brando, V. E., & Schaepman, M. (2012). Review of constituent retrieval in optically deep and complex waters from satellite imagery. *Remote sensing of environment*, *118*, 116-126.

Raitsos, D. E., Lavender, S. J., Maravelias, C. D., Haralabous, J., Richardson, A. J., & Reid, P. C. (2008). Identifying four phytoplankton functional types from space: An ecological approach. *Limnology and oceanography*, *53(2)*, 605-613.

Rodofili, E. N., Lecours, V., & LaRue, M. (2022). Remote sensing techniques for automated marine mammals detection: a review of methods and current challenges. *PeerJ*, *10*, e13540. doi: 10.7717/peerj.13540

Sadeghi, A., Dinter, T., Vountas, M., Taylor, B., Altenburg-Soppa, M., & Bracher, A. (2012). Remote sensing of coccolithophore blooms in selected oceanic regions using the PhytoDOAS method applied to hyper-spectral satellite data. *Biogeosciences*, *9(6)*, 2127-2143.

Sagarminaga, Y., Borja, Á., & Fontán, A. (2024). “Extreme Highest” and “Extreme Anomalous”: Proposed indices for chlorophyll-a extreme events in European seas between 2003 and 2021. *Remote Sensing of Environment*, *300*, 113885. doi: 10.1016/j.rse.2023.113885

Santos, A. M. P. (2000). Fisheries oceanography using satellite and airborne remote sensing methods: a review. *Fisheries Research*, *49*(1), 1–20. https://doi.org/10.1016/S0165-7836(00)00201-0

Wang, M., & Hu, C. (2021). Satellite remote sensing of pelagic Sargassum macroalgae: The power of high resolution and deep learning. *Remote Sensing of Environment*, *264*, 112631. doi: 10.1016/j.rse.2021.112631

Xi, H., Losa, S. N., Mangin, A., Soppa, M. A., Garnesson, P., Demaria, J., ... & Bracher, A. (2020). Global retrieval of phytoplankton functional types based on empirical orthogonal functions using CMEMS GlobColour merged products and further extension to OLCI data. *Remote Sensing of Environment*, *240*, 111704.

3. Citizen Science

Citizen Science (CS) usually labels research activities conducted by citizens, and citizens doing research are called citizen scientists. In this section, we present the findings of a Scopus literature review carried out to elucidate the state of the art of CS in marine biology for biodiversity (MBB) and extrapolate further insights and knowledge about the use of technologies and levels of engagement in CS practices aimed at collecting scientific data with an emphasis on pelagic (and benthic) observations. We briefly introduce different forms of CS and outline current scholarly discussions and research themes on CS, including summarising recent debates regarding CS in MBB as addressed by illustrative review articles. In our following Scopus literature review, the applied methodology and findings on trends and patterns concerning (self-declared) CS practices in MBB are explored and explained. Similarly, we expand on technological tools used by citizen scientists for pelagic observations. The literature review also informed a review of existing and emerging technologies for benthic observations presented in Forsblom et al. (2024).

3.1. What is Citizen science?

Although a clear definition is still lacking, Citizen Science (CS) typically refers to research activities conducted by citizens or engaging citizens at different levels and to a different degree (as data collectors, co-analysts, co-researchers, policy co-developers, or co-disseminators) to deliver scientific knowledge. CS is originally inspired by the principles of co-creation, co-operation, sustainability and/or social impact (Strasser et al., 2019; Kasperowski and Kullenberg 2019; Kasperowski et al., 2019; Jaeger et al., 2023). CS is a response to increasing pressure on universities to open their research processes and scientific activities to explain the benefits of their results to citizens (e.g., via public communication or outreach activities) or to justify public spending on them (Heckers et al., 2018; Vohland et al., 2021).

CS has used as an 'umbrella' term to describe a variety of ways in which citizens can participate to scientific activities leading to scientific knowledge production. The CS concept is related to other notions such as participatory science, post-normal science, civic science, crowd science or open science (Kullenberg and Kasperowski 2016; Kieslinger et al., 2018). However, the main peculiarities of CS distinguishing it from other concepts are: (1) Citizens are actively involved in research, in partnership or collaboration with scientists or academic professionals; and (2) there is a specific outcome such as new scientific knowledge, conservation action or policy changes. An international community of researchers and practitioners – led by the *European Citizen Science Association* (ECSA) – has characterized CS and established the standards for good CS practices in several disciplines (Robinson et al., 2018; Haklay et al., 2020, 2021). These efforts gave origin to a framework of ten principles (10 Principles of Citizen Science – ECSA 2015), which is available in several languages, wherein the CS community established a set of distinctive characteristics for "good" CS practices.

The debate on CS has evolved from more broad concerns regarding democratization of research and the role of citizens in research (Bonney 1996; Irwin 1995) to identify instead, which projects, activities or initiatives

constitute CS, so that CS can easily be distinguished by other forms of participatory research. This novel approach to CS covers research activities to verify if projects are misconceived or mislabelled as CS by its initiators or participants since examples of overuse of the term CS are frequent (Zambon et al., 2023). It responds to pleas for the democratization of science and knowledge production that are common also in later CS studies (Jaeger et al. 2023). While recent research attempts to create a general, comprehensive definition of CS, including specific scientific practices belonging to it (Heigi et al., 2019), this task is made difficult by the broad, interdisciplinary endeavour, and its placement at the intersection of several scientific fields. In this light, we argue that there might be a need for more discipline- or context-specific definitions able to receive broad consensus by practitioners and affiliated scientific communities, and to describe the common features of what scientists expect from CS activities in particular fields. We will attempt this undertaking by focusing on CS in MBB for (benthic and) pelagic observations. Precisely, by an exploration of existing literature – self-labelled as CS – in this field and to observe/highlight which specific patterns CS in this area assumes.

When the ideal of CS has been discussed in relation to specific disciplines and research areas, slightly different ways to conceive CS emerge. Yet, boosting transparency and accountability of research processes delivering policy-relevant knowledge in the broad sense of "science democratization" and active "lay citizens' participation/public involvement" are regularly seen as necessary elements and leading criteria to brand science and scientific activities as CS; particularly relevant is the level of non-expert citizen engagement in higher-level scientific tasks (Haklay et al., 2020; Haklay et al., 2021). Following Goulombic et al. (2017), three fundamental aspects of CS are: a) Inclusion (i.e., how researchers deal with activities built for public participation); b) contribution (i.e., how a project contributes to science, and at the same time, how it impacts the public) and, c) reciprocity (i.e., what citizens and researchers can do for each other in the context of knowledge elicitation and dissemination). Similarly, existing scales to evaluate CS projects by Bonney (2009a, 2009b, 2016) point to the degree of citizen contribution to research results and so, CS studies are defined either merely as contributory (i.e., research questions and/or protocols are established by powerful researchers, institutional promoters and the like with little or no inputs by citizens) or genuinely collaborative (i.e., the citizen involvement in the creation of the project and its protocols is significant and established via genuine collaborations amongst citizens and researchers institutional promoters). In the same line, Haklay at al. (2013a, 2013b) offer a four-level scale of citizen involvement where CS implies voluntary participation at all levels: From the level one (Crowdsourcing: citizens act mainly as sensors) moving up to level two (Distributed Intelligence: citizens act as basic interpreters) and level three (Participatory Science: citizens participate in the problem/aims definition and/or data collection) until the fourth level (Extreme CS: where the research processes are controlled by citizens, and scientists merely facilitate and help out) that is rather difficult to achieve, since it entails that citizens manage collaborative science practices in which all tasks from previous levels (1-2-3) take place almost simultaneously. Haklay et al. (2021) also come up with a set of further specification for classifying CS projects. The most important ones are: 1) the level of cognitive engagement of participants (beyond data collection and gathering), 2) the expertise and training required for participants, and 3) the conditions for data sharing. Evidently, leading conceptions of CS, so as its principles, scales and desiderata imply a wide-ranging participatory science ideal and indeed,

classifications are mainly made according to the extent of participation of lay people, non-expert citizens in scientific research and knowledge production.

In targeting CS in MBB, a central challenge is thus to figure out if and to what extent this participatory ideal is fulfilled by (self-defined) CS studies, due to obvious limitations that non-expert citizens can encounter to actively participate in research and observational tasks in the (benthic and) pelagic environments.

In general, CS is considered a valuable practice in situations where data are lacking or temporally and spatially insufficient or funding is sparse (Andrews et al. 2019, García-Soto et al., 2021). Citizens can make observations at several locations simultaneously and collect data that may not otherwise be possible to obtain (Matear et al. 2019). Yet, uses of CS in MBB have been limited to estimating specific species' distributions, while neglecting the aspects of biodiversity more broadly, e.g., valuing the ecosystem services these species deliver (Chandler et al., 2017; Turbé et al., 2019; Embling et al., 2015; Wege et al., 2020). Furthermore, there is a lack of "marine CS" projects compared to the terrestrial environment and current marine CS largely confines itself to specific approaches (Fulton et al., 2019; Dalby et al., 2021; García-Soto et al., 2021). Citizens involved have variously been depicted as motivated by social engagement, level of enjoyment, the feeling of contributing to something, and participating in a project with minimal effort (Dalby et al., 2021). Still, little work on CS in MBB is done on the more subjective and personal aspects of citizen scientists' motivations and barriers to participation, and the inclusivity of CS projects. Existing research tends to focus on data collection/data output (Dalby et al. 2021), and we suggest that more research could be done focusing on the determinants and conditions of citizen scientist engagement. A way to enclose these concerns could be allowing citizens to contribute to the project with the potential to discover the interesting or unexpected, and not just as a tool for data collection (Lukyanenko et al., 2016). In our systematic SCOPUS review (next section), we have used these remarks as a starting point and to frame the search on recent CS studies in MBB to narrow down and further map methods and tools/technologies used in observational tasks in the (benthic and) pelagic environments. Our findings on CS for pelagic observations is presented in this report. Findings on CS used to study benthic environments are presented in the WP3 review on existing and emerging techniques for benthic habitats (Forsblom et al. 2024).

3.2. Methodology

Following the specific requirements of this report and its focus on technological tools for pelagic observations, we define the main research question that guided our literature review in SCOPUS:
RQ: What are the technologies employed in MBB pelagic studies that exploit CS in their research?

Other repositories such as ACM Digital Library and Google Scholar were tested but were not used in our analysis as their findings frequently lacked relevance. SCOPUS was thus selected as the repository of reference for our study due to its performance when considering our interest in technologies for CS research in MBB.

The search was structured in four and five blocks:

Table: SCOPUS search strategy for literature on CS in MBB.

Block	Search string	Justification
1: Citizen science	(LIMIT-TO (EXACTKEYWORD , "Citizen Science")	We wanted articles that had CS as a central focus, and exclude those that only mention CS.
2: in Marine biology	"Marine Biodiversity"	This was found to be the best way to find articles within the topic of OBAMA-NEXT
3: for pelagic or benthic observations	"Benthic" OR "Pelagic"	We decided to find papers for both pelagic and benthic observations and later manually separate them.
4: Published in the period 2013 to 2023 (10 years)	PUBYEAR > 2012 AND PUBYEAR < 2024	Publications from the latest 10 years seemed appropriate as we were not interested in obsolete technologies.
5: With a focus on automatization	"Automatic" OR "Automated"	We added this fifth block to ensure a focus on automation.

The SCOPUS review was conducted in the last half of September 2023. The PRISMA 2000 guidelines were followed for paper selection and screening. More than 1000 articles were found when we only searched on CS as a keyword. Adding the additional three blocks gave 103 total articles. This, together with 13 additional papers from the OBAMA-NEXT application, gave 116 papers meeting our criteria. We then screened papers for references to specific CS tools/technologies and and a focus on automation (block 5). This lowered the total to 112 papers.

To be included in this study, articles needed to explicitly refer to CS initiatives and introduce the tools as either employed in CS projects or as providing data to "citizen scientists" (due to the peculiarity of the research settings, here the term is understood broadly to include non-academic experts as well, e.g., scuba divers). During this process a further five articles were not retrievable and so were excluded.

After an additional screening phase, ten more papers were excluded as they failed to provide enough information to assess whether the related research/projects could fit the dominant CS definitions. Moreover, eight articles were removed from this study since they did not report who took part in the project, making it impossible to assess the features of the participants and their degree of involvement in some undefined tasks (vital CS criteria). This last screening left 89 papers.

Next, we manually categorized the papers according to whether the reported technologies were used for pelagic observations, benthic observations, both benthic and pelagic observations, or were difficult to classify. Louise Forsblom assisted us in the task. 42 papers reported on technologies used for benthic observations, 21 papers for pelagic observations, and 13 papers for both benthic and pelagic observations. The remaining 13 papers could not be classified. Thus, 76 papers were relevant for further analysis.

The 76 selected papers were used in the subsequent categorization phase. Each of the papers was thoroughly reviewed to assess the papers relevance to our research aims manually (by quickly reading all papers' abstracts and conclusions) and further classified based on predetermined criteria, including year of publication (YEAR),

tools used for data gathering (TOOL), geographical area of interest of the research (AREA), species the research is focusing on (SPECIES).

3.3. Findings

In reporting the findings, we mainly focus on CS publication trends in MBB and further highlight the technologies used by "citizen scientists" (citizens, non-academic experts) for pelagic observations. We also briefly report on the geographical areas of the identified studies and the species targeted by the CS papers.

Figure 1 shows that, overall, there is a steady growth in the number of articles published per year addressing CS in MBB (both pelagic and benthic). The number of identified articles seems to stagnate in 2023, but this is because the search was conducted in the last half of September 2023. Additional articles have most likely been published in the last quarter of 2023 resulting in continued increase in publications regarding CS in MBB. This might underscore a beginning enthusiasm within the scientific community of MBB for collaborative and community-driven research endeavours. As the years has progressed, this positive trend not only seems to reflect an increasing recognition of the value of CS in MBB research but also may signify a significant shift towards more inclusive, participatory approaches to scientific inquiry and scientific knowledge production. This upsurge of published works dedicated to exploring marine ecosystems with the engagement of citizen scientists might demonstrate a collective commitment to harnessing diverse perspectives and expertise in addressing complex ecological challenges.

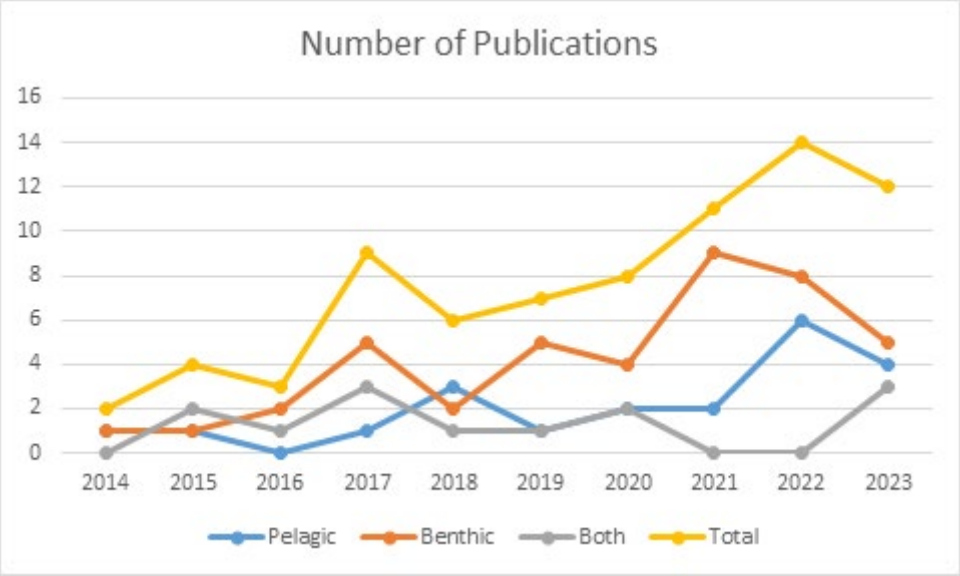

Figure 2: Publication trends for CS in MBB.

Furthermore, these results indicate that collaboration between (ordinary) scientists and citizens for advancing our understanding of marine environments and fostering sustainable stewardship practices is increasingly taking place. In it, this increase in academic publications on CS likely documents both its progress, but also, its ability to foster critical discourse and knowledge exchange in non-academic scientific circles (enhancing science

dissemination and outreach). In our view, our dataset/review can be taken as an example and repository of practices, methodological innovations, and empirical findings enriching the collective understanding of CS in MBB, including its caveats and potential limits.

There are typically more CS studies published on benthic data than on pelagic data. One possible explanation is that non-academic experts, such as scuba divers, are often involved in CS in MBB, which may explain why there are more CS publications for studies of benthic habitats.

3.3.1. Technologies for CS in MBB for pelagic observations

In the following section, we report on the characteristics of the 34 articles related to pelagic observations (21 articles) and to both pelagic and benthic observations (13 articles).

As shown in Figure 2, the most frequently represented technologies used for pelagic observations are cameras (10), followed by online tools (7), tools for doing surveys (6), custom and standard toolkits (4), laboratory technologies (3), satellite (2), and sampling technologies (2).

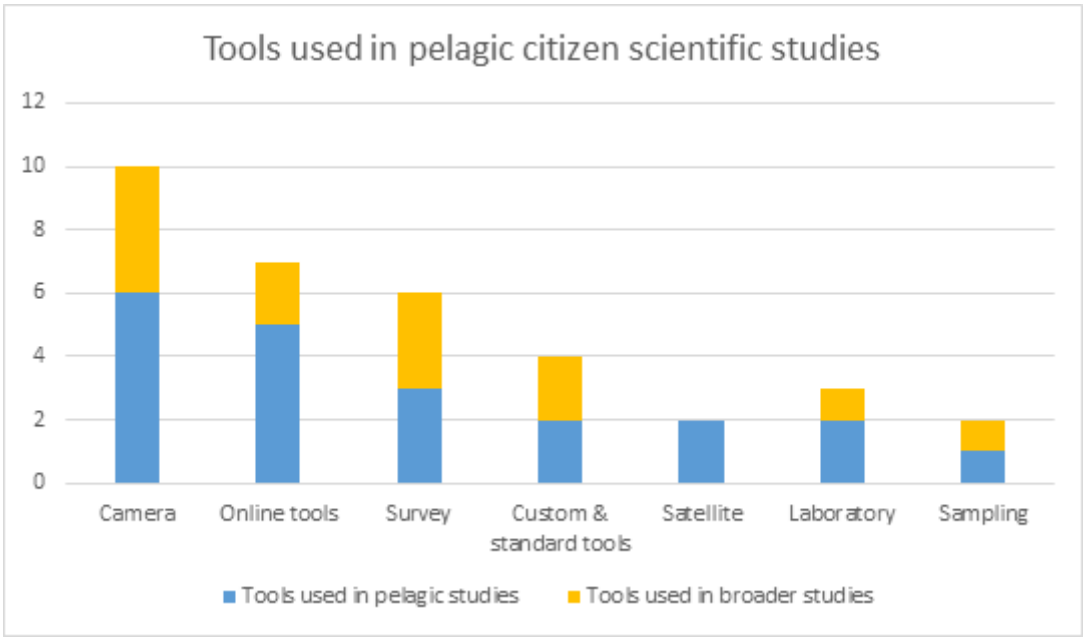

Figure 3. Technologies used in CS for pelagic observations.

A smaller portion of projects focus on data storage and data analysis (re-using existing data – not collection of new data) to accumulate and produce new knowledge. If any camera (different models and types) were used with the intent of producing a picture, we report the general "camera" term for later analysis. Increasing levels of automation are also observed in later studies. These are the categories of most relevance for citizen scientists contributing to the OBAMA-NEXT project, since the project aims to not only identify new methods, technologies for MBB and benthic/pelagic observations, but also to provide new data/knowledge from established learning sites (LSs). For instance, in the EU-consortium Marco-Bolo (marcobolo-project.eu), where new data are not collected, storage platforms or similar biodiversity repositories would be more relevant CS technologies.

The survey suggests that the technologies used for different observational tasks in benthic and pelagic settings are very similar. Yet, while for benthic observations primacy is given to cameras (likely due to the characteristics of the setting, CS practices involve scuba diving), in pelagic observations seasonal surveys and metadata repositories were particularly important.

The contours of different archetypal CS projects also emerge across the review: one type of project is based on collections of photos taken by citizens, and sometimes also by researchers, employees of public monitoring institutions, as well as other professionals like scuba divers or fishers. Photos may also be collected on social media (such as Facebook) or other digital platforms (such as http://www.digitalglobe.com). Sometimes photos are uploaded to a digital platform (such as http://www.seawatchers.org or www.redmap.org.au) or in other databases (such as https://data.npolar.no/sighting/). These publications do not include details about the camaras used to obtain the photos.

Another archetypal CS project engages observers (often trained) to collect data for a specific project. Here data can be photos, samples and/or written observations. Photos, samples and/or written observations are registered in apps (e.g., MedusApp), platforms or surveys. The latter requires species identification skills among citizen scientists.

An example of a CS project conducted within the OBAMA-NEXT project is carried out by the Finnish Environment Institute (SYKE), who is collecting data on algal blooms using the Järvi-Meriwiki (page in Finnish: https://www.jarviwiki.fi/wiki/Sinilev%C3%A4tilanne. The name can be translated as “lake-sea wiki”). Citizens can either use the app to submit single observations, or they can create an observation point if they want to submit observations throughout the year (this is intended for, e.g., people who spend their summers at their summer cottage by the sea and want to send more or less regular observations from same place). In the summer months the regional authorities regular give alerts concerning possibly harmful algal blooms in the sea and lakes, and these data are used to supplement the monitoring done by the authorities. From a CS point of view, this is primarily an activity belonging to the crowdsourcing of data acquisition type.

3.3.2. Location and species

In terms of location, most of the 34 articles were centred in Europe (12), Oceania (7), and North America (4). Most identified articles use CS to observe one type of species (29) – different types of fish including sharks (12), mammals including whales (8), turtles (2), and other species (7). Only a few articles report on data on a wider biodiversity spectrum.

3.4. Discussion of survey findings

Our findings indicate a growing interest in CS in MBB. Yet, the papers in our review frequently do not provide enough information for the reader to assess the nature and scope of CS, and moreover, if the participatory ECSA’s principles of CS are fulfilled and to what extent. Likewise, activities involving “citizen scientists” beyond data collection are poorly described or not described at all. Thus, according to Haklay’s (2013a, 2013b) taxonomy

to evaluate CS projects/studies the status of CS in MBB partly, belongs to CS Level 1-3 (citizens are mainly data collectors/data gatherers).

Even though technological advancements apparently facilitate greater connectivity and accessibility, the barriers to participation in CS initiatives appear difficult to navigate. Our survey also shows that there is a lack of information provided by authors on the definition of CS adopted to frame the studies. Descriptions of citizen scientists, who use the tools mainly for data collection and data gathering processes, are frequently missing in the papers. Existing MBB studies seem to struggle to include non-expert citizens in their activities. For instance, participants are often "expert" divers, fishers, or even retired scientists, which is somewhat expected considering the necessity of specific expertise to access the pelagic environments or to performs certain task (e.g., eDNA sampling). Even so, we wish to stress the need for a more detailed description of participants in (self-labelled) CS projects/studies to allow the readers to understand or further evaluate which principles of CS are fulfilled by actual scientific practices in the field of MBB, or similarly, how such adherence to them can be improve (and CS be promoted accordingly). In its current state, CS in pelagic studies is mainly carried out by non-academic experts and largely does not rely on non-expert citizens (a typical requirement for CS in other fields).

Differently from CS in other fields, and likely depending on the specific conditions of tasks involved, there seems to be greatest focus on specific technologies for observations as directly associated to CS (something that is not common to CS in other fields). We argue that this can be taken as a peculiarity of CS and CS practices in the MBB field, and thus, this finding can indicate that redefining the ideal of CS specific for MB and related fields might be fundamental to properly embrace the reality of actual scientific practices and individuate viable paths to advance CS accordingly. While redefining the CS ideal for MBB is an endeavour that cannot be done properly in this brief research report due to its complexity, it seems essential that any new account should give central attention to the technologies used when doing CS in benthic/pelagic environments. As our survey clearly demonstrate, and further informal discussions across the OBAMA-NEXT project work packages (mainly, WP2, WP3 and WP6) indicate, it is a clear trend in MBB and related disciplines to associate specific technologies to CS tasks.

To conclude, our literature review has shown that what is usually intended as "democratization" of science and CS principles and desiderata in terms of active participation of lay citizens in higher order scientific tasks (Haklay et al., 2021) still seem difficult to fulfil completely in MBB, likely due to the nature of tasks and tools/techniques required to carry out specific scientific activities in the benthic/pelagic environments. In reference to Haklay's taxonomy (2013a, 2013b), CS in MBB seems to belong partly to the activities described at CS Levels 1-3 (mainly, crowdsourcing and data collection), but the papers in the review do not provide enough precise information or envision further levels of engagement for citizen scientists or their participation to higher-level tasks (e.g., definition of research goals or data analysis). Namely, authors self-define their practices as CS but the extent to which the CS ideal is fulfilled remains unclear, since participants (expertise, their role) and their tasks in CS projects are not explained in sufficient detail. Some notable exceptions can be found in recent studies in MBB that are not included in the literature survey presented here, as they were published after September 2023 (e.g.,

Brodie et al., 2023; Vazquez-Delfín et al., 2024), which are more precise about the characteristics of the participants. Nonetheless, the extensive agreement within the scientific community of reference (i.e., marine biologists) on what constitutes CS and CS tasks, as well as the impressive similarity of tools/technologies used in rather different contexts (i.e, similarities across Oceania, Europe, etc.), seems (to us) a good reason to re-thinking the ideal of CS in MBB and related fields. That is, to tailor definitions of CS to the reality of current scientific practices in the field but also, to promote further viable CS advancements. Primarily, by giving central importance not only to the extent of involvement of citizen scientists (here broadly understood to include non-academic experts as well) but also, to devote greater attention to technologies for observations (automated and non-automated) used by citizen scientists in different undertakings. Evidently, such an ambitious endeavour exceeds the scope of this report.

3.5. References

Andrews, K., Nichols, K., Harvey, C., Tolimieri, N., Obaza, A., Garner, R., & Tonnes, D. (2019). All Hands on Deck: Local Ecological Knowledge and Expert Volunteers Contribute to the First Delisting of a Marine Fish Species Under the Endangered Species Act. *Citizen Science: Theory & Practice*, *6*(1)

Bonney R, Ballard HL, Jordan R, McCallie E, Phillips T, Shirk J and Wilderman CC (2009). Public Participation in Scientific Research: Defining the Field and Assessing Its Potential for Informal Science Education. A CAISE Inquiry Group Report. Washington, D.: Center for Advancement of Informal Science Education (CAISE).

Bonney R, Phillips TB, Ballard HL, and Enck JW (2016). Can Citizen Science Enhance Public Understanding of Science? *Public Understanding of Science (Bristol, England)*, *25*(1): 2–16.

Bonney, R., Cooper, C. B., Dickinson, J., Kelling, S., Phillips, T., Rosenberg, K. V., & Shirk, J. (2009b). Citizen science: A developing tool for expanding science knowledge and scientific literacy. *BioScience, 59*(11), 977-984. https://doi.org/10.1525/bio.2009.59.11.9

Brodie, J., Kunzig, S., Agate, J., Yesson, C., and Robinson, L. (2023). The Big Seaweed Search: Evaluating a citizen science project for a difficult to identify group of organisms. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *33*(1), 44-55. DOI 10.1002/aqc.3903

Chandler, M., See, L., Copas, K., Bonde, A. M., López, B. C., Danielsen, F., Legind, J. K., Masinde, S., Miller-Rushing, A. J., & Newman, G. (2017). Contribution of citizen science towards international biodiversity monitoring. *Biological Conservation*, *213*, 280-294.

Dalby, O., Sinha, I., Unsworth, R. K., McKenzie, L. J., Jones, B. L., & Cullen-Unsworth, L. C. (2021). Citizen science driven big data collection requires improved and inclusive societal engagement. *Frontiers in Marine Science*, *8*, 610397.

ECSA (2015). The 10 Principles of Citizen Science. DOI: 10.17605/OSF.IO/XPR2N

European Citizen Science Association (2020). ECSA's Characteristics of Citizen Science. Version 1. (Haklay, M., Motion, A., Balázs, B., Kieslinger, B., Greshake Tzovaras, et al). Berlin, Germany, European Citizen Science Association (ECSA), 6pp. DOI: https://doi.org/10.5281/zenodo.3758668

Embling, C. B., Walters, A., & Dolman, S. J. (2015). How much effort is enough? The power of citizen science to monitor trends in coastal cetacean species. *Global Ecology and Conservation*, *3*, 867-877.

Forsblom, L., Azhar, M., Barkved, L. J., Berov, D., Borsen, T., Cenci, A., ... & Zambon, T. (2024). OBAMA-NEXT Deliverable 3.1. Review of existing and emerging techniques for benthic habitats. 90.

Fulton, S., López-Sagástegui, C., Weaver, A. H., Fitzmaurice-Cahluni, F., Galindo, C., Fernandez-Rivera Melo, F., Yee, S., Ojeda-Villegas, M. B., Fuentes, D. A., & Torres-Bahena, E. (2019). Untapped potential of citizen science in Mexican small-scale fisheries. *Frontiers in Marine Science*, *6*, 517.

Garcia-Soto, C., Seys, J. J., Zielinski, O., Busch, J. A., Luna, S. I., Baez, J. C., Domegan, C., Dubsky, K., Kotynska-Zielinska, I., & Loubat, P. (2021). Marine citizen science: Current state in Europe and new technological developments. *Frontiers in Marine Science*, *8*, 621472.

Golumbic, Y. N., Orr, D., Baram-Tsabari, A., & Fishbain, B. (2017). Between vision and reality: A study of scientists' views on citizen science. *Citizen Science: Theory and Practice*, *2*(1), 6-6.

Haklay, M. (2013a). Citizen science and volunteered geographic information: Overview and typology of participation. *Crowdsourcing geographic knowledge: Volunteered geographic information (VGI) in theory and practice*, 105-122.

Haklay, M. (2013b). Neogeography and the Delusion of Democratisation. *Environment and Planning A, 45*(1), 55–69.

Haklay, M., Fraisl, D., Greshake Tzovaras, B., Hecker, S., Gold, M., Hager, G., ... & Vohland, K. (2021). Contours of citizen science: a vignette study. *Royal Society Open Science*, *8*(8), 202108. https://doi.org/10.1098/rsos.202108

Hecker, S., Haklay, M., Bowser, A., Makuch, Z., Vogel, J. & Bonn, A. (2018). Citizen Science: Innovation in Open Science, Society and Policy. UCL Press, London.

Heigl, F., Kieslinger, B., Paul, K. T., Uhlik, J., & Dörler, D. (2019). Toward an international definition of citizen science. *Proceedings of the National Academy of Sciences*, *116*(17), 8089-8092. doi:10.1073/pnas.1903393116

Irwin A. 1995 *Citizen science: a study of people, expertise and sustainable development*. London, UK: Routledge.

Jaeger, J., Masselot, C., Greshake Tzovaras, B., Senabre Hidalgo, E., Haklay, M., & Santolini, M. (2023). An epistemology for democratic citizen science. *Royal Society Open Science*, *10*(11), 231100.

Kasperowski, D., & Kullenberg, C. (2019). The many modes of citizen science. *Science & Technology Studies*, *32*(2), 2-7. doi: 10.23987/sts.74404.

Kasperowski, D., Kullenberg, C., & Rohden, F. (2019). The epistemology of mobilising citizens in the sciences: tensions in epistemic cultures of contribution and ideals of science. In *Designs for Experimentation and Inquiry* (pp. 158-176). Routledge.

Kieslinger, B., Schäfer, T., Heigl, F., Dörler, D., Richter, A., & Bonn, A. (2018). Evaluating citizen science: Towards an open framework. In Hecker et al. (eds).

Kullenberg, C., & Kasperowski, D. (2016). What is citizen science?–A scientometric meta-analysis. *PloS one*, *11*(1), e0147152. doi:10.1371/journal.pone.0147152

Lukyanenko, R., Wiggins, A., & Rosser, H. K. (2020). Citizen science: An information quality research frontier. *Information Systems Frontiers*, *22*, 961-983.

Matear, L., Robbins, J. R., Hale, M., & Potts, J. (2019). Cetacean biodiversity in the Bay of Biscay: suggestions for environmental protection derived from citizen science data. *Marine Policy*, *109*, 103672.

Robinson, L. D., Cawthray, J. L., West, S. E., Bonn, A., & Ansine, J. (2018). Ten principles of citizen science. In *Citizen science: Innovation in open science, society and policy* (pp. 27-40). UCL Press.

Strasser, B., Baudry, J., Mahr, D., Sanchez, G., & Tancoigne, E. (2019). " Citizen science"? Rethinking science and public participation. *Science & Technology Studies*, *32*(2), 52-76. https://doi.org/10.23987/sts.60425

Turbé, A., Barba, J., Pelacho, M., Mugdal, S., Robinson, L. D., Serrano-Sanz, F., ... & Schade, S. (2019). Understanding the citizen science landscape for European environmental policy: an assessment and recommendations. *Citizen Science: Theory and Practice*, *4*(1).

Vázquez-Delfín, E., Galindo-De Santiago, C., Paredes-Chi, A., Ríos-Vázquez, A., Benavides-Lahnstein, A., Khatun, K., & Brodie, J. (2024). Marine macrophyte strandings in the Yucatán peninsula: Citizen science as a potential tool for long-term monitoring. *Aquatic Botany*, *190*, 103728. https://doi.org/10.1016/j.aquabot.2023.103728

Vohland, K., Land-Zandstra, A., Ceccaroni, L., Lemmens, R., Perelló, J., Ponti, M., ... & Wagenknecht, K. (2021). *The science of citizen science* (p.529). Springer Nature. https://doi.org/10.1007/978-3-030-58278-4_1

Zambon, T., Bassetti, C., & Prandi, C. (2023). A Close Look at Citizen Science Through the HCI Lens: A Systematic Literature Review. In *IFIP Conference on Human-Computer Interaction* (pp. 414-435). Springer, Cham.

4. General conclusion

The different methodologies, techniques and analytical approaches described in this study, deployed together with abiotic environmental sensors in different platforms with varying degrees of autonomy, provide the opportunity to comprehensively study biodiversity at multiple levels, from the smallest microbes to marine mammals. They facilitate the collection of data on the effects of climate change and on the effectiveness of biodiversity conservation measures over time. They help to manage proliferation and potentially harmful events at different timescales and spatial resolutions, in response to a variety of pressures (including extreme short-term events). Given the uneven distribution and dynamics of species in marine systems and the vast size, variability and complexity of marine environments, extensive sampling is necessary for accurate biodiversity assessments. Relying solely on research vessels and discrete cruises, while essential for the most comprehensive and integrated ecosystem studies of biodiversity, is insufficient. Therefore, the widespread use of innovative emerging technologies on moorings, fixed automated platforms and commercial vessels such as ferries, merchant ships and cruise ships for automated sampling and *in vivo* measurements is essential. It is also important to maintain long-term reference monitoring, which will increasingly be complemented by some relevant innovative measurements. This collaborative approach will allow for more comprehensive data collection on marine biodiversity, improving our understanding and ability to effectively protect marine ecosystems.

2024