



Biodiversity in Marine Ecosystems – European Developments toward Robust Assessments

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Sustainability of marine ecosystems and their services are dependent on marine biodiversity, which is threatened worldwide. Biodiversity protection is a major target of the EU Marine Strategy Framework Directive, requiring assessment of the status of biodiversity on the level of species, habitats, and ecosystems including genetic diversity and the role of biodiversity in food web functioning and structure. This paper provides a summary of the development of new indicators and refinement of existing ones in order to address some of the observed gaps in indicator availability for marine biodiversity assessments considering genetic, species, habitat, and ecosystem levels. Promising new indicators are available addressing genetic diversity of microbial and benthic communities. Novel indicators to assess biodiversity and food webs associated with habitats formed by keystone species (such as macroalgae) as well as to map benthic habitats (such as biogenic reefs) using high resolution habitat characterization were developed. We also discuss the advances made on indicators for detecting impacts of non-native invasive species and assessing the structure and functioning of marine food-webs. The latter are based on indicators showing the effects of fishing on trophic level and size distribution of fish and elasmobranch communities well as phytoplankton and zooplankton community structure as food web indicators. New and refined indicators are ranked based on quality criteria. Their applicability for various EU and global biodiversity assessments and the need for further development of new indicators and refinement of the existing ones is discussed.

Keywords: indicators, food web, good environmental status, invasive species, pelagic ecosystem, benthic ecosystem, marine strategy framework directive

INTRODUCTION

Sustainability of marine ecosystems and their services are dependent on marine biodiversity, which is threatened worldwide (Narayanaswamy et al., 2013; Bennett et al., 2015). Biodiversity is fundamental to sustain marine ecosystem services, such as food, maintenance of water quality, and recovery from perturbations (Beaumont et al., 2007; Lique et al., 2016). Despite its important

role and contribution to human wellbeing, its loss has been reported world-wide. The main threats to marine biodiversity include habitat loss, overexploitation, pollution by hazardous substances, eutrophication, and invasions by non-indigenous species (Kappel, 2005; Venter et al., 2006). Efforts to reduce these pressures for halting the biodiversity loss, a commitment of the signatory countries of the Convention on Biological Diversity (CBD, 1992), is therefore essential for global food security, coastal water quality, ecosystem stability, and buffering the resistance and recovery of ecosystem services, thus enabling different types of future economic valuations and management (Reker et al., 2015). Restoring marine biodiversity through sustainable fisheries management, pollution control, maintenance of essential habitats, and the creation of marine reserves, are some of the opportunities for investments that can support the productivity and reliability of goods and services that the ocean provides to humanity (Worm et al., 2006; Palumbi et al., 2009; Cressey, 2016). Marine management should ensure sustaining all of an ecosystem's biological parts at functioning levels—via conservation of biodiversity at all different levels (from genetic to ecosystems)—in order to maintain ecosystem integrity and stability (Palumbi et al., 2009). The objective of ecosystem-based management of marine environment is to ensure healthy, functional and diverse ecosystems by managing the key drivers of adverse impacts. Biodiversity indicators need to measure variables that are documented to respond to pressures, using methods that can distinguish the anthropogenic impact from natural variability (Borja et al., 2016).

In order to understand the current biodiversity status and its conservation needs (including restoration and prevention), it is imperative to monitor fundamental parameters of biodiversity, both structural and functional (Strong et al., 2015). As biodiversity is such a multifaceted concept, monitoring may need covering genetic variability and physiological or life history diversity within species, surrogate taxa such as habitat-forming species (e.g., seagrasses, kelps), pollutant-recycling species (e.g., marsh grasses, macroalgae), and species diversity all the way from megafauna to microbes, planktonic prokaryotes and micro-eukaryotes, and energy flow hubs (Strong et al., 2015). In addition to skilful taxonomists, the monitoring process might strongly benefit from information-based tools designed for quicker assessments of taxonomy (Pittman et al., 2007), long-term monitoring sites, new tools for remote and continuous measurement of different biological components (e.g., microbial diversity, oceanic, and coastal phytoplankton and zooplankton, and meio- and mega-fauna in the benthos). Such tools include (i) genomics (Bourlat et al., 2013), (ii) robust marine biosensors (e.g., automated aerial, surface, and underwater drones equipped with sonar or acoustic monitoring), (iii) underwater cameras for detection of ocean fauna, and (iv) improved mathematical models to chart energy flow within food webs amounting to creation of marine life observatories (Palumbi et al., 2009).

The EU Marine Strategy Framework Directive (MSFD; 2008/56/EC), one of the major legal frameworks for the protection of marine biodiversity together with the EU Biodiversity Strategy 2020 (COM/2011/0244) and the Convention on Biological Diversity (CBD, 1992), highlights

setting programs for monitoring and assessing the environmental status of the marine waters. According to MSFD, the status of the marine environment is evaluated using 11 descriptors, that comprise both biodiversity related descriptors (D1, biological diversity; D4, food-webs; and D6, seafloor integrity) and pressure descriptors (D2, non-indigenous species; D3, fisheries; D5, eutrophication; D7, hydrological conditions; D8 and D9, contaminants in the environment and in seafood; D10, litter; D11, energy and noise). These are further detailed in the EU Commission Decision 2010/477/EU providing 29 criteria and 56 associated “indicators” that should be monitored for the assessment of the environmental status.

The MSFD puts biodiversity in the center of the assessment of marine environmental status (Borja et al., 2010). The descriptor (D1) on biodiversity has the following target to contribute to the achievement of the Good Environmental Status (GES): “*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.*” The background and definitions, the key attributes (biological components, predominant habitat type, and ecotypes for mobile species) as well as the suggested indicator classes for the descriptor (D1) criteria of the attributes are presented by Cochrane et al. (2010). While this descriptor directly targets biodiversity, MSFD Descriptor 4 (Marine food-webs), which calls for maintenance of the normal functioning of marine food-webs, and some aspects of MSFD Descriptor 6 (Seafloor integrity) are also closely related to the assessment of biodiversity (Borja et al., 2010).

The assessment of the ecological status of coastal waters is also required by the EU Water Framework Directive (WFD; 2000/60/EC) which does not specifically address biodiversity. Nevertheless some of the indicators for biological quality elements under WFD, such as phytoplankton, macrophytes, zoobenthos, and additionally indicators for fish community structure for transitional waters (e.g., Heiskanen et al., 2004), can be also applied to the MSFD (Borja et al., 2010). Some of these indicators include parameters for species composition, community structure and abundance, and are thus also applicable for assessing biodiversity at the community level. For example, zoobenthos indicators such as AMBI (AZTI Marine Biotic Index; Borja et al., 2000) and BBI (Brackish waters Benthic Index; Perus et al., 2007) and macrophyte indicators such as eelgrass depth limit, all describe aspects of those communities (or biogenic habitats) and can be used in the biodiversity assessment for the MSFD (Rice et al., 2012) and have been included in the initial assessments of the marine environment carried out by EU member states in 2012.

The analysis of indicators and assessments applied in the MSFD initial assessments during the first phase of the MSFD implementation revealed some problems regarding the degree of development and operationality of the biodiversity assessment within the EU member states compared to the requirements of the MSFD (Berg et al., 2015; Hummel et al., 2015). Data availability and regional specificities influenced the number of methodologies used and reported by member states (Pali Alexis et al., 2014). Some ambiguity in the EU Commission Decision

(Berg et al., 2015) introduced discrepancies and increased the potential for non-harmonized approaches in the assessment of the marine environment even between member states of the same regional sea.

In addition to the assessments performed by EU member states, marine biodiversity assessments have also been carried out at regional level by the Regional Sea Conventions (RSC), which have identified a number of biodiversity indicators representing the different trophic levels of the marine ecosystem as well as the relevant habitats and ecosystems. OSPAR and HELCOM, the relevant RSC for the North East Atlantic and Baltic Sea regions, respectively, have agreed to develop common indicators for the major elements and species groups of marine biodiversity: benthic and pelagic habitats, seabirds, marine mammals, fish and food webs (HELCOM, 2016; OSPAR, 2016). Existing monitoring programmes in these regions were originally set up to assess pollution effects in the marine system and commercially exploited fish stocks. Therefore, these did not originally cover biodiversity assessment needs as specified by the MSFD. Many of the common indicators currently being defined under OSPAR and HELCOM are new indicators to the regions, specifically developed for the forth-coming environmental assessment in 2018 and many of them have not been properly validated yet.

In order to complement the on-going work for biodiversity indicator development for the MSFD environmental status assessments in the EU, the EU FP7 project DEVOTES (DEVELOPMENT OF innovative TOOLS for understanding marine biodiversity and assessing good Environmental Status; www.devotes-project.eu) carried out a comprehensive overview of existing biodiversity-related indicators used in previous marine assessments carried out under different policy frameworks, in the MSFD initial assessment, and elsewhere (Teixeira et al., 2014). The DEVOTES inventory of the existing biodiversity indicators was compiled in the form of a catalog, which includes over 600 entries. In this paper, we present an overview of the indicator development and refinements in DEVOTES, which were aimed to address some of the identified gaps and further development needs for the next phase of the MSFD assessment (by 2018).

MATERIALS AND METHODS

DEVOTES biodiversity indicator catalog is available via a database software (DEVOTool), which allows navigating the metadata (<http://www.devotes-project.eu/devotool>) and to make specific queries to find existing indicators depending on the needs of the user. The catalog currently includes over 600 indicators of marine biodiversity, food web status, sea floor integrity, and alien species, used and proposed to be used in marine assessments. We queried the database to find out how many indicators there are for each ecosystem component, and how they relate to the MSFD Descriptors and criteria (Table 1). The exact DEVOTool query used here is presented in the Supplementary Material. Table 1 presents an overview of the number of indicators included in the DEVOTool Indicator Catalog and representing the different biodiversity components that these indicators cover

(i.e., Microbes, Phytoplankton, Zooplankton, etc.), and their applicability to the MSFD Descriptors and criteria.

At the early stages of the project, after the initial indicator catalog compilation, similar data was used to analyse the gaps in the available indicator coverage, and to prioritize the indicator development taking place within the project (Teixeira et al., 2014). The gap analysis was carried out comparing how well the indicators in the catalog cover the requirement of the MSFD Commission Decision (2010/477/EU) criteria and indicators for the biodiversity related descriptors D1, D2, D4, and D6. Also it was evaluated how well the indicators in the catalog covered the biodiversity components, and habitats, as listed in Table 1 of Annex III to the Directive (MSFD; 2008/56/EC) and specified by Cochrane et al. (2010). The number of indicators refined and new indicators developed for various biodiversity components are also included in Table 1. Overview of the methodologies for development of new indicators and refinements of existing indicators are compiled in Berg et al. (2016) and referred in the text.

RESULTS

Gap Analysis of Biodiversity Indicators

Despite the high number of marine biodiversity indicators available, there were important gaps in terms of the MSFD requirements (Table 2; Berg et al., 2015). Additionally, there is insufficient information regarding the quality and confidence of the indicators (Queiros et al., 2016). Most indicators lack regional targets or quality threshold values, and few have a measure of confidence and a demonstrated link to pressures (Teixeira et al., 2014). Thus, although the indicators were operational in the sense that they were used in previous marine assessments, their applicability to fulfill the criteria of MSFD is less evident (Berg et al., 2015).

Major gaps observed for the Descriptor 1 were indicators to assess the biodiversity at ecosystem level and the genetic composition of populations, indicators for microbes, pelagic invertebrates (Cephalopods), and reptiles (Table 2). Abyssal and bathyal zones totally lack indicators addressing those depths (Hummel et al., 2015; Teixeira et al., 2014). Moreover, habitats with restricted distribution in the regional seas (like ice-associated species and communities) had a low overall number of indicators (Teixeira et al., 2014). Indicators for the MSFD biodiversity criterion 1.7 “Ecosystem structure” and the associated processes and functions are relatively scarce (Berg et al., 2015), and those few addressing this criterion focused essentially on communities, often in isolated components. Only few biodiversity indicators related to ecosystem processes and function were reported (Table 1), all of them for the North-Eastern Atlantic (Teixeira et al., 2014).

MSFD Descriptor 2 addresses the threat on natural biological diversity caused by the non-indigenous species (NIS), which are the taxa introduced outside their natural range and natural dispersal potential as result of human activities. Particularly the invasive NIS are recognized as a global threat to biodiversity (Olenin et al., 2010). Despite the requirements of MSFD Descriptor 2, there were no reported indicators in Europe

TABLE 1 | Total number of indicators for specific biological components and MSFD criteria of descriptors 1, 2, 4, and 6 (according to Commission Decision 2010/477/EU) included in the DEVOTool Catalog prior to the new developments (database version 7).

MSFD Criteria	fauna										Benthic habitat			
	General	Microbes	Phytoplankton	Zooplankton	Angiosperms	Macroalgae	Benthic	Fish	Birds	Mammals		Reptiles	Cephalopods	Water column habitat
1.1 Species distribution	-	0 (0/1)	10	4	21	31 (1/0)	23 (1/0)	18	9 (0/1)	10	5	4	18	71
1.2 Population size	-	0	15 (3/3)	7 (2/0)	16	20	29	26	13	13	3	6	32	62
1.3 Population condition	-	0	2	2	2	0	13	21 (0/2)	22	18	5	2	18	15
1.4 Habitat distribution	27	-	4	3	18	22	8	2	1	3	1	1	3	60 (1/0)
1.5 Habitat extent	22	-	4	1	18	18	5	3	1	1	1	1	5	49 (1/0)
1.6 Habitat condition	30	1	35	25	30	47	62	17	8	5	3	2	72	136
1.7 Ecosystem structure	15	-	17	10	19	37	50	27	6	4	3	7	37	95
Ecosystem processes & functions	1	-	2	1	2	1	4	3	2	2	2	2	3	6
2.1 Abundance and state of non-indigenous species	3 (2/1)	0	9	11	8	9	9	9	5	5	-	2	10	9
2.2 Environmental impact of invasive non-indigenous species	0 (0/1*)	0	3	5	2	3	3	3	2	2	-	0	5	3
4.1 Productivity (per unit biomass) of key species or trophic groups	1	0	2 (0/1)	0	1	0	2	5	4	6	0	1	3	2
4.2 Proportion of selected species at the top of food-webs	-	-	-	2	-	-	2	9	1	0	-	1	6	2
4.3 Abundance/distribution of key trophic groups/species	0	0	26 (3/3)	22 (2/0)	16	27	30	32 (0/1)	9	9	0	7	54	60 (1/0)
6.1 Substrate characteristics — physical damage	41	-	-	-	24	33	20	2	1	1	-	-	1	97 (1/0)
6.2 Condition of benthic community	5	0 (0/1)	2	1	25	47	47 (1/3)	2	-	-	-	-	1	107

In parenthesis are the number of indicators refined or developed during the DEVOTES project (refined/new). A dash (-) means that the MSFD criteria is not relevant for the biological component or habitat (or general indicators) in question. Note that many of the indicators could be applied to more than one MSFD criterion (such as "phytoplankton community composition" or "phytoplankton functional groups" which are applicable to both MSFD criteria 1.2 and 4.3). "General" indicators are not associated to a specific biological component (such as biotopes, habitats or areas, chemical or physical status or indicators using the abundance of functional groups), or apply to any biological component. See Supplementary Material for the explanation how the data was obtained from the DEVOTool Indicator Catalog (<http://www.devotes-project.eu/devotool>).

TABLE 2 | Overview of the identified gaps in the suite of existing indicators (as included in the DEVOTool Catalog v. 7) for MSFD environmental status concerning Descriptors 1, 2, 4, and 6 (Annex 1) with respect to MSFD criteria (as identified in Commission Decision 2010/477/EU) and development of new and refined indicators in the DEVOTES project, as well as the identified future research needs.

MSFD descriptor	Identified gaps and MSFD criteria	Developed/refined in DEVOTES	Future research needs
D1: 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 climate conditions.	<p>Indicators addressing different organizational levels of biodiversity:</p> <p>1.1. Species level</p> <ul style="list-style-type: none"> • Microbes • Pelagic invertebrates (cephalopods) and reptiles <p>1.2. Populations:</p> <ul style="list-style-type: none"> • Genetic structure <p>1.4–1.6. Habitat level</p> <ul style="list-style-type: none"> • Deep-sea habitats • Sea ice- habitats <p>1.7. Ecosystem level:</p> <ul style="list-style-type: none"> • indicators biodiversity criterion “Ecosystem structure” and the associated processes and functions 	<p>New indicators for biological components: species and population levels:</p> <ul style="list-style-type: none"> • Microbes • Genetic indicators <p>New indicators for habitat and ecosystem level:</p> <ul style="list-style-type: none"> • Indicators describing habitat extent and biomass of key stone species (e.g., extent and biomass of seagrass meadows coupled with the abundance of water fowl) • Habitat mapping (high resolution habitat characterization) 	<p>Indicators for Cephalopods and reptiles (where relevant)</p> <p>Research on genetic structure of populations</p> <p>Habitats:</p> <ul style="list-style-type: none"> • Indicators for deep sea and sea ice habitats (where relevant) • Indicators based on high resolution habitat mapping <p>Ecosystems:</p> <ul style="list-style-type: none"> • Indicators for ecosystem processes and functions
D2: Non-indigenous species (NIS) introduced by human activities are at levels that do not adversely alter the ecosystem	<ul style="list-style-type: none"> • Indicators addressing Impacts of non-indigenous invasive species at the level of species, habitats, and ecosystem, where feasible” (MSFD criterion 2.2., indicator 2.2.2) 	<ul style="list-style-type: none"> • 2.2. Abundance and distribution range (ADR) of NIS indicating magnitude of impact • 2.2. Cumulative IMPact index of Invasive ALien Species (CIMPAL) 	<ul style="list-style-type: none"> • Testing and validating ADR and CIMPAL in other regional seas
D4: 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	<p>Low number of indicators for</p> <ul style="list-style-type: none"> • Criterion 4.1 “Productivity (production per unit biomass) of key species on trophic groups” both for primary and secondary producers • Criterion 4.2 “Proportion of selected species at the top of food-webs” 	<ul style="list-style-type: none"> • 4.1. Phytoplankton primary production: Time series of annual gross primary production (AGPP), combined with phytoplankton biomass estimation with remote sensing, ferry box, CHEMTAX • 4.2. indicator of size composition in fish and elasmobranch communities • Non-Declining Exploited Species’ (NDES) • 4.3. Phytoplankton community composition as indicator for food web structure and functioning 	<p>Further development of indicators with special focus on primary and secondary producers.</p>
D6. 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	<ul style="list-style-type: none"> • Lack of indicators for criterion 6.1 Substrate characteristic—physical damage • No indicators developed for criterion 6.2 Condition of benthic community for Cephalopods 	<ul style="list-style-type: none"> • Further refinement of the Multivariate AZTI Marine Biotic Index (AMBI) and Benthic quality index (BQI) using signal detection theory as harmonized way to set target values for GenS and to evaluate the specificity and sensitivity of the indices. • Genetic tools for macrobenthic community condition and functionality assessment • High resolution habitat characterization for benthic integrity assessment 	<ul style="list-style-type: none"> - Better integration of visual/acoustic tools for new indicators of sea-floor integrity - Definition of targets for structural habitat/ecosystem
Other identified deficiencies of existing indicators	<ul style="list-style-type: none"> • Quality/confidence of existing indicators • Targets/GES threshold levels missing • Low regional coverage 	<ul style="list-style-type: none"> • Indicator quality criteria and scoring system for MSFD applicability (Queiros et al., 2016) • Refinement of 13 existing indicators regarding quality/confidence, targets • Novel approaches/ methods for indicator target setting (e.g., signal detection theory) 	<ul style="list-style-type: none"> - Indicator quality/confidence testing and scoring system as operational part of DEVOTool - Testing and validation of indicators in further regions

specifically assessing the “*Impacts of non-indigenous invasive species at the level of species, habitats and ecosystem, where feasible*” (MSFD criteria 2.2., indicator 2.2.2; **Tables 1, 2**; Teixeira et al., 2014). In most cases, the MSFD initial assessments of the Member States did not include reporting of the adverse effects in biodiversity or the magnitude of impacts caused by NIS (Palialexis et al., 2014; Berg et al., 2015). RSCs (e.g., Barcelona and Bucharest Conventions, HELCOM, and OSPAR) have not included indicators for NIS impacts within their agreed set of indicators. Only recently there have been research activities to develop practical proposals to help managers assess these requirements in standardized ways (e.g., Zaiko et al., 2011; Katsanevakis et al., 2016; Nentwig et al., 2016; Rabitsch et al., 2016).

Most of the MSFD food web indicators (Descriptor 4) are related to “*Abundance or distribution of key trophic groups or species*” (MSFD criteria 4.3), and are thus simultaneously D1 (MSFD criteria 1.1) indicators (Berg et al., 2015). There is a need to develop indicators related to MSFD criterion 4.1 (“*Productivity (production per unit biomass) of key species on trophic groups*”) especially focusing on primary and secondary producers, as well as for the criterion 4.2 (“*Proportion of selected species at the top of food-webs*”; **Table 2**).

Finally, the remaining biological descriptor of the MSFD (Descriptor 6 on sea-floor integrity) is rather well developed in terms of indicators, having a high number of them (≥ 20 indicators), both for criterion 6.1. (“*Substrate characteristics—physical damage*”) and 6.2. (“*Condition of benthic community*”) for angiosperms, macroalgae, and benthic fauna. However, there is a need for developing indicators especially for cephalopods, but also for other biological components. Also there is a gap in the development of targets at the level of benthic habitats and ecosystems.

Biodiversity Indicator Refinement and Development in DEVOTES

In order to address the identified gaps in the indicator availability for marine assessments, 16 new indicators were developed and 13 indicators were refined (**Table 3**) to better fulfill the MSFD requirements (Berg et al., 2016). The indicators were scored according to the eight indicator quality criteria listed by Queiros et al. (2016) in order to evaluate their fitness as potential indicators for the MSFD assessments (**Figure 1**). The quality criteria are: (1) Scientific basis, (2) Ecosystem relevance, (3) Responsiveness to pressure, (4) Possibility to set targets, (5) precautionary capacity, (6) Quality of sampling method, (7) Cost-effectiveness, and (8) Existing and ongoing monitoring data. In general, the newly developed indicators covered both pelagic and benthic ecosystems and addressed several biological components and habitats that were identified as lacking indicators or as being under-represented in several marine regions (Teixeira et al., 2014). In addition, 13 pre-existing indicators were further refined in order to improve their performance and confidence. For example, they were tested for responsiveness to pressures, or using new data sets to validate their applicability in wider sea regions.

Indicators for Genetic Diversity of Microbial and Benthic Communities

The genetic structure of a population (MSFD criteria 1.3, and indicator 1.3.2) was the least covered of the MSFD D1—biological diversity indicator requirements (**Table 1**; Teixeira et al., 2014). This highlights the need for new developments addressing the genetic component of biodiversity and introducing such new methods to marine monitoring programmes. Emerging assessment tools based on molecular techniques have received strong attention from the scientific community (Bourlat et al., 2013), which might increase their potential for contributing to assessments of GES.

Genetic diversity is an aspect of biodiversity that has recently gained increased attention, but operational indicators addressing genetic structure of the populations are still scarce. Instead of using traditional sampling, taxonomic data can be obtained using a DNA metabarcoding technique. Microbial indicators were identified as one of the gaps and, consequently, further work to advance bacterial community indicators using nucleic acid microarrays was initiated in DEVOTES. Microbial abundance and biodiversity variables are relevant for several MSFD descriptors i.e., D1 Biodiversity, D4 Food webs, and also D5 Eutrophication (Caruso et al., 2015). Specifically, MSFD indicators 1.2.1 (*Population abundance and/or biomass, as appropriate*), and 1.3.1 (*Population demographic characteristics (e.g., body size or age class structure, sex ratio, fecundity rates, survival/mortality rates)*), and 1.3.2 (*Population genetic structure, where appropriate*) were targeted in the development work.

Micro-organisms present in the sediments were also included in the analysis addressing the MSFD indicator 6.2.1. *Presence of particularly sensitive and/or tolerant species*; in addition to D1 indicators. The microbial sediment indicator followed the approach of gAMBI (genetic AMBI; Aylagas et al., 2014, 2016), and scored quite high in the fitness as an operational indicator, particularly on ecosystem relevance, concreteness, early warning capacity, and cost efficiency (**Figure 1**).

The AMBI indicator for benthic invertebrates (Borja et al., 2000) was modified by applying simultaneous amplification of a standardized DNA fragment from the total DNA extracted from an environmental sample (gAMBI). This allows the rapid, accurate and cost-effective identification of the entire taxonomic composition of thousands of samples simultaneously (Aylagas et al., 2014, 2016). Such DNA data do not provide accurate estimations about the abundance of the taxa in a certain sample, so current use is restricted to presence/absence estimations. Nonetheless, a high proportion of the taxa visually identified can be detected using the DNA metabarcoding technique. The benthic gAMBI indicator scored also high in the evaluation of the indicator fitness (**Figure 1**).

Habitats of Key Species as Indicators of Biodiversity on Ecosystem Level Functions

Vegetated marine areas, such as seagrass meadows and kelp forests, are important habitats for a wide diversity of algae, invertebrates and fish (Steneck et al., 2002, Schmidt et al., 2011, Boström et al., 2014, Sheaves et al., 2015, Thormar et al., 2016) including economically important fish such as cod (Lilley and

TABLE 3 | New and refined indicators developed during the DEVOTES project.

Name of the indicator	Code	Description	MSFD GES Descriptor	MSFD GES Criteria/indicator	Ecosystem component	New/refined	(1) Type of indicator	(2) Quantitative or qualitative	(3) Relevant spatial coverage	(4) Relevant to MSFD	(5) Established indicator	References
Microbial community indicator	MCI	Microbial abundance and biodiversity indicators using nucleic acid microarrays	D1	1.2.1; 1.3.1; 1.3.2.	Microbes	New	I	Qualitative	Depends on monitoring	NA	No	Berg et al., 2016
Microbial sediment indicator	MSI	Genetic based benthic microbial community condition and functionality assessment -	D1, D6	1.2.1; 1.3.1; 1.3.2; 6.2.1.	Microbes	New	S	Qualitative	Depends on monitoring	Trends	No	Berg et al., 2016
Lower Depth distribution Limit of Macrophytes	LDLM	Lower Depth distribution Limit of Macrophytes (LDLM) (Furcellaria lumbricoides)	D1, D5	1.1.1; 1.4.1.; 5.3.1	Macroalgae	Refined	P, S	Qualitative	Depends on monitoring	Targets	Latvia, Lithuania	Berg et al., 2016
Waterfowl and eelgrass indicator	WEI	Distribution of herbivorous waterfowl in relation to eelgrass biomass distribution	D1, D4	1.1.2; 1.4.2; 4.3.1	Seabirds, Angiosperms	New	S	Quantitative	Depends on monitoring	Targets	No	Berg et al., 2016
High resolution habitat characterization	HRHC	Mapping of biogenic reef forming species by multibeam echosounders	D1, D4, D6, D7	1.5; 1.7; 1.7.1.	Habitat	New	S	Quantitative	Depends on monitoring	NA	EMODNET	Berg et al., 2016
Abundance and distribution range of NIS	NIS-ADR	Abundance and distribution range of established Non-Indigenous Species	D2	2.1.; 2.2.	Various	Refined	S	Quantitative	Regional	No targets	No	Zaiko et al., 2015
NIS-Arrival trends	NIS-AT	Trends in the arrival of new non-indigenous species	D2	2.1.1.	Various	Refined	P	Quantitative	Europe	Trends, targets	No	Berg et al., 2016
NIS-Entry pathway	NIS-EP	Trends in the arrival of Non-Indigenous Species by pathway of entry	D2	2.1.1.	Various	Refined	P	Quantitative	Europe	Trends	No	Berg et al., 2016
NIS-cumulative impacts	CIMPAL	Cumulative impact index of Invasive Alien Species (CIMPAL)	D2	2.2.	Various	New	P	Quantitative	Regional	Targets, trends	No	Katsanevakis et al., 2016
Annual gross primary production	AGPP	Time series of annual gross primary production (AGPP)	D4, D5	4.1.	Phyto-plankton	New	S	Quantitative	Depends on monitoring	Trends	No	Berg et al., 2016

(Continued)

TABLE 3 | Continued

Name of the indicator	Code	Description	MSFD GES Descriptor	MSFD GES Criteria/indicator	Ecosystem component	New/refined	(1) Type of indicator	(2) Quantitative or qualitative	(3) Relevant spatial coverage	(4) Relevant to MSFD indicator	(5) Established indicator	References
Satellite Chlorophyll-a	S-Chl	Surface Chlorophyll-a concentration from satellite measurements	D5, D4	5.2; (4.1)	Phyto-plankton	Refined	P	Quantitative	Europe	Trends	Yes	Berg et al., 2016
Typical length of fish	TLF	Size composition in fish and elasmobranch communities	D4	4.2.1.	Fish	New	S	Quantitative	Depends on monitoring	Targets	No (ICES)	Berg et al., 2016
Large fish indicator	LFI	Large Fish Indicator (LFI) of the demersal fish and elasmobranch community	D1, D4	1.7; 4.2.	Fish	New	S	Quantitative	Depends on monitoring	Targets	Yes	Berg et al., 2016
Fish Mean Maximum Length	MML	Mean maximum length (MML) of demersal fish and elasmobranchs	D1	4.2.1.	Fish	New	S	Quantitative	Depends on monitoring	Targets	Yes	Berg et al., 2016
Phytoplankton food-web indicator	PFW	Phytoplankton community composition as a food web indicator	D4	4.3.1.	Phyto-plankton	New	S	Quantitative	Baltic Sea	Tentative target	No	Berg et al., 2016
Phytoplankton food quality traits	PFQ	Phytoplankton community composition based on food quality traits indicator for food web effects	D4, D1	1.6.2; 1.6.3; 1.7.1; 4.3.1;	Phyto-plankton	New	S	Quantitative	Baltic Sea	No targets	No	Berg et al., 2016
Phytoplankton Shannon95	PSh	Phytoplankton taxonomic diversity (Shannon95)	D1, D4	1.6.2; 1.6.3; 4.1	Phyto-plankton	Refined	S	Quantitative	Baltic Sea	No targets	No	Uusitalo et al., 2013
Phytoplankton evenness	PE	Phytoplankton taxonomic (Pielou's) evenness indicator	D1, D4	1.6.2; 1.6.3; 1.7.1.	Phyto-plankton	New	S	Quantitative	Baltic Sea	No targets	No	Berg et al., 2016
Phytoplankton seasonal succession	PSS	Seasonal succession of phytoplankton functional groups	D1, D4	1.6.1; 1.6.2; 4.3.1	Phyto-plankton	Refined	S	Quantitative	Baltic Sea	No targets	No	Berg et al., 2016
Diatom/Dinoflagellate index	DDI	Diatom/Dinoflagellate index	D4, D1	1.6.2; 1.7.1; 4.3.1	Phyto-plankton	Refined	S	Quantitative	Baltic Sea	No targets	No	Berg et al., 2016
Spring Diatom/Dinoflagellate ratio	SDD	Spring diatom/dinoflagellate biomass ratio (Black Sea)	D4, D1, D5	1.6.2; 1.7.1; 4.3.1; 5.2.4.	Phyto-plankton	New	S	Quantitative	Depends on monitoring	Trends	Bulgaria	Berg et al., 2016

(Continued)

TABLE 3 | Continued

Name of the indicator	Code	Description	MSFD Descriptor	MSFD GES Criteria/indicator	Ecosystem component	New/refined	(1) Type of indicator	(2) Quantitative or qualitative	(3) Relevant spatial coverage	(4) Relevant to MSFD indicator	(5) Established indicator	References
N2-fixing cyanobacteria	N2C	Biomass of N2-fixing cyanobacteria indicating nitrogen transferred in food webs	D4	Tentively 4.3.1.	Cyano-bacteria	New	S	Quantitative	Baltic Sea	No	No	Berg et al., 2016
Share of cyanobacteria	Scy	Share of cyanobacteria from total phytoplankton biomass indicator for food web effects	D4, D1	Tentively 4.3.1; 1.6.2.	Oyano bacteria/Phyto-plankton	New	S	Quantitative	Baltic Sea	No	No	Berg et al., 2016
Copepod biomass	CoB	Contribution of the Copepoda species to the total mesozooplankton biomass	D1, D4	1.6.2; 4.3.1	Zooplankton	Refined	S	Quantitative	Black Sea	Targets	No	Berg et al., 2016
Mesozooplankton biomass	MezB	Total mesozooplankton biomass in the zooplankton community	D1, D4	1.6.2; 4.3.1	Zooplankton	Refined	S	Quantitative	Depends on monitoring	Targets	Bulgaria	Berg et al., 2016
AZTI's Marine Biotic Index (AMBI)	AMBI	AZTI's Marine Biotic Index (AMBI)	D6	6.2.1.	Macro-invertebrates	Refined	I	Quantitative	Depends on monitoring	Targets, trends	Yes	Berg et al., 2016
Genetic AMBI	gAMBI	Genetic AMBI — macrobenthic community condition and functionality assessment based on genetic	D6	6.2.1.	Macro-invertebrates	New	I	Qualitative	Depends on monitoring	Trends	Yes	Aylagas et al., 2016
Multivariate AMBI	mAMBI	Multivariate AZTI Marine Biotic Index	D6	6.2.2.	Macro-invertebrates	Refined	I	Quantitative	Depends on monitoring	Targets, trends	Yes	Borja et al., 2015
Benthic quality index	BQI	Benthic quality index—multimetric indicator of benthic community condition and functionality	D6, D1	6.2.2; 1.6.2; 1.7.1; 4.3.1	Macro-invertebrates	Refined	S	Quantitative	Depends on monitoring	Trends	Baltic	Berg et al., 2016; Chuševé et al., 2016

The table summarizes which of the Marine Strategy Framework Directive (MSFD), good environmental status (GES), Descriptors (D), criteria, indicators, and ecosystem components were addressed. The columns from the left are following: "Code" means the abbreviated name of the indicator; "Description" is the indicator description; "MSFD Descriptor" and "MSFD Criteria/indicator" refer to the Descriptor, criteria, and indicators according to the notation of the EU Commission Decision 2010/477/EU; "Ecosystem component" specifies which ecological or biological component associated to the indicator; "New/refined" specifies, if the indicator is newly developed or an existing refined indicator. Next five columns summarize the evaluation of the indicators according to the following OSPAR criteria: (1) type of the indicator (I = impact, S = state, P = pressure); (2) whether the indicators were quantitative or qualitative; (3) relevant spatial coverage of the indicator; (4) if the indicator was relevant to MSFD assessment purposes ("Targets" means that the indicator methodology includes setting of quantitative targets, while "No targets" means that those are not yet developed for the indicator; "Trends" means that target setting is not part of the indicator methodology), and (5) whether the indicator is already established, i.e., used in established management frameworks such as the OSPAR system of Ecological Quality Objectives (EcoQOs) for the North Sea. The last column refers to the latest publication, where the indicator development is described in detail.

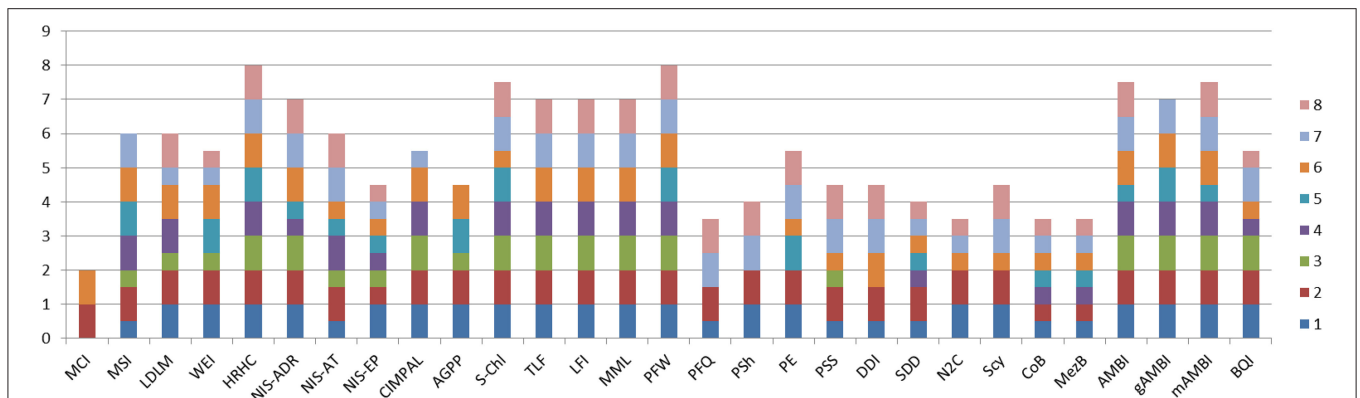


FIGURE 1 | Cumulative quality scoring of the indicators developed and refined during the DEVOTES project. Indicator quality scores are based on the framework to test and evaluate indicator quality (Queiros et al., 2016). Each indicator was evaluated based on eight quality criteria: (1) Scientific basis; (2) Ecosystem relevance; (3) Responsiveness to pressures; (4) Possibility to set targets; (5) Early warning capacity; (6) Concreteness; (7) Cost-Efficiency; (8) Existing and on-going data. For each indicator the quality criteria were evaluated and scored using three evaluation scores: 1, criteria is fully met; 0.5, criteria is partially met; and 0, criteria is not met. The scoring principles for each indicator and criteria are described in detail by Berg et al. (2016). Indicator codes are same as in **Table 3**.

Unsworth, 2014). The vegetation stimulates biodiversity by being a habitat-forming ecosystem component, hugely increasing the colonizable area while also providing shelter and food for a wealth of organisms.

In turn, the organisms of the habitat exert a feed-back effect on the meadows and their functioning. Hence, the presence of fish exerts an important role in contributing to maintaining healthy vegetated ecosystems via top-down control of nuisance algae (Baden et al., 2012). The extent and biomass of sea grass meadows may also couple with the abundance of waterfowl in terms of bottom-up as well as top-down effects, thereby showing an additional link to biodiversity. Accordingly, Berg et al. (2016) propose that “the idea of an indicator is that extended eelgrass cover/biomass in combination with large populations of foraging birds reflects good environmental status” which “requires a suitable balance between bottom up control of eelgrass meadows on bird populations and top-down control of the birds on eelgrass meadows.” Testing of the relationships between eelgrass cover/biomass and the abundance of herbivorous waterfowl was carried out in order to develop a new indicator “*Distribution of herbivorous waterfowl in relation to eelgrass biomass distribution*” (Berg et al., 2016). This indicator is relevant for several GES criteria, D1 (biodiversity) and D4 (food webs), 1.1. *Species* and 1.4. *Habitat distribution*; and 4.3. *Abundance/distribution of key trophic groups/species*. The indicator scored relatively high (**Figure 1**), and thus it is considered as a promising indicator for MSFD. On this basis we suggest that indicators describing habitat extent and biomass of key stone species may serve as indicators of biodiversity both on habitat as well as on species level and concurrently enabling coupling with other biological components, and providing proxies also for ecosystem level of structural and functional biodiversity indicators.

Moreover, one of the commonly used macrophyte indicators is the Lower Depth distribution Limit of Macrophytes (LDLM), which indicates the distribution and abundance of habitat forming macrophyte species. This indicator informs about

the following MSFD criteria 1.1. *Species*, and 1.4. *Habitat distribution*, as well as 5.3. *Indirect effects of nutrient enrichment*, particularly the indicator 5.3.2. *abundance of perennial seaweeds and seagrasses adversely impacted by decrease in water transparency*. In DEVOTES, the target setting of the LDLM indicator for a perennial red alga species, *Furcellaria lumbricalis* was refined and harmonized (**Table 3**).

A promising tool to characterize and map marine habitats is to use multibeam echosounders on vessels (systematic high resolution habitat characterization) which provide high resolution and georeferenced technology and allow continuous and direct mapping of biogenic reef-forming species (e.g., Harris and Baker, 2011). This methodology can be used to derive operational indicators for many MSFD biodiversity indicators under the criteria 1.5 *Habitat extent*, and (1.6) *condition*, as well as 1.7 *Ecosystem structure*, 1.7.1. *Composition and relative proportions of ecosystem components (habitats and species)* (Berg et al., 2016).

Non-indigenous Species Indicators (D2)

One of the identified gaps in all regional seas was the lack of indicators for measuring the ecological impact of non-indigenous species in the marine ecosystems. The early detection of invasive species, using eDNA and metabarcoding was addressed by Ardura et al. (2015) and Zaiko et al. (2015). The abundance and distribution range (ADR), a semi-quantitative characteristic of the extension of a non-indigenous species population within the biopollution assessment framework (Olenin et al., 2007), can be used as measure of the bioinvasion impact. It was tested on the zebra mussel studying ecosystem-level impacts (Minchin and Zaiko, 2013; Zaiko et al., 2014) and the results showed that ADR of zebra mussels generally corresponded with the overall impact score and might be indicative of the particular invasion phase (establishment, expansion, outbreak, accommodation). ADR could thus serve as a proxy for the overall magnitude of impact of the species. Since data on species abundance and distribution can

be retrieved from the regular biodiversity monitoring records, delivering ADR is a cost-effective solution for environmental status assessment. Determining the cumulative impact of invasive non-indigenous species is another approach toward assessing their role in biodiversity. The recently developed Cumulative IMPact index of Invasive ALien Species (CIMPAL) uses a spatially explicit conservative additive model based on the distributions of invasive species and ecosystems, including the reported magnitude of ecological impacts and the strength of such evidence (Katsanevakis et al., 2016).

Indicators for Food Webs (D4)

Productivity of key species on trophic groups

Indicators for phytoplankton primary production (PP), provide information on the vigor of an ecosystem (energy fluxes and ability to recover from disturbance) and thus health of the pelagic ecosystem (Tett et al., 2007). Phytoplankton photosynthesis produces organic matter which is then utilized by organisms at higher trophic levels and provides the base on the pelagic food web. There are currently different methods adopted for measuring PP (e.g., oxygen evolution, ^{14}C method, PAM fluorimeters, models, remote sensing). Traditional methods for measuring PP (e.g., ^{14}C method) are reliable but time-consuming, expensive and localized. Other methods (e.g., remote sensing, models) can investigate a wider area but require validation or may have limited applicability to certain water types. Time series of annual gross primary production (AGPP) at different ecohydrodynamic regions of the North Sea (based on van Leeuwen et al., 2015) were calculated using an empirical model (Cloern, 1987) from measurements of chlorophyll (proxy of phytoplankton biomass), light attenuation coefficient (Kd), and surface irradiance. Currently there is no ongoing monitoring that would provide data for AGPP indicator calculation and target setting, thus the indicator had a medium score (**Figure 1**), and it requires further development to be operational.

Phytoplankton blooms in coastal and open marine waters are characterized by high temporal and spatial fluctuations. Therefore remote sensing and continuous fluorometric measurements are promising tools to detect and measure phytoplankton phenomena in the surface layers of marine waters (e.g., Kutser, 2009; Kahru and Elmgren, 2014; Cristina et al., 2015). The phytoplankton biomass assessment method based on surface chlorophyll-a concentration measurements using cost-effective remote sensing data (Gohin, 2011a,b; Novoa et al., 2011; Cristina et al., 2015), builds on the indicator assessment approach developed for *in situ* samples (Revilla et al., 2009) and gained a high score in the indicator evaluation matrix (**Figure 1**).

The joint use of remote sensing biomass observations and ship-of-opportunity fluorescence measurements are a powerful combination to detect changes both in the phytoplankton biomass and composition. Remote sensing, bio-optics, microscopy, and CHEMTAX results provide a combination of analytical tools that can be used to develop a phytoplankton biomass (chlorophyll a) index for marine and coastal waters off

the Iberian peninsula, in Portugal (Gohin, 2011b; Cristina et al., 2015, 2016a,b; Goela et al., 2015).

Proportion of selected species at the top of food-webs: fish and elasmobranch indicators

A number of promising indicators that capture the effects of fishing on marine biodiversity has been tested (Fu et al., 2015; Lynam and Mackinson, 2015; Coll et al., 2016). Fishing targets particular species and through the inherent selectivity of the fishing gears, often regulated by mesh size restrictions, larger individuals of populations are preferentially caught. So community level indicators that focus on changes in abundance (Kleisner et al., 2015), species composition (such as the mean maximum length of fish and elasmobranchs), trophic level (Shannon et al., 2014), or the relative biomass across a size spectrum (Engelhard et al., 2015; Thorpe et al., 2016) of fish and elasmobranch assemblages can respond strongly to direct fishing pressure. DEVOTES also developed an indicator of size composition in fish and elasmobranch communities (Berg et al., 2016), the biomass weighted geometric mean length of fish, known as Typical Length (ICES, 2014) since changes in size structure has been shown to represent change in trophic level (Jennings et al., 2007).

Shannon et al. (2014) made a comparison of the performance of trophic level (TL) indicators to demonstrate fishing impacts in 9 marine ecosystems and showed that the information content of these indicators differed depending on the data source, the previous changes in state and the historical development of fishing in the system. Catch-based TL indicators calculated using landings statistics represent the pressure on the system, while survey based TL indicators show unbiased state changes at the surveyed community level. In order to gain a complete picture of the wider effects of fishing on food web structure, complementary model derived TL indicators can be examined. While a meta-analysis of the 9 ecosystems revealed a significant pattern of low TL (for either catch-, survey-, or model-based indicators) under high fishing mortality, the relationship varied greatly at the level of the single ecosystem (some positive and negative relationships in addition to non-significant relationships). To understand why a particular trajectory in TL occurred in any single ecosystem, a good knowledge of changes in fisheries management and environmental change specific to the system is necessary.

Kleisner et al. (2015) tested the “Non-Declining Exploited Species” (NDES) indicator across 22 marine ecosystems, where the indicator is the proportion of species with survey catch-rates that have a positive monotonic temporal trend assessed with a significance test determined using a distributional test for the community given the length of time series data available (see Lynam et al., 2010). The authors conclude that the indicator can provide a valuable and relatively easy-to-understand measure of change in the ecosystem. The authors compared their evaluation of changes within ecosystems by the NDES to similar evaluations using community indicators derived from survey data (i.e., the proportion of predatory fish, mean trophic level, and mean life span; see Coll et al., 2016). In many, but not all cases, a decline in NDES was mirrored by the community metrics. In other cases, fishing pressure was found to be impacting only part of

the community and this was not reflected well in the overall community metrics. Thus, the scale of fishing impacts on the ecosystem and thus the responsiveness of community metrics to pressure are dependent on the level of fishing pressure relative to other drivers including natural environmental change as shown by Fu et al. (2015). Similarly, spatial patterns in indicators and their response to drivers are often evident. Engelhard et al. (2015) demonstrated that the Large Fish Indicator of the demersal fish and elasmobranch community has responded to decreases in fishing pressure in those parts of the North Sea where demersal fishing effort was once high but the response was not uniform across the area. This study along with Marshall et al. (2016) demonstrates that the strength of different drivers of fish community structure varies across the North Sea. As a result, the outcomes of management measures are likely to vary in different localities.

Abundance/distribution of key trophic groups/species

Phytoplankton community composition. Phytoplankton community composition can be used as an indicator for food web structure as well as being an early warning indicator for subsequent effects on the food web. Food web indicators are an important part of biodiversity assessment because the food web delivers energy to all trophic levels thus sustaining the biodiversity components, and chl-*a* alone is not an applicable indicator for the dynamic processes in food webs. To this end, a phytoplankton community composition index was developed (Suikkanen et al., 2013). The application to areas in the Baltic Sea revealed that late summer communities in the Gulf of Finland, the Åland Sea, and the northern Baltic proper have shifted toward more microbial, less energy-efficient food webs consisting of more mixotrophic and lower food-quality phytoplankton. This may lead to a decreased availability of energy for herbivorous zooplankton and planktivorous fish, despite an observed increase in chl-*a* and phytoplankton biodiversity. The food web indicator “Phytoplankton community composition as a food web indicator” scored high in the indicator quality evaluation and is currently a candidate indicator for HELCOM holistic ecosystem assessment (Lehtinen et al., 2015).

Novel indicators focusing on the role and impact of N₂-fixing cyanobacteria in the pelagic food web were also considered. Cyanobacterial nitrogen is efficiently assimilated and transferred in Baltic food webs (Karlson et al., 2015). On the other hand, high abundance of cyanobacteria may harm the copepod reproduction and exert negatively on the food web (Engström-Öst et al., 2015). However, the tested indicators did not show a clear and coherent response to pressures, and scored low in ranking of the potential indicators (**Figure 1**).

Also phytoplankton community composition based on food quality traits could potentially be used as an early warning indicator for food web effects on higher trophic levels, as the quality of different phytoplankton taxa as food source for higher trophic levels varies (e.g., Daniëlsdottir et al., 2007). The same idea was also behind the indicator based on diatom/dinoflagellate ratio that has implications for zooplankton community composition and further in the food web. The different functional properties of diatoms and dinoflagellates

have an influence on the fate of the organic matter produced and thus have consequences for the overall biogeochemical cycles (e.g., Klais et al., 2011). Diatoms and dinoflagellates are proposed as a life form indicator in the OSPAR area (Gowen et al., 2011), considered as a supplementary indicator in the Baltic Sea (HELCOM, 2012a,b; Klais et al., 2011), and in the Black Sea (Sahin et al., 2007).

Revision of other existing indicators for phytoplankton diversity were also considered and developed further where feasible. The Shannon95 indicator (Uusitalo et al., 2013) and phytoplankton taxonomic evenness, that has been shown to correlate with the resource use efficiency and stability of the community (Ptacnik et al., 2008), were tested but did not show a clear and coherent response to pressures. The indicator on seasonal succession patterns of phytoplankton groups (Devlin et al., 2009), describes the normal or established seasonal succession patterns of phytoplankton groups and suggests that major deviations from this pattern indicate impairment of environmental status. However, sufficiently frequent sampling is seldom available through monitoring programmes.

Zooplankton community composition

Zooplankton has a crucial role in the pelagic food web, as it transfers energy from phytoplankton to higher trophic levels (Checkley et al., 2009), and changes in the zooplankton community's abundance and composition are related to the functioning of the aquatic ecosystem (Jeppesen et al., 2011). Changes in the eutrophication status of aquatic systems impacts composition of zooplankton community (Gliwiz, 1969; Pace, 1986), and the growth of planktivorous fish is regulated by composition and biomass of the mesozooplankton that they feed upon (Cardinale et al., 2002; Rajasilta et al., 2014). A number of zooplankton indicators have been proposed to assess the status and functioning of marine food webs (Teixeira et al., 2014; Berg et al., 2016).

Long-term monitoring of mesozooplankton composition has been conducted in the Baltic Sea as well as in the Black sea, and based on these data, zooplankton metrics have been proposed as indicators of environmental status both in the Baltic (HELCOM, 2012b) and in the Black Sea (Bulgarian Initial Assessment report, 2013, and Black Sea Commission zooplankton expertise group).

Biomass of mesozooplankton includes information of major key groups, forming the structure of the planktonic fauna, particularly the groups Copepoda, Cladocera, Meroplankton, and the species *Oikopleura dioica* and *Parasagitta setosa*. Copepods are present all year round and distributed within the coastal, shelf, and open sea habitats. They are a key group of mesozooplankton that reflects the food availability for zooplanktivorous fish (particularly sprat and anchovy, partly horse mackerel). Mesozooplankton community composition is indirectly impacted by eutrophication (via changes in primary productivity and phytoplankton community composition), whereas climatic changes, predation, introduction of synthetic compounds (from point sources), and predation of invasive species, result in direct impacts. Relatively high copepod biomass implies food availability for fish and consequently is considered to represent good status of the food web structure (D4

criterion 4.3.1 *abundance trends of functionally important selected groups/species*).

The response of the zooplankton indicators with respect to environmental variables was tested using Signal Detection Theory (SDT; Murtaugh, 1996). Phytoplankton total biomass and chl-a values were used as “Golden Standard” meaning that the indicator was considered to represent good status when these metrics were in good status. Zooplankton indicators received relatively low grading using the quality criteria ranking. Zooplankton is patchily distributed and seasonal variations in biomass and species structure result in a large variation in the data. Therefore the failure to detect a response to pressure patterns might partly be a problem in the different spatial and temporal scales of zooplankton and pressure data.

Approaches for Setting Thresholds/Targets for the Biodiversity Indicators

Indicator boundaries (thresholds) or target values are necessary to decide whether management action is required. A numeric definition for GES, i.e., the GES boundaries, can be defined by several alternative approaches (HELCOM, 2012a): (a) as an “acceptable” deviation from a reference condition (i.e., reference conditions representing natural conditions with minimal impact of anthropogenic pressures, that is the EU WFD approach), (b) as an “acceptable” deviation from a fixed reference point (i.e., fixed or depending on other variables), (c) as an “acceptable” deviation from a desired hypothetical condition (e.g., based on models), (d) as a threshold derived from ecological or physiological models (e.g., carrying capacity of a system, critical depth for photosynthesis, etc.), (e) as temporal trends, or tipping points (e.g., an analysis for changes in status), and (f) as biological effects on the condition of an organism (e.g., thresholds for contamination effects).

In the Baltic Sea, HELCOM has coordinated development of core indicators, which also included setting up the GES boundaries. In practice, the core indicators’ GES boundary is not only a single threshold but can be a range (with a lower and upper thresholds), a direction of a trend, or based on a class-scale. Also it appeared to be practical to apply several approaches in parallel, when setting GES boundaries for the HELCOM core indicators (HELCOM, 2013). The trend-based targets are heavily debated, as they do not address whether the status is GES or not, but only show the direction. An alternative to this could be a class-scale assessment, which could be given under high uncertainty of more definite GES threshold. This has not, however, yet been applied to any of the core indicators in practice.

SDT was tested for setting the threshold values for indicators (Chuševé et al., 2016). SDT was applied to the “Benthic quality index” (BQI; Rosenberg et al., 2004) in order to check its accuracy, sensitivity and specificity. In general, the SDT was found to be a robust and scientifically sound approach to set boundaries for indicator values, and to be helpful for planning environmental monitoring.

Finally, a new approach to address the target setting of the indicators in relation to ecosystem resilience (i.e., the ability to recover rapidly and predictably from pressures) and to select indicators and their target ranges has been introduced (Rossberg

et al., 2017). The idea is to simply choose the target range for any ecosystem state indicator as the range of values from where, when all pressures were hypothetically removed, the mean time to reach the indicator’s natural range of variation was no longer than the “acceptable recovery time R,” which is a societal choice. Based on examples, an acceptable recovery time was settled to 30 years. Where this criterion was applied, Rossberg et al. (2017) showed that this definition naturally leads to (1) related criteria for pressure indicators, and (2) selection criteria for important indicators among a range of candidates and for suites of indicators.

This approach implies that it is not always necessary that the targets of MSFD indicators aim at restoring natural or near-natural ecosystem states. Deviations from natural states are acceptable if recovery to natural states is not too slow. It is acknowledged that some ecosystem components are naturally much less resilient than others; and therefore the focus is paced on indicator-based assessments of these low-resilience components that recover slowly after pressures have been removed or decreased. Rossberg et al. (2017) then argued that state indicators and pressure indicators should always be used jointly in assessments of sustainable use, because due to the slow recovery of low-resilience ecosystem components, there is no immediate relationship between states and pressures. If an ecosystem component recovers quickly after the relaxation of pressures, there is little concern that it might be used unsustainably. The approach by Rossberg et al. (2017) aims to define status boundaries that ensures sustainable use of ecosystem services. It is therefore focused primarily on protecting the interests of future generations. Moreover, the status assessments (within these boundaries) should be complemented by considerations of their suitability for current societal needs. They recommend that these two kinds of assessment, and management decisions based on these, should be carried out by separate management bodies to avoid potential conflicts of interest.

DISCUSSION

Indicator Development and Gaps Addressed

The new developments and refined indicators by DEVOTES addressed some of the gaps identified with respect to the MSFD criteria and indicators in different marine regions where possible. In addition, DEVOTES indicator development focused on those biological components and habitats where monitoring data and the expertise in the DEVOTES research consortia were available.

Some of the indicators developed and refined were already established indicators, meaning that those are applied in the management frameworks such as EcoQO indicators (i.e., the OSPAR System of Ecological Quality Objectives—EcoQO—for the North Sea), or in the national MSFD monitoring of the EU member states (Table 3). However, many of the new indicators (such as new genetic indicators for microbes) are not yet used in the marine assessments meaning that those need to be tested in different marine regions and approved by the national

and regional managers to be part of the MSFD monitoring and assessment programmes. Many of the NIS indicators were evaluated as relevant for MSFD, but those are not yet included in the marine monitoring programmes. Most of the fish and macrozoobenthic indicators developed and refined are used for food web and benthic integrity and biodiversity assessments being more mature for assessment purposes, while many of the phytoplankton and zooplankton indicators were judged not to be particularly useful for MSFD purposes, besides the new indicator on Phytoplankton food quality traits, that is currently considered as a candidate for a HELCOM core indicator.

Two of the identified gaps were the absence of indicators for biodiversity on a genetic level (Descriptor 1, criteria 1.3. *Genetic structure of populations*) as well as the lack of indicators for microbial communities. A DNA metabarcoding technique was applied to develop indicators to assess both microbial communities (Berg et al., 2016) and benthic invertebrate biodiversity (Aylagas et al., 2014, 2016). Genetic methods such as nucleic acid microarrays were considered a suitable methodology to quickly determine diversity and abundance of microbial communities (DeSantis et al., 2007). Marine prokaryotes respond rapidly to environmental changes and anthropogenic pressures and are thus considered as useful components for the assessment of microbial biodiversity, and impacts of eutrophication and toxic substances (Caruso et al., 2015). When the genetic methods were applied to identify microbial and benthic organisms in already established and tested indicator methodologies such as AMBI (Borja et al., 2000), they appeared to provide a cost-effective and robust methodology for biodiversity assessment. Development of genetic tools is expanding rapidly and more effort on benchmarking and standardization will be needed to enable the use of genetic tools in biodiversity assessments in the future (Aylagas et al., 2016).

We also developed indicators to assess habitats of key species as indicators of biodiversity on ecosystem functions. Keystone species, such as species of seagrasses, kelps, and intertidal algae are recognized as effective ecosystem engineers forming vegetated habitats with multiple ecosystem functions including the stimulation of biodiversity (Gutiérrez et al., 2011) and the mitigation of climate change (Duarte et al., 2013). As increasing human pressures on coastal ecosystems threaten the continued supply of essential functions and services, the protection of marine vegetated habitats should be a management priority (Duarte et al., 2013). The high number of indicators available for vegetated habitats (Table 1) suggests that this is already well recognized and that these indicators (such as lower depth limit of macrophytes) are used in practice in marine assessments for policy purposes. In a recent review on seagrass indicators globally, Roca et al. (2016) carried out a meta-analysis and compared the applicability of different types of seagrass indicators to detect environmental improvement. They concluded that physiological and biochemical indicators are more suitable due their fast response to changes of environmental stressors than structural indicators (e.g., shoot density or biomass). Moreover, there is global and local evidence that biodiversity and top-down control strongly influences the functioning of threatened vegetated ecosystems, and indication

that biodiversity is comparably important to global change stressors (Duffy et al., 2015; Matheson et al., 2016). Therefore key stone species prove well suited as operational indicators of biodiversity at the levels of species, habitat and ecosystems.

One of the major global threats to marine biodiversity is the spreading of invasive NIS (Costello et al., 2010). There are a number of regulations for controlling NIS, including the MSFD that aim to restrict the spreading of new NIS (Ojaveer et al., 2014). However, the intrinsic complexity of NIS for being detected on time implies the need to provide early warning indicators for tracking potential vectors of invasions as well as to assess the impacts of NIS on native species, habitats, and ecosystems. Therefore, DEVOTES developed and refined indicators to assess the impacts of invasive species which can be used to identify vulnerable areas, environmental targets and to prioritize management actions.

The MSFD also calls for indicators that address the deviations from the normal structure and functioning of the marine food webs. Such indicators would reflect distortions in the top-down control or bottom-up regulation of the food webs. The cascading impacts of top-down control reach into all trophic levels of food webs. As an example, removal of large predatory species can cause a relief on lower trophic levels causing increase on zooplanktivorous fish and thus, change composition and biomass zooplankton communities. Likewise, the bottom-up regulation based on the changes in the phytoplankton composition and quality of algae as a food source for zooplankton can change productivity and species composition of zooplankton impacting the fish communities. D4, food web, was identified to have relatively few indicators (Table 1), and DEVOTES developed and refined indicators that can be used to assess different aspects of food web structure and functioning, such as the abundance and distribution of fish indicators, productivity of key trophic groups (phytoplankton primary production) and phytoplankton composition as food web indicator providing an indication of the palatability of phytoplankton for zooplankton.

Some of the indicators developed or refined in the DEVOTES project were evaluated to show poor responsiveness to pressures (Table 3, Figure 1). Nevertheless, we suggest maintaining these ecologically relevant indicators in the assessment suites, particularly if they are collected on the side of other monitoring programmes and the monitoring would not be very costly. They could then be used as surveillance indicators and applied to complement the indicators with clear pressure-state links (ICES, 2014). Some factors used in the assessment of marine ecosystem health do not have a very clear pressure-state relationship (ICES, 2014). Many foodweb components are simultaneously affected by multiple pressures and processes, showing response to cumulative and synergistic effects, and singling out the effect of any one of those may be impossible. These surveillance indicators would be supplementary and provide information of the overall “health status” of the ecosystem even in the absence of clear pressure-state relationships.

Despite the current work to supplement the indicator suite already included in the DEVOTool catalog, important gaps still remain; many of the biological components and habitats are not adequately monitored to allow development and testing of

potential indicators. Reptiles, such as the European sea turtles, *Caretta caretta*, *Chelonia mydas*, and *Lepidochelys kempii*, are all either endangered or vulnerable species (IUCN Red List), and thus require conservation measures through marine protected areas. There are specific regional or local programmes for sea turtle protection (such as Sea Turtle Protection Society of Greece ARCHELON) that provide information of the status of local populations and advocate conservation measures. Likewise, Cephalopods represent a group of species with only few existing indicators included in the DEVOTool Indicator Catalog. A recent ICES report on Cephalopod biology and fisheries stated: “[*Despite the importance of several species for European fisheries, there is limited management of the fisheries and no routine assessment; data collection is often either not part of routine fishery data collection or the data are inadequate for assessment. Increasingly, however, cephalopods are seen as alternative target species to replace overexploited finfish stocks, and the growing fishing effort means that management will almost certainly be needed within the next few years. Also on the horizon is the development of commercial aquaculture.*]” (Jereb et al., 2015).

One of the major oceanic ecosystems lacking indicators is the deep-sea habitats and their respective communities (Teixeira et al., 2014). The deep pelagic ocean and deep seabed ecosystems represent the largest biomes of the global biosphere, but still the knowledge of their biodiversity, habitats and processes is scarce (Webb et al., 2010; Danovaro et al., 2014). The deep-sea physical, biogeochemical and ecological processes present distinctive features from other marine ecosystems (Danovaro et al., 2014; Thurber et al., 2014), and the specific habitats or ecosystems host very specific communities (Danovaro et al., 2010). Also many deep-sea invertebrates are exceptionally long-lived and grow extremely slowly (Clark et al., 2016). Current evidence indicates that cumulative stressors from e.g., fishing and resource exploitation will cause important and largely unpredictable ecological changes in these biotopes (Gramling, 2014; Clark et al., 2016). Removal of habitat-forming species, decline in diversity, change in abundance and biomass, reduction in distribution, change in community structure, namely its composition and food web structure are a few of the expected impacts (Clark et al., 2016). Climate change will further expose these already vulnerable ecosystems to combined stresses of warming, ocean acidification, deoxygenation, and effects of altered food inputs (Levin and Le Bris, 2015; Rogers, 2015). Due to the attributes mentioned, the recovery capacity of these deep-sea ecosystems is highly limited and predicted to take much more time to recover after pressures have ceased (Clark et al., 2016). Specific metrics of sensitivity of deep-sea fauna and habitats are therefore urgently needed for assessing the risk stemming from impacts and for identifying vulnerable ecological units (Clark et al., 2016). Baselines need to be established for diversity, abundance, and biomass of deep-sea ecosystems, particularly for the less studied pelagic realm and an understanding of ecological processes needs to be developed (Danovaro et al., 2014; Rogers, 2015). Such indicators will allow prioritizing areas for protection and designing more efficient monitoring programmes for the deep-sea realm.

There were not any indicators for the sea ice habitats, as these represent quite marginal habitat in the European regional seas. In the Polar Regions, sea ice habitats are important for the productivity of the sea and harbor rich biological communities and food webs associated with those (see Thomas and Dieckmann, 2010, for further references). Due to the climate change and warming of the Polar Regions, the extension of the seasonal, and permanent ice cover is shrinking with an alarming pace (Dieckmann and Hellmer, 2010). In the Baltic Sea, the seasonal ice cover is mostly restricted to the northern parts, and similarly as in the Arctic regions, it is an important habitat with rich community of ice-associated algae and micro-organisms as well as migrating birds, and as a primary breeding ground for the two seal species (Granskog et al., 2006). Climate change is projected to change further the biology and ecology of the Baltic Sea, including the diminishing duration and extent of the sea ice cover with its consequences to the ice associated biota (Viitasalo, 2012). However, due to the lack of monitoring and indicators for the sea ice habitat and biota, it is not possible to assess their impacts on biodiversity status in the ice-covered sub-basins of the Baltic Sea.

Linking to Other Indicator Based Assessments of Biodiversity

The conservation initiatives worldwide often share common assessment elements and make use of similar baseline information (e.g., Duffy et al., 2013; Pereira et al., 2013). Versatile use of indicators across environmental policies, geographical regions, and spatial scales is apparent as many of methods and biodiversity indicators are applied in several assessment or monitoring programmes (Teixeira et al., 2014). Approximately 30% of the indicators suggested to be applied for MSFD were already used for assessment needs of the other EU Directives or regulations (e.g., Birds and Habitat Directives, Water Framework Directive or Common Fisheries Policy). The need to economize marine monitoring, but yet maintain and enhance operational monitoring networks (Borja and Elliott, 2013) can benefit from careful planning of interoperable monitoring and assessment where the same indicators could be used for several purposes, and combined depending on the needs of each respective assessment purpose.

There are indicators used for MSFD assessments targeting threatened marine species included in the IUCN Red List, as well as several MSFD indicators miming the candidate metrics to Essential Biodiversity Variables proposed by Pereira et al. (2013, e.g., *Abundances and distributions, Taxonomic diversity, Habitat structure, Allelic diversity*). Also the nine Essential Ocean Variables (EOVs) on Biology and Ecosystem health of marine ecosystems under discussion can benefit from operational indicators included in the DEVOTool Catalog (Teixeira et al., 2014) or those refined and developed by DEVOTES (GOOS, 2016). These relate to the “Status of functional groups” and the “Health of living ecosystems” (*Phytoplankton biomass and productivity, Incidence of harmful algal blooms, Zooplankton diversity, Fish distribution and abundance, Apex predator distribution and abundance, Seagrass*

cover, Macroalgal cover, Live coral cover, Mangrove cover). The wider use of environmental indicators applicable for various marine EU legislation and international agreements such as the RSC or the CBD (Zampoukas et al., 2012; Pereira et al., 2013) promotes harmonization between the different assessment systems and allows effective use of monitoring data for different reporting purposes, provided that the indicators satisfy specific quality criteria (Tittensor et al., 2014; Queiros et al., 2016) that should be common for all programmes.

European status assessments of marine biodiversity have chosen an ambitious path, where data-driven indicators with numeric thresholds should be used to depict definite status classifications. Moreover, the purpose is to link indicators to anthropogenic pressures and further to the human activities. Comparison with the assessment approaches in the marine and coastal areas of the United States (U.S.) shows that there is a conceptual difference, which is mainly due to the different understanding of the indicator concept and approaches for setting the assessment thresholds. A general trend in the U.S. assessments is to give scores for different indicators (or assessment questions) and the scoring is based on descriptive definitions. For instance, the National Oceanographic and Atmospheric Administration (NOAA) Marine Sanctuary Programme¹ makes status assessments based on expert analysis. The experts make the status interpretation based on 17 questions and the descriptions of status classes which are elaborated on the basis of monitoring data. Similarly, the NOAA fish stock assessments² and the Sea Turtle Assessment (National Marine Fisheries Service, 2013) are based on four criteria scored by experts and guided by information from the monitoring data. The National Estuarine Eutrophication Assessment (Bricker et al., 2007) uses quantitative thresholds for some eutrophication symptoms (e.g., percentage change in vegetation coverage) but the class boundaries are defined qualitatively using expert knowledge. The NOAA Marine Mammal Assessment (Carretta et al., 2015) is based on monitoring data but the assessment is descriptive, no specific status class is given, and the state of the populations is determined based on the viability analysis of the population. The U.S. National Park Service³ assesses the country's intertidal zone within protected areas based on expert interpretation of data variability and trends. The indicators in the U.S. Environmental Protection Agency (EPA) National Coastal Condition Report (US EPA, 2012) are closest to the European indicator concept, containing some biodiversity aspects, similar to EU MSFD, and using numerical indicator thresholds to define status classes. Due to the relatively strong European consensus on the indicator concept, the US indicators would not likely be applicable in the MSFD context. Though being used successfully in the US-wide assessments such as the National Coastal Condition Report, the US indicators do not have similar marine assessment framework as in the EU which aims at covering all marine elements, regions and pressures in a coherent and coordinated way.

¹<http://sanctuaries.noaa.gov/science/monitoring/welcome.html>

²<http://www.st.nmfs.noaa.gov/stock-assessment/reports>

³<http://science.nature.nps.gov/im/units/nccn/monitor/intertidal.cfm>

Further Research Needs, and Way Forward

The EU MSFD depicts a cyclical implementation and the next assessment of the environmental status of the marine environment is planned to be completed in 2018. Thus, the EU member states and RSCs are currently on the way in planning this assessment. Based on the experiences from the previous MSFD initial assessment (completed in 2012), the EU Commission is on its way to revise the earlier Commission Decision (2010/477/EU) to advice the on-going initial (MSFD Article 8) assessment that is due to 2018. Different aspects of biodiversity will be in the focus of the assessment, as the ecosystem services provided by the living part of the marine ecosystems are strongly dependent on structural and functional status of biodiversity. Monitoring and managing the health of the seas and oceans is highly relevant for the sustainable use of the marine resources particularly in the light of the recent Blue Growth initiatives in Europe (EU's Blue Growth Strategy⁴) and worldwide (e.g., FAO's Blue Growth Initiative⁵) that emphasize the sustainable use of marine resources, but with the expectation that more seafood, energy, and other living and non-living resources can be extracted from the seas. Full ecosystem approach, with concise cover of the marine ecosystem components, is needed for the managers to evaluate that Blue Growth is carried out sustainably, i.e., not threatening the future potential of delivering marine living resources and ecosystem services.

There is a need to have a comprehensive set of indicators, in order to cover all important multifaceted components of marine biodiversity assessments; ideally including both surveillance indicators and those with a clear pressure-state link. Some of the remaining gaps and further specific research needs are presented in **Table 2**. Further development and validation of marine biodiversity indicators requires improved data with better spatial and temporal coverage based on novel monitoring methods. In addition to the tests carried out in the DEVOTES project, more experimental indicator testing is needed to ensure their ecological relevance, robustness, and responsiveness to pressures, and also to enable incorporation into models in order to extrapolate marine assessments for into larger spatial regimes and temporal scales. With the help of combination of different tools, indicators covering both early warming and long-term assessment needs across different spatial scales can be combined into a holistic assessment of marine environment.

CONCLUSIONS

Despite the large number of indicators available for the assessment of marine biodiversity there are needs for further development in order to ensure (1) full ecosystem approach (covering all components of marine ecosystem, and all levels of marine biodiversity), (2) improved indicator confidence and responsiveness to pressures, and (3) consistent approach and methodology for setting thresholds for environmental status assessment. DEVOTool provides a comprehensive state-of-the-art compilation of biodiversity related indicators developed for

⁴http://ec.europa.eu/maritimeaffairs/policy/blue_growth/

⁵<http://www.fao.org/zhc/detail-events/en/c/233765/>

assessment of coastal and marine ecosystems in Europe and elsewhere. This tool is publicly available for marine managers, experts, and NGOs to rank, evaluate, and choose biodiversity related indicators and to find those that fit best to the needs of the regional and local environmental assessments in Europe and worldwide. There is a relatively concise set of indicators for the second phase of the MSFD implementation, however, some important areas like the deep sea habitats, and trophic levels of marine food webs (e.g., microbes) or taxonomic groups (i.e., reptiles) have fewer indicators operational. Moreover, an assessment of ecosystem processes and functions, i.e., the overall status of ecosystem functioning is an area that requires attention in order to understand interrelations between various ecosystem components and how those impact each other under changing anthropogenic manageable and non-manageable external pressures.

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Conceived and designed the paper: AH, TB, LU. Data was analyzed by HT, AB, AH. All authors participated in the interpretation of the data, reviewing the literature, and drafting the paper. Final approval of the version to be published: AH.

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SUPPLEMENTARY MATERIAL

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