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A Process-Based Approach to Guide the Observational Strategies for the Assessment of the Marine Environment

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Abstract: Ocean sustainability entails the management of marine ecosystems and their services. Monitoring and evaluation of the health of the sea is challenged by the complexity of the marine environment, whose multitude and interconnected aspects, together with the lack of comprehensive models, make the understanding of its functioning a very arduous endeavour. Observations are costly and time-consuming. For this reason, a European joint action, named Science for Good Environmental Status, tested a new approach to monitor and evaluate effectively the state of health of the sea. This approach is based on the identification of driving physical processes that are present in the sea basins and directing the observation strategy to be designed on the basis of preliminary space–time information and patterns. The proof-of-concept of this approach has been implemented offshore of the Belgian coast in an attempt to achieve ecosystem assessments with targeted data collection methods requiring a reduced combination of variables. The proposed approach can impact monitoring activities implemented by those countries aiming to fulfil the requests of the European Marine Strategy Framework Directive. A map of EU marine areas to further test this process-based approach is also provided.

Keywords: knowledge-based support to ocean sustainability; Marine Strategy Framework Directive; Good Environmental Status; observation strategy

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1. Introduction

Ocean sustainability addresses a multitude of interconnected aspects and dimensions.

Understanding and tackling the impacts of human activities on the environment involves different agents at social, economic, legislative and environmental levels. The oceans and, in particular, their coastal areas are fundamental components of the Earth's ecosystem, hosting millions of species that provide a wide range of ecosystem services. The oceans can also be considered a key asset for the economies in the transportation, energy and tourism sectors. Marine ecosystems are, therefore, relevant aspects in most of the global challenges, providing an essential buffer to global climate warming and to the decline of biodiversity, too.

Seas and oceans constitute, per se, a complex system, even if we discount any anthropogenic disturbances. Their functioning embodies physical and biological aspects. Unfortunately, complexity is a concept that cannot be framed within a unique mathematical formulation, such as for the fluid dynamics of quantum mechanics. This implies that modelling and simulating marine ecosystems is still a scientific challenge [1,2]. It is also

well known that complex systems cannot be linearised, and forecasting their dynamics is mainly effective only with strong assumptions or in limited cases [3].

In order to address the challenge of achieving the sustainability of marine resources and maintaining or restoring a Good Environmental Status (GES) of European Seas, the European Union adopted, in 2008, the Marine Strategy Framework Directive, hereafter referred to as the MSFD [4,5]. The MSFD aims to coordinate Member States in establishing criteria for the exploitation of the sea with a precautionary approach to prevent irreversible changes caused by the cumulative effects of human activities. GES is defined as "the environmental status of marine waters where these provide ecologically diverse and dynamic oceans and seas which are intrinsically clean, healthy and productive, and the use of the marine environment is at a level that is sustainable, thus safeguarding the potential for use and activities by current and future generations" [4].

To implement the MSFD, a set of qualitative Descriptors (D1–D11, Table 1) were defined by the European Commission, including related criteria that are meant to guide the assessment of the status of the seas and, thus, the achievement of the GES.

Table 1. MSFD Descriptors, their respective achievements, which describe what the environment will look like when GES has been achieved, and their related number of criteria (environment.ec.europa.eu/topics/marine-environment/ URL accesses on 19 September 2024).

ID	Type	Descriptor	Achievement	n. of Criteria
D1	state	Marine biodiversity	Biological diversity is maintained. The quality and occurrence of habitats and the distribution and abundance of species are in line with prevailing physiographic, geographic and climatic conditions	6
D2	pressure	Non-indigenous species	Non-indigenous species introduced by human activities are at levels that do not adversely alter the ecosystems	3
D3	state	Commercial fish and shellfish	Populations of all commercially exploited fish and shellfish are within safe biological limits, exhibiting a population age and size distribution that is indicative of a healthy stock	3
D4	state	Food webs	All elements of the marine food webs, to the extent that they are known, occur at normal abundance and diversity and levels capable of ensuring the long-term abundance of the species and the retention of their full reproductive capacity	4
D5	pressure	Eutrophication	Human-induced eutrophication is minimised, especially adverse effects thereof, such as losses in biodiversity, ecosystem degradation, harmful algae blooms and oxygen deficiency in bottom waters	8
D6	state	Seabed integrity	Sea-floor integrity is at a level that ensures that the structure and functions of the ecosystems are safeguarded and benthic ecosystems, in particular, are not adversely affected	5
D7	pressure	Hydrographical conditions	Permanent alteration of hydrographical conditions does not adversely affect marine ecosystems	2
D8	pressure	Contaminants	Concentrations of contaminants are at levels not giving rise to pollution effects	4
D9	pressure	Contaminants in seafood	Contaminants in fish and other seafood for human consumption do not exceed levels established by Union legislation or other relevant standards	1
D10	pressure	Marine litter	Properties and quantities of marine litter do not cause harm to the coastal and marine environment	4
D11	pressure	Energy, including underwater noise	Introduction of energy, including underwater noise, is at levels that do not adversely affect the marine environment	2

In this context, the MSFD recognises the complexity of marine ecosystems and promotes a holistic approach [6]. It defines targets and monitoring strategies, also introducing a set of 11 descriptors which explicitly address what are considered to reflect the main characteristics or processes embedded in marine ecosystems. The Descriptors are formulated to describe how the systems should function and evolve, though very qualitatively, and therefore to evaluate their status. Unfortunately, since the presence of each living organism depends not only on the abiotic (temperature, nutrients, pollutants, hydrodynamics, etc.) or biotic (metabolic capacity, plasticity, range of tolerance to variations, etc.) boundary conditions but also on its interactions with the whole set of external agents, the evolution of one species cannot be understood only in terms of linear and prevalently abiotic–biotic interactions [7]. Moreover, the network of acting agents on the localised scale may even be impacted by the whole ecosystem [8], hence requiring us to go beyond the integration of singular aspects [9].

The scientific community has reflected on the framework needed for the definition of GES and the challenges to coupling human activities and their impact on the marine environment [10–15]. Specific efforts have addressed aspects related to the metrics for the estimation of the different indicators and the salient criteria for the assessment of the GES [16].

In late 2019, the European Joint Programming Initiative "Healthy and Productive Seas and Oceans" (JPIO) launched an action jointly coordinated by Belgium, Italy and Malta and the official participation of 11 countries (BE, DE, EE, FR, GR, HR, IE, IT, MT, NO, UA). This Joint Action, named "Science for Good Environmental Status" (S4GES), has designed and tested a complementary approach towards GES to assess marine environmental health. It relies on a sampling strategy that targets the actual processes within the system based on near real-time observations of deviations or events in the system. A key aspect of S4GES is to support the implementation of more efficient and effective observation strategies. The ultimate goal of S4GES is to lay the foundations for a paradigm shift by piloting marine observation strategies in different settings [17].

S4GES began to test the innovative approach in a proof-of-concept sampling campaign aboard RV Belgica in the summer of 2022 within the tide-driven system in front of the Belgian and Dutch coasts. During the campaign, sampling positions were chosen on the basis of near real-time satellite observations, comparing this approach to the traditional sampling taken at historical fixed sampling sites, i.e., the measurements were aligned to the prevailing marine environmental patterns compared to climatology. Both sample sets were analysed for an exhaustive set of chemical, physical and biological parameters. The interconnection of the physical, chemical and biological characteristics of the system was analysed with a model of the trophic web and by taking into account the contributions of data on e-DNA and contaminants.

In this paper, we describe the scientific rationale that guided the design and implementation of the sampling strategy and the preliminary results of the observational campaign. We also provide a map of EU marine areas to further test the proposed process-based approach. The outcomes of the action have already suggested that Belgium revise its sampling strategy for the implementation of MSFD, and we promote the tested method as a milestone towards a knowledge-based approach to monitoring the marine environment.

2. Methodology

With the aim to first design and then operationally develop a plan for testing a new approach to monitoring the status of the marine ecosystem, four main scientific arguments were selected to identify emerging properties of the dynamics of different variables influencing the health of the ecosystems and to define the sampling activity:

(i) physical oceanography, (ii) biodiversity, (iii) trophic web and (iv) contaminants.

Those arguments mainly approach the ecosystem, looking at the coupling between physical and biological aspects. They include directly or indirectly all the Descriptors of MSFD, except D11, on acoustic underwater noise.

2.1. The General Approach to the Sampling Strategy

Detailed identification of the sampling stations was based on full-resolution, site-specific satellite and modelling climatological information analysed by the physical oceanography activity (see Section 3). The first-order sampling stations (in the area reported in Figure 1) were defined according to the ensembles of information, ensuring (i) an available and consistent time series useful as a robust reference for data management and interpretation and (ii) consistent and coherent pelagic ecosystem features and dynamics. In particular, climatological analyses of remote sensing ocean colour products defined the spatial and temporal biogeochemical patterns that would help to recognise the best areas that represent different ecosystem regimes, i.e., biogeochemical areas (Figure 1). Such an approach was refined during the cruise by processing and using near real-time satellite imagery directly on board (Figure 2). The main oceanographic features (fronts, mixed layer patterns, current dynamics, etc.) were also taken into consideration through the available information from time series data.

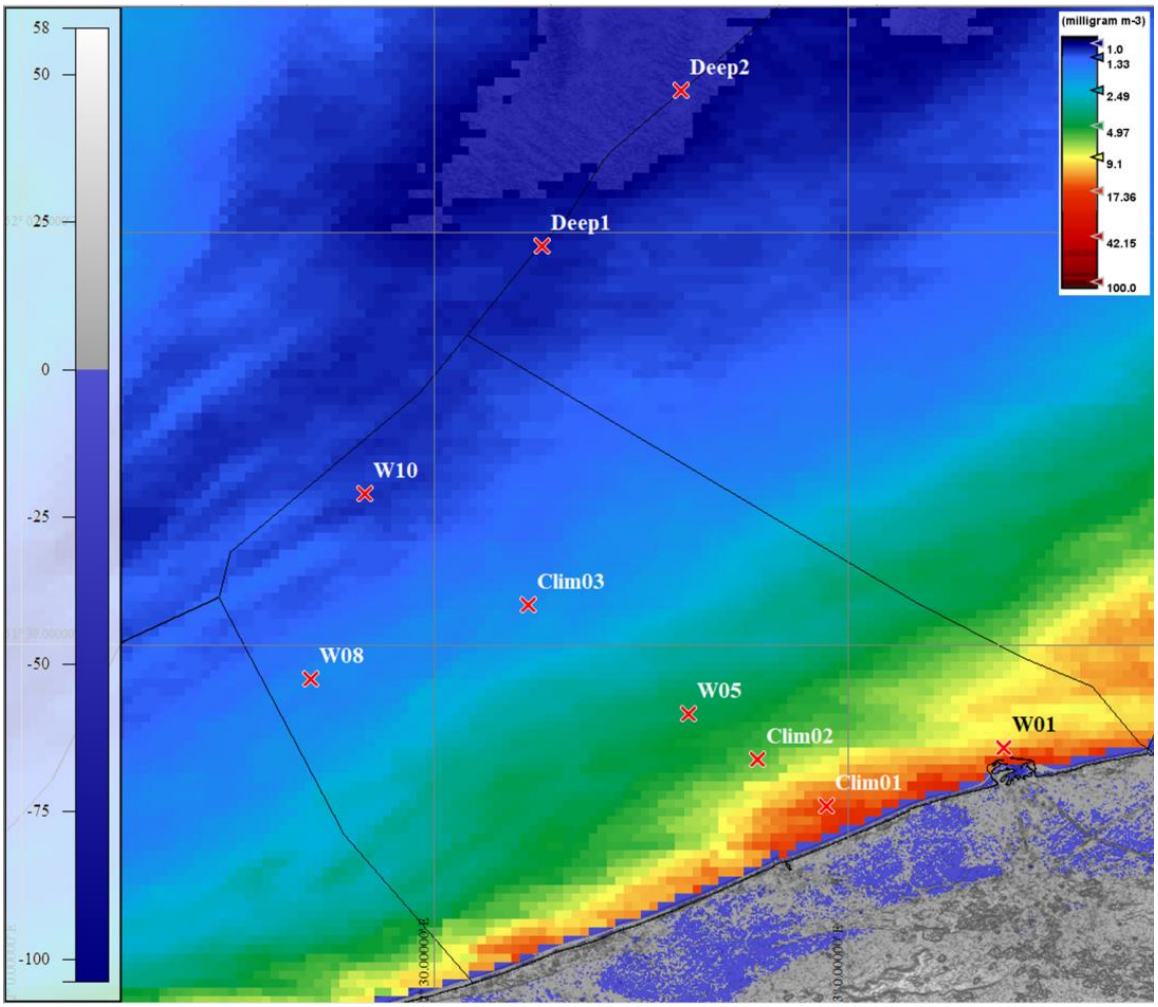

Figure 1. Summer climatological pattern of Chlorophyll-a concentration (2010–2021) superimposed on the planned bio-hydrological stations. Satellite product trimmed and downloaded from the Copernicus Marine Service.

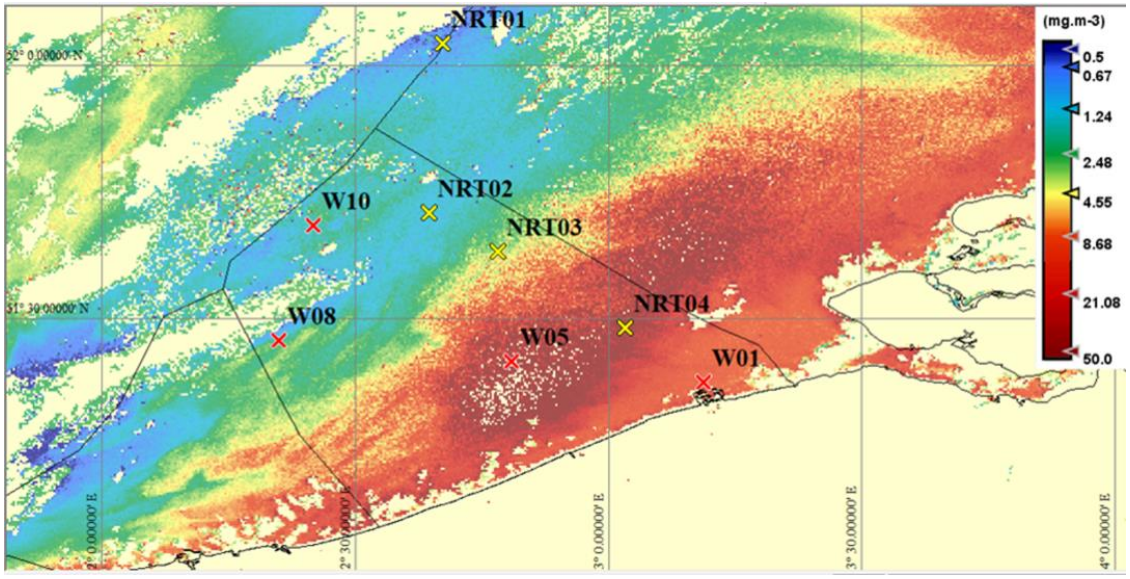

Figure 2. Example of near real-time satellite Chlorophyll-a concentration that was used for the refined definition of sampling stations. Satellite products trimmed and downloaded from EUMETSAT for the day 11 July 2022.

Belgium offered the use of one week on board the RV Belgica to host scientists and implement an observing campaign. A letter of commitment from 12 institutions from five countries (BE, IT, MT, FR, NO) was signed to frame a 2-year activity project. The costs for the engagement of experts, provision of the vessel and instrumentation, and workshops' organisation have been estimated at approximately EUR 700 000.

The cruise was successfully carried out from 9 to 15 July 2022. Data and samples were archived and made accessible to the experts.

From each sampling site, the following suite of parameters were measured:

- Physical and biogeochemical parameters (Sea water temperature and salinity along the water column, Beam Transmission, Chlorophyll-a absorption and concentration, POC, DOC, CDOM, Inherent Optical Properties, Ocean Colour radiometry);
- Biodiversity (environmental DNA for metabarcoding, bottle and net samples for quantitative analysis at various trophic levels via microscopic counts, flow cytometry, optical scanners);
- Contaminants (concentration: dissolved and particulate, bioavailability (by analysis of free and complexed forms of the various pollutants in seawater and also adopting passive sampling) [18], and distribution at the various plankton trophic levels to investigate contaminants accumulation and transfer quantify along the web;
- Biomarkers (sentinel molecules for detecting cumulative and/or specific impacts of contaminants) at the individual and/or trophic level;
- Plankton sampling (as required for trophic web analyses).

2.2. Physical Oceanography

The sampling strategy and the subsequent support to sampling activities during the S4GES oceanographic cruise consisted of first recognising those physical and biogeochemical patterns that highlight the spatial and temporal ecosystem dynamics of the Southern Bight of the North Sea. This implied an extensive use of satellite imagery, such as Ocean Colour (OC) and Sea Surface Temperature (SST) products from the Copernicus Marine Service (CMEMS) and the European Organisation for the Exploitation of Meteorological Satellites (EUMETSAT). In particular, a climatological (monthly and seasonal) satellite analysis of Chlorophyll-a concentration (Chl) provided a "first guess" to recognise the biogeochemical patterns that can drive the sampling strategy [19] (Figure 1). This analysis

was based on the ESA Ocean Colour CCI surface Chl (1 km resolution), which uses the regional OC5CCI chlorophyll algorithm [20] and merges the data from SeaWiFS, MODIS-Aqua, MERIS, VIIRS and OLCI-3A sensors, with realignment of the spectra to those of the MERIS sensor. The OC5CCI algorithm was tested and selected through an extensive calibration exercise that analysed the quantitative performance against in situ data for several algorithms in these specific regions. The multi-decadal L4 (interpolated) monthly composites are available on CMEMS from 1997 to the present (accessed on 19 September 2024). The climatological analysis from these satellite products was complemented by additional analyses comprising sea currents, wind field, and the mixed layer depth available from numerical models, also available on CMEMS. Such a holistic, observational approach further provided additional information that revealed, along with the chlorophyll pattern, insights into the spatial and temporal distribution of primary production. These patterns, and, thus, the observation of emerging coastal fronts and filaments, represented the basis of the sampling strategy: sampling stations planned within and away from the recognised climatological coastal patterns, as well as in the transitional zones.

The climatological, satellite-based patterns were then refined on board a few hours before the actual measurements, based on near real-time satellite imagery, by downloading, trimming and processing Sentinel3-OLCI OC Level-2 full resolution (300 m) data from the EMETSAT data centre (Figure 2). Indeed, Sentinel-3 OLCI offers daily-based OC products (e.g., Chl, Diffuse attenuation coefficient at 490 nm, Total Suspended Matter) at a 300 m spatial resolution. These L3 non-interpolated products, trimmed and downloaded from EU-METSAT, enabled the identification of the actual biogeochemical (spatial and temporal) patterns that characterised the marine ecosystem of the area during the cruise. This additional near real-time analysis was therefore used as a "probe" to detect the actual biogeochemical variability and thus to place the sampling stations in a more precise way, along filaments and orthogonal to fronts. The OC information was assessed by a match-up activity, i.e., in situ bio-optical measurements, including profiles of Inherent Optical Properties and ocean colour radiometry [21,22]. In particular, absorption phytoplankton spectra obtained from Hobi Labs a-sphere [23] were collected for each station and for each tidal phase (see below).

Moreover, during the cruise, the Physical Oceanography also performed high-frequency observations of plankton community properties with a water-following device, i.e., a bio-Lagrangian drifter that essentially provides a frame of reference moving with the plankton itself. This represents a satellite-tracked Surface Velocity Program (SVP) Lagrangian drifter with a drogue configured to collect high-frequency observations of plankton community properties with the aid of a bio-optical sensor. With this configuration, the SVP drifter measured the particulate backscattering coefficient (Bbp), which is related to the particle concentration in seawater, their composition, size distribution, shape and structure, and it is mostly influenced by submicron non-algal particles. The bio-Lagrangian drifter assessed and complemented the satellite information, allowing the mesoscale and sub-mesoscale dynamics of biogeochemical patterns (e.g., coastal filaments and fronts) to be explored, as well as their bio-optical evolution in space and time, in a region that is strongly characterised by small-scale dynamics due to strong winds and tides.

All samples were collected during a specific tidal phase (maximum eastward or westward tidal current and/or slack current). When it was not possible to collect samples during all tidal phases (for each station), the sampling was carried out during the slack current phase, taken as a reference tidal phase. At each station, tidal barotropic currents were obtained from the Oregon State University TOPEX/Poseidon Global Inverse Solution tidal model (TPXO). TPXO uses satellite altimetry to constrain solutions to the shallow water Laplace tidal equations on a 1/30 bathymetric grid [24].

Finally, the PO group focused on scientific analyses of the coastal and inland waters variability, taking advantage of the satellite, in situ and modelling information collected before, during and after the oceanographic cruise. This implies a subsequent diagnosis of

the observed biogeochemical patterns in light of physical forcings, such as winds, storms, tides, marine currents, river runoffs, etc. [25–28].

2.3. Biodiversity

Biomonitoring is essential for marine ecological assessment, providing correct biodiversity estimates, including cryptic diversity and non-indigenous species (MSFD Descriptor 2). Classic marine biomonitoring is achieved through targeted campaigns sampling fish, invertebrates and phytoplankton with various devices, followed by time-consuming classic taxonomic identifications. Recent developments in high-throughput DNA-sequencing techniques now provide alternatives as they allow us to directly analyse the DNA of water or sediment samples with so-called environmental DNA (eDNA) with little to no impact. Here, we define eDNA in the broad sense, according to [29], including both extracellular DNA from the water column and cellular DNA from (small) intact organisms. In combination with metabarcoding, eDNA techniques can simultaneously characterise the diversity of entire marine communities. eDNA has been shown to detect more fish and phytoplankton species than traditional methods [30–32] and can provide information on rapid temporal [33] or spatial [34] changes. Even if certain aspects of eDNA-based techniques are still awaiting further validation, their great potential for biomonitoring has been recognised [32,35,36] and has started to be applied to marine ecosystems (see, for example, [37–39]) and also for the support of policy and management decisions [40–42].

Previous studies have successfully used eDNA metabarcoding techniques on free-living marine bacteria, marine and toxic phytoplankton [43], zooplanktonic communities [44–46] and marine fish in the North Sea [47,48]. For the S4GES cruise, metabarcoding eDNA techniques were targeted towards analysing biodiversity patterns of four ecological groups: bacteria, phyto- and zooplankton and fish. Sea water was taken at different locations and during different tidal cycles with Niskin bottles for various analyses from the same samples, including eDNA. Water samples from each sampling point and type were pumped through filters with different pore sizes according to the target organisms, including negative field and filter controls and the filters were snap-frozen for subsequent DNA extractions in dedicated eDNA laboratories. Different metabarcoding regions were amplified by PCR, including part of the ribosomal 16S region for bacteria, part of the 18S region for phyto- and zooplankton and of the 12S region for fish [49]. Short 12S amplicons were sequenced with Illumina sequencing techniques, as in other studies, while longer fragments of 16S and 18S were sequenced with Oxford Nanopore technology to obtain better taxonomic identifications. The latter, long-read sequencing technique is still relatively new for eDNA studies of bacteria [50] and zooplankton [51] and novel for phytoplankton.

With bioinformatic pipelines like dada2 [52] or Kraken2 [53], raw DNA sequencing reads were processed, followed by taxa identification to the lowest taxonomic level by comparisons with published and custom reference databases. Rarefied data were used in statistical analyses in R to estimate alpha and beta diversities and to identify which abiotic factors drive biodiversity patterns.

2.4. Trophic Web

Marine systems are undergoing ecological changes worldwide, as in coastal and urbanised regions. For instance, the biomass of trophic players in pelagic communities is generally distributed across size classes following a power law, with the smallest organisms being more copious than the largest ones, but anthropogenic pressures, hydrodynamic changes, fisheries and pollution are breaking this natural pattern, potentially modifying the structure of trophic networks and the ecological state of marine systems [54]. A task to support the sampling strategy aboard RV Belgica was to select and test those indicators (for the assessment of Good Environmental Status) that explicitly consider trophic networks. The latter were investigated in different environmental conditions, at different trophic states (roughly, eutrophic vs. oligotrophic states), at different levels of chemical contamination and over coast-to-offshore gradients. A standard procedure to study marine trophic networks

by optimising the balance between the detail of the ecological description of the system under investigation and the workload required in terms of sampling, sample processing and data analyses was defined.

Addressing the trophic web aims to integrate information from oceanography and biodiversity and provide a background to explore contaminant spreading in the pelagic community as vehiculated by trophic interrelationships. Trophic networks were investigated, and we integrated plankton, benthos and nekton within a common framework. This operation is highly constrained by the level of detail gathered in the description of the different trophic players residing at different positions in the water system and whose sampling requires different categories of observations. The pelagic environment lying over continental shelves encompasses huge biodiversity, including both unicellular and multicellular organisms whose sizes span several orders of magnitude. Several trophic steps connect primary producers, which are mainly microbic, and top predators, that is, larger metazoans [55,56]. Planktonic organisms sit in the middle of such a 'chain' and play a pivotal role in driving fluxes of matter and energy.

Definition of plankton nodes

Traditional knowledge emphasised the importance of primary producers in regulating pelagic systems: that is, the higher the primary production, the higher the secondary one, with bottom-up regulation being a primary driver of energy and matter fluxes. Yet, knowledge of the functional diversity of plankton has advanced in the last decade, leading to (i) the detailed description of the trophic habit of microscopic organisms, (ii) the drawing of the convoluted trophic webs amongst them, and (iii) the assessing of the role of top-down control in pelagic systems. An important regulative role is played by planktonic consumers, such as mixotrophs (protists capable of both photosynthesis and phagocytosis) and copepods (crustaceans playing as switchers of trophic pathways, thanks to their feeding flexibility) [55–57]. Trophic pathways involving these organisms stabilise the efficiency of matter transfer towards larger animals in the face of the oscillations that abiotic conditions undergo in coastal systems. Plankton is a pivotal component of pelagic communities, that is, it can respond quickly to environmental shifts and propagate the effect of these changes to higher trophic levels [57]. To study plankton networks, it is necessary to gather quantitative information for a minimum number of functional nodes representing the main trophic roles in the plankton trophic web. This need can be matched in many ways, but the final operational choice must also be pondered based on the actual spectrum of reliable information that can be gathered from the cruise. Collecting information about the carbon biomass of each functional node was considered mandatory.

The study of the plankton trophic networks requires the estimation of the biomass of the following functional nodes (FNs) (according to 55):

1. Detritus (better if split into dissolved and particulate components);
2. Heterotrophic bacteria;
3. Pico-phytoplankton (cell size < 2 μm);
4. Nano-phytoplankton (cell size 2–20 μm);
5. Micro-phytoplankton (better if split into different groups, such as diatoms and dinoflagellates) (cell size 20–200 μm);
6. Protozooplankton (better if split into the nano and micro size classes, and/or hetero- and mixotrophic) (cell size 2–20-200 μm);
7. Juvenile herbivorous crustaceans (e.g., copepods) (individual size > 200 μm);
8. Adult herbivorous crustaceans (copepods, cladocerans) (individual size > 200 μm);
9. Adult omnivore crustaceans (copepods) (individual size > 200 μm);
10. Adult detritivores crustaceans (copepods) (individual size > 200 μm);
11. Gelatinous filter feeders (pelagic tunicates) (individual size ca. 2000 μm);
12. Carnivorous zooplankton (individual size ca. 2000 μm).

The biomass of FNs #1–6 was obtained with good approximation using biogeochemical data, such as the concentration of Particulate Organic Carbon (POC), Dissolved Organic

Carbon (DOC), Chlorophyll-a (Chl), other photosynthetic pigments characteristic of the main phytoplanktonic groups (i.e., fucoxanthin and peridinin for microphytoplankton, 19-butanoyloxyfucoxanthin, 19-hexanoyloxyfucoxanthin and alloxanthin for nanophytoplankton and zeaxanthin and chlorophyll b for picophytoplankton), and Primary Production (PP). The biomass of nodes #7–13 was obtained by counting samples taken from vertical nets (WP2 or Bongo nets). All variables were quantified all over the water column and expressed as integrated values. Standard procedures were applied to sample and analyse these variables during the cruise. Appropriate and standardised conversion methods were applied to derive the biomasses of FNs #1–6 from biogeochemical data [58,59]. PP is necessary to derive the biomass of detritus within POC.

Trophic network models

Trophic webs, being complex adaptive systems, derive their configurations in terms of trophic links and the related intensity, and this is largely dependent on initial conditions. Therefore, different trophic states would trigger different trophic networks. The structure of trophic networks at different trophic conditions was derived by the Ecopath and Tracepath approaches [60], that is, ecological network models having the following as inputs:

(a) The biomass of FNs;
(b) Production, consumption, assimilation and respiration rates of FNs (estimated from published metrics and from measured rates, if available, i.e., PP, bacterial and secondary production);
(c) Diets of FNs (based on expert knowledge and implemented by isotope analysis, if available).

As for the actual diet of consumer FNs (at the specific eutrophic and oligotrophic conditions found in the area), this could be refined by comparing biogeochemical data (i.e., the amount of phytoplankton biomass and its partition among different photosynthetic nodes) and diversity data obtained from eDNA metabarcoding. This operation would allow the most probable trophic links between consumers (mainly metazoans) and producers (unicellular organisms) to be derived based on the site-specific taxonomic composition.

The output of such a model is a weighted trophic network in which the edge weight is the amount of carbon biomass transferred among nodes, i.e., predators and prey. This network was analysed to derive the network indicators as follows:

i. Maximum number of trophic levels (max TL) in the network.
ii. Number of trophic cycles in the network (TC).
iii. Detritivory/herbivory ratio
iv. Topological importance (TI) and trophic overlap (TO) of each FN.

These indicators provided hints on the ability of the network to transfer matter to larger animals, the level of dissipation/recirculation of matter in the trophic web and the occurrence of shifts in trophic roles in consumer FNs due to changes in the trophic state of the system (iv). Trophic network indicators were calculated based on the Ecopath approach [60], TI, i.e., a measure of the centrality of a species that takes into consideration both direct and indirect interactions [61], and TO, i.e., a measure of the trophic uniqueness of a species [62]. The networks with a resolution such as that listed above can capture the different ways the trophic web functions at different amounts of primary resources available. Inferences from Eco-path models were primarily compared with other observations, too, as follows.

Biomass–size distribution

The size of the organisms composing the investigated trophic web embraces five orders of magnitudes. This fact allowed us to apply some methods specifically developed to capture ecological in-formation from the way by which biomass is distributed across the whole community size-range. One of these methods is the Normalized Biomass Size Spectra (NBSS), which is a proxy of TTE [63]. The NBSS method can be applied using size distributions derived from the same biomass data used in the Ecopath approach, plus data

from underwater visual profiling (if available). We expected that estimations of TTE from different methods co-vary with the trophic state of the system at the spatial scale.

Isotope analysis. We expected that TLs determined with different methods (C, N stable isotopes on POC and lipids) co-vary with the trophic state of the system at the spatial scale.

Nekton trophic network from eDNA data. This procedure is based on inferences based on the taxonomic composition derived from eDNA metabarcoding. A meta-trophic network was derived by annotating the list of detected taxa with information on their trophic behaviour and diets, as available in the literature and from public databases, such as Globi [64–66]. The trophic networks derived from such a trophic annotation were analysed to characterise network structure and node role but using unweighted networks as an input. The network indicators for the plankton trophic network and for the nekton trophic network co-varied with the trophic state of the system at the spatial scale.

2.5. Contaminants

Plankton represents an important gateway for contaminants into marine food webs [67–69]. Various processes modulate the dynamics of contaminants flux through the plankton food web. In particular, bioconcentration of contaminants in phytoplankton is driven mainly by partition equilibrium processes between the cells and the surrounding water with the octanol–water partitioning coefficients (log K_{ow}) representing a suitable index for determining pollutant distribution between the abiotic and biotic systems. On the other hand, bioaccumulation processes in zooplankton are modulated by the entry of contaminants by both the water aqueous phase (bioconcentration) and diet, trophic interactions between phytoplankton and zooplankton, and contaminant removal. Such complex interactions driving fluxes of pollutants within the trophic web appear significantly influenced by the size-fraction distribution, the biochemical/energetic content, the trophic interactions and fluxes in organic matter [70,71].

From this perspective, the S4GES observational campaign also aimed to measure the concentration, distribution patterns, accumulation and transfer of inorganic contaminants through the plankton food webs (phyto-, zoo- and bacterio-plankton, using as proxies grain size classes) in the areas of scientific and economic interests of the North Sea and to contribute to assessing the role of plankton as a biological pump of contaminants.

The aims of the sampling were (1) to determine the concentration levels of trace elements (Cu, Pb, Cd, Zn, Cr, Mo, V, Ni and Hg) and auxiliary data (major elements, alkalinity, DOC, POC) in seawater (dissolved and particulate phase) and selected plankton size classes (from 63 to 2000 µm); (2) to investigate pollutants accumulation and transfer mechanisms in the plankton communities with potential influence on trophic interactions and community structures. Specifically, the sampling strategy for contaminants in plankton particles separated planktonic organisms into various size classes (over the range 0.2–2000 µm) by sieving or filtration. This was intended to discriminate pico-, nano- and micro-phytoplankton, micro-, meso- and macro-zooplankton, as well as heterotrophic prokaryotes (total, free or bound to particles) and unicellular heterotrophic eukaryotes. The strategy was to carry chemical and biological analyses as far as possible on the same size fractions/planktonic groups to reconstruct the puzzle of the accumulation and transfer of anthropogenic compounds within the plankton network. The methodology and sampling strategy followed [69].

3. Results

The results were describing a scenario that did not behave as expected.

They showed that environmental conditions were driving a response of the biological communities that deviated from historical records. Tides were expected to act as a washing machine, and the traditional fixed-stations approach would have led to an entirely different assessment of the system or have caused an unexplained increase in the variability of the observations, something that is not altogether uncommon. It can mean that something

significant is happening in the system but would probably be missed with a classical point-sampling approach.

The satellite-based climatological analysis revealed three distinct biogeochemical regions, with a decreasing Chl from onshore to offshore (Figure 1). In particular, two sharp Chl gradients highlighted a nearshore region of Chl ~ O(10) mg/m^3, a shelf region of Chl ~O(1) mg/m^3, and an offshore region where Chl ~ O(10^{-1}) mg/m^3. Such a pattern suggested the investigation of five potential sampling stations located within the biogeochemical regions and around the Chl fronts, i.e., Clim01, Clim02, W05, Clim03 and W10 in Figure 1 (W05 and W10 stations are pre-existing monitoring stations). Note that the Sentinel3 satellite images and their related analysis refer to a specific time of the day (around noon, i.e., the time the satellite passes over the investigated area) and, therefore, the climatological patterns we recognised (Figure 1) could not capture the daily Chl evolution due to tidal currents that might advect different waters and/or resuspended biogeochemical loads form the near-bottom layer.

Indeed, once on board the research vessel, near real-time images showed a slightly different biogeochemical pattern, characterised by one single, sharp Chl gradient (Figure 2) and with a Chl-rich area that extended over the shelf region. Such a shift was likely due to the actual tidal condition, which should alter the Chl spatial distribution over the tidal cycle. This was confirmed by the in situ measurements. By considering both climatological and near real-time analyses and also taking into account the water depth, we finally choose to sample station DEEP2 (Figure 1) and Station W05 (Figures 1 and 2). DEEP2 guarantees oligotrophic conditions and, due to the water depth, it was not supposed to be largely affected by the tidal cycle (a hypothesis that is not fully confirmed), while W05 represents the Chl-richest station. Both stations need to be sampled during the tidal cycle. Such a choice simplified a lot the sampling strategy, making it effective and efficient, avoiding useless replicas and saving time. From in situ sampling, we find that patterns of phytoplankton diversity differ significantly between phases of the tidal cycle at both the deep water and more shallow sampling stations, and this is true for both the estimates of diversity, species richness and the Shannon diversity index (Figure 3).

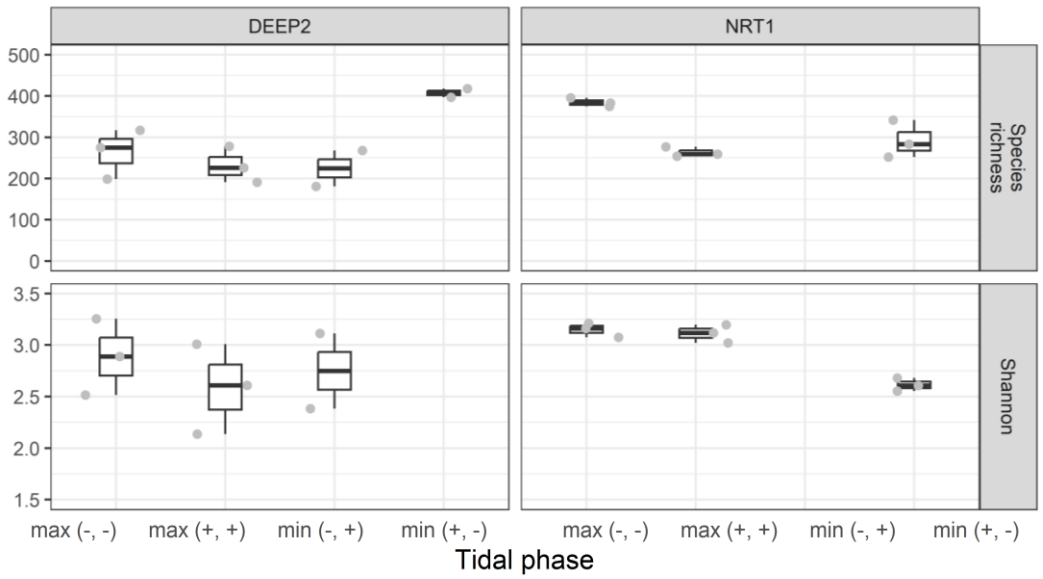

Figure 3. Biodiversity patterns of phytoplankton species estimated as the number of species (species richness) or as the Shannon index for alpha diversity. On the *y*-axis is the index value (grey dots). The horizontal lines inside the box plots indicate the median, and the boundaries of the box plots indicate the 25th and 75th percentiles. ANOVAs show that diversity varies significantly among the tidal phases at station DEEP2 (p = 0.029 for species richness and 0.048 for Shannon) and also among

the tidal phases at station NRT1 (p = 0.0002 for species richness and p = 0.002 for Shannon index). Horizontal axes indicate whether the tidal current was in its maximum or minimum magnitude. In brackets, we indicate the sign of zonal (u) and meridional (v) components of the tidal velocity: (-,-) = south-westward; (+,+) = north-eastward; (-,+) = north-westward; (+,-) = south-eastward.

These differences are well represented by bio-optical phytoplankton spectra collected at these two stations at different tidal phases. Figure 4 shows the difference in amplitudes of the secondary peak at 670 nm, a consequence of the different Chl in station W05. Moreover, increasing spectral magnitudes from cast A to C (from low to high and then back to low tidal current) indicates a decreasing average phytoplankton size. The relatively coherent spectral shapes indicate constant pigment proportions among casts (lower panel of Figure 4), which makes all curves overlap. On the other end, the phytoplankton spectra in DEEP2 (Figure 3) display similar values, indicating similar phytoplankton sizes (Figure 4), but the second derivative analysis highlights fine-scale differences in the spectra. This is caused by differences in secondary pigments across all the temporal cycles (lower panel of Figure 5).

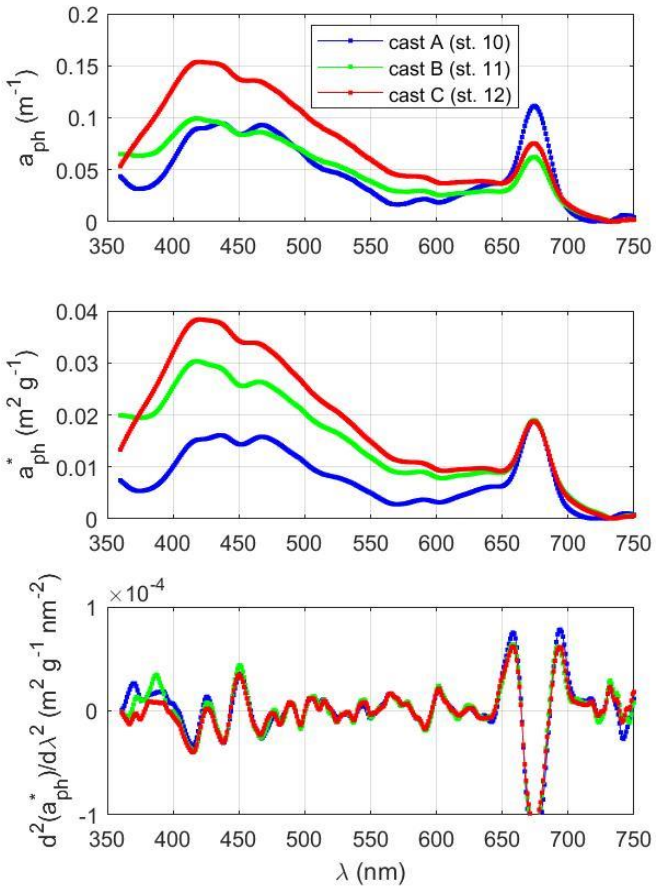

Figure 4. Bio-optical characteristic of station W05 (i.e., NTR1 in Figure 3) obtained from Hobi Labs a-sphere. Difference amplitudes of the secondary peak at 670 nm are a consequence of the different CHL (upper panel). To investigate the phytoplankton types, all spectra were normalised by an estimate of Chl-a, as a power law, given by a_ph(670) = A·Chl-a·E, where A = 0.019 and E = 0.96 (middle panel). Increasing spectral magnitude of a*ph from cast A (max(-,-)) to cast B (min(+,-)) to cast C (max(+,+)) indicates a decreasing average phytoplankton size. The relatively coherent spectral shapes indicate constant pigment proportions among casts. This is confirmed with the second derivative analysis of a*ph (lower panel), which makes all curves overlap.

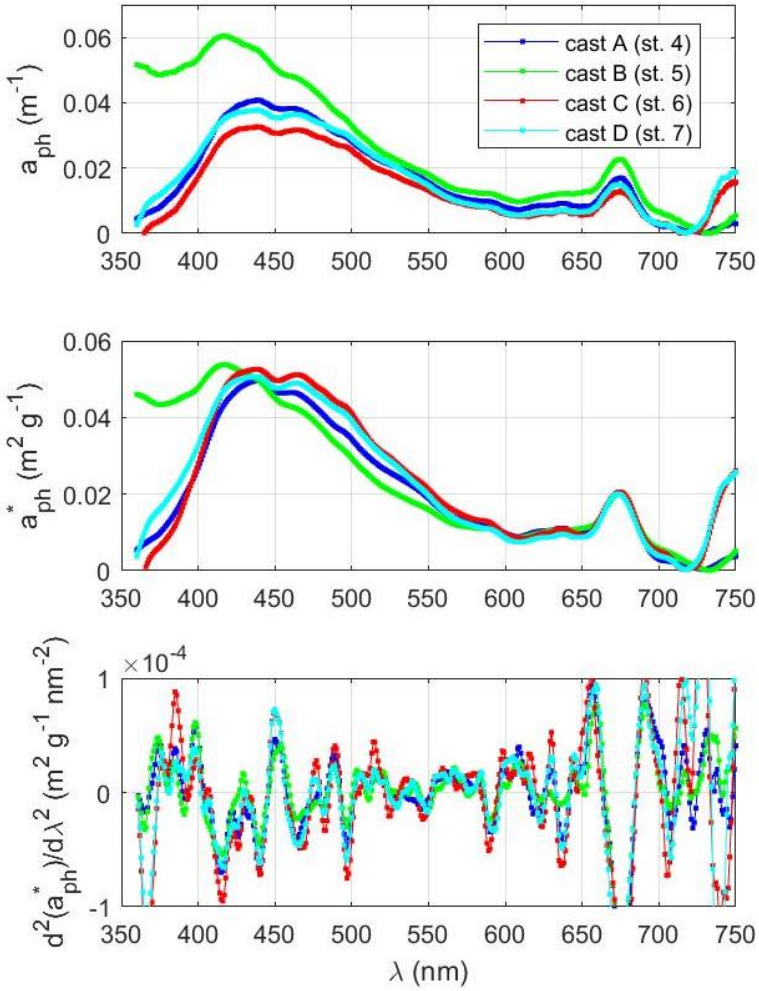

Figure 5. Bio-optical characteristic of station DEEP2, as obtained from Hobi Labs a-sphere. As for Figure 4, all spectra were normalised by an estimate of Chl-a, as a power law, given by a_ph(670) = A·Chl-a·E, where A = 0.019 and E = 0.96 (middle panel). The relatively coherent spectral shapes indicate different pigment proportions among casts. This is confirmed with the second derivative analysis of a*ph (lower panel), which shows no match among the curves.

A detailed description of the results associated with the identification and evolution of the status of the system has been recently reported [72]. Moreover, remote observations have been demonstrated to provide a probe for characteristics of the trophic web when analysed through e-DNA and water sampling.

4. Discussion

The preliminary results of the observational campaign were shared with many representatives of regional conventions and responsible authorities of the MSFD. The main messages were focused on evaluating the opportunity for a different use of funds when observations are carried out to assess the marine environment. As an immediate outcome, Belgium seriously investigated a thorough modification of its monitoring strategy and applied it in the next MSFD cycle. Patrick Roose, one of the authors of this paper and a person responsible for the management of the Belgian observation strategy for MSFD, communicated that Belgium will adopt the new approach for MSFD purposes by reallocating time and means to set up a process-based approach to its monitoring.

The validity of the approach was also recently emphasised by the COMPEAT process used by the OSPAR commission for its 2023 QSR assessment. In this, satellite remote-sensing observations were combined with in situ measurements and numerical modelling

data, providing a much more balanced assessment of the eutrophication status that can be linked to environmental factors such as currents, subsurface geology, etc.

The results shown by S4GES do not identify a unique set of data and analysis towards the assessment of the marine status. Other variables and samples can be collected, as well as different processes that are considered relevant for the dynamics of the ecosystems. S4GES, in fact, demonstrated that some observational strategies adopted to fulfil the requests of MSFD can be efficiently and effectively substituted by a process-based approach.

Preliminary identification of targets for the process-based approach in EU marine environments has been provided (see Figure 6) to facilitate national authorities, in collaboration with scientists, to implement additional observations to validate an observation strategy based on a process-based approach and consequently save money and increase the effectiveness of the analysis when providing clues on the status of the marine environment.

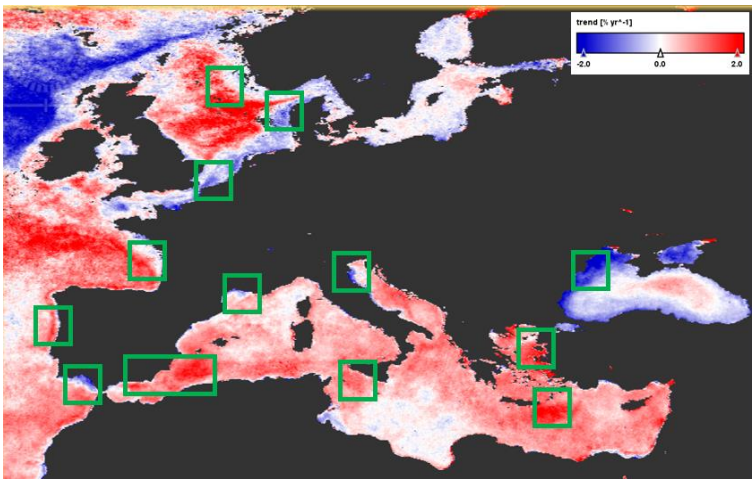

Figure 6. Map of the statistical trend of satellite-based Chl-a concentration processed from the global climate-quality chlorophyll time series produced by the ESA Ocean Colour Climate Change Initiative (ESA OC-CCI: 1997–2021). The green boxes highlight particular negative and positive trends over crucial regions where significant biogeochemical changes occur.

5. Conclusions

An observing campaign, framed within a European joint action named S4GES, has been implemented offshore of the Belgian coast as a proof-of-concept of a new approach to assessing the health of the marine environment.

The adoption of an observational strategy based on the dynamics of the driving physical processes, therefore abandoning the traditional fixed-stations approach, had impacts at the scientific and policy levels.

Scientifically, the results showed an unexpected distribution of populations within the trophic web during the tidal cycles. Moreover, a probe for the trophic web from the analysis of remote observations was identified.

The results shown by S4GES do not identify a unique solution towards the assessment of the marine status. In fact, they were built on a selection of the processes acting in the sampling area and on a specific design of the data sampling and analysis. What we show suggests that the adopted approach would increase the effectiveness and efficiency of observations, optimising the selection in space and time of the sampling stations and the use of resources.

When dealing with the implementation of the MSFD in terms of the very costly investments for monitoring, Belgium has already communicated the adoption of the proposed new approach by reallocating time and funds for the assessment of the marine environment.

In order to promote the adoption of the proposed new approach to the observational strategies, we also reported a map for possible targets in EU marine environments.

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